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INVESTIGATE THE NATURE OF VIRAL PERSISTENT INFECTIONS USING Hz-1 BACULOVIRUS AS A MODEL SYSTEM

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INTRODUCTION

Essentially most of the viruses have two phases of replication in their life cycle- acute (productive) and persistent infections. It is true not only for many important human viral diseases, such as herpes simplex virus, Epstein-Barr virus, human immunode ficiency virus, and heptitis B virus, but also for many other animals and plants. In insects, it is generally believed that most insect populations in the field or in the laboratory harbor viruses. Under stress, for examples, lack of food, sudden change in temperature, over populated, and infection by other viruses, the persistently infected viruses may be activated and subsequently wipe out most of the insect in that area. Podgwaite and Mazzone (1986) believed that these activated virus could be responsible to the observations of epizootics and the often unexplained sudden collapses of dense insect population in the field.

Viral persistent infection is a long been recognized phenomenum. It is among the most interesting and complex host: parasite interactions in the nature. In fact, any virus, or more generally any obligate parasite that is very destructive to some host must have established in nature a strategy in order to ensure its own survival. Partial persistency, then is almost a necessity for every virus in some hosts.

In this article, we will use Hz-1 insect baculovirus as a model system to investigate the nature of viral persistent infection(s). The physical map of this virus had been constructed. This map was subsequently used to locate the regions where viral RNA were transcribed. More then one hundred viral specific transcripts had been detected during viral acute infection and very interestingly, only one detectable viral specific transcript exists during viral persistent infection.The implication and significance of such observations will be discussed.

BACULOVIRUS CLASSIFICATION

Baculovirus is classified as a family-Baculoviridae (Matthews, 1982). The characterization of this family is as following: an enveloped, rod-shaped virion, approximately 250-420 nm in length, containing a 80-230 kb circular double-stranded DNA genome. Baculoviridae contains only a single genus, *Baculovirus*. Members of this genus are divided into three subgenera 1) Subgenus A consists of the nuclear polyhedrosis virus. The characteristic feature of this group is that many virions are embeded in the inclusion body. 2) Subgenus B consists of the granulosis virus. The inclusion body of this grous is much smaller and each virion is occluded in the individual inclusion body. 3) Subgenus C consists of the viruses which do not have polyhedrin gene and free with inclusion body.

THE DISCOVERY OF HZ-1 BACULOVIRUS

Back to 1970, Hink and Ignoffo established a cell line from adult ovarian tissue of *Heliothis zea*, and named this line as IMC-Hz-1 (Ignoffo *et al.* 1971). In 1973, this cell line was inoculated with the *Autographa californica* NPV (AcNPV) by Vail *et al.* (1973). They reported that this cell line appeared nonsusceptible to most insect viruses or gave unexpected cytopathic effects.

Since viral persistent infection is suspected to be existed in IMC-Hz-1 cell line, Granados and coworkers decided to investigate the properties of this line more closely. They examined the cells with electron microscope and found enveloped, rod-shaped nucleocapsid structures existed in the nucleus. They transmitted this virus to *Porthetria dispar*, *H. zea*, *Spodoptera frugiperda* (SF 21), and *Trichoplusia ni* (TN 368) tissue culture cells. These lines became granulated and died soon after inoculation. No occlusion bodies were observed as typically found late in subgenus A or B baculovirus infection. Through these studies, it was discovered that the IMC-Hz-1 cells were persistently infected.

Hz-1 virus is a nonoccluded baculovirus and does not share significant sequence homology with the HzNPV and many other baculovirus. Smith and Summers (1982) performed an extensive comparison of DNA homologies among subgenus A, B, and C baculovirus. They found that the DNA sequence homology between Hz-1 virus and that of most other baculovirus are minimum except that of *Heliothis armigar* and *Plodia interpunctella* granulosis virus. Since Hz-1 virus does not have inclusion body, and, according the experiment of Wood and Burand (1986), this virus also does not have significant homology with baculovirus polyhedrin gene argues it is a member of subgenus C baculovirus. Nevertheless, the detectable sequence homology between Hz-1 virus and granulosis virus indicates that they may be related evolutionally.

HZ-1 BACULOVIRUS AS A MODEL SYSTEM FOR STUDYING VIRAL PERSISTENT INFECTION

Hz-1 virus has long been recognized as the best model to study insect viral persistent infection (Burand *et al.* 1986; Wood and Burand, 1986). If Compare this virus with two model mammalian viral persistent infection systems- herpes simplex and Epstein-Barr viruses, Hz-1 gains more advantages over the other two.

The disadvantage of herpes simplex virus system is obvious. So far, there is no cell culture system to support the persistent infection of herpes simplex virus. Epstein-Barr virus is another commenly used system, also gains some disadvantages: 1. It is not easy to produce large quantity of viral particle for doing experiment. 2. It is difficult to perform plaque assay using this virus, if not completely impossible. Hz-1 virus, in constrasts to those two afore-mentioned model systems, the advantages are as following: 1. This virus is not a mammalian pathogen. Thus, it will not threat the helth of the people working in the laboratory. 2. It can be propagated into large quantity easily. 3. It can infect many lepidopteral insect cell lines acutely and persistently. The last feature is very useful for studying the interactions between the virus and different hosts.

THE PHYSICAL PROPERTIES OF THE VIRUS

Viral particles

From electron microscopy, two types of viral particles can be observed routinely (Fig. 1). Uniformed virus particle with size 420 nm can be isolated using plaque purification. This virus is referred as standard virus. If such virus is

Fig. 1. Electron micrograph of Hz-1 viral particles. Single arrows indicate standard virual particles. Double arrows indicate defective interference viral particles. bar=500 nm

passed with high moi through cell lines, gradually, virus with heterogenous sizes can be found. These viruses are referred as defective interference (DI) particles (Wood and Burand, 1986). DI particles basically contain the same genome as what contains in the standard virual particles except that their DNA may be deleted as much as 49% of the total genome (Burand and Wood, 1986; Chao and Wood, 1989, manuscript in preparation).

Viral physical map

Hz-1 virus contains an enomous double stranded circular DNA genome. By summing up the restriction fragments, Burand *et al.* (1983b) calculated the size of this virus to be 233 kb. A similar value, 230 kb, was determined by Huang *et al.* (1982) using reassociation kinetic analysis method.

The regulation and switch of Hz-1 gene expression during persistent and productive infections have not been investigated. As a first step towards a more complete understanding of these processes, we decided to generate a physical map of the Hz-1 genome. We reasoned that a map is necessary for finding all the transcripts and finally locates the gene(s) which is/are important for switching acute:persiotent infections.

Since the virus is enomous the work became a big task. When the viral DNA is digested with frequently used enzymes, multifragments are generated (Fig. 2). The approach we used to construct physical map was as following: As the first step, viral DNA was partially digested with restriction enzymes XhoI and HindIII. These fragments were then ligated into a cosmid vector pVK102 (Knauf and Nester, 1982; and Leisy *et al.*, 1984). Since the original DNA fragments were partially digested, the isolated cosmid clones should contain overlapping restriction fragments. These overlapping fragments were subsequently lined up to construct the physical map of the whole genome.

Figure 3A shows HindIII cosmid clones which contain the HindIII restriction fragments from Q to K, b to c, W to V and H to G. The derived HindIII library contained four unconnected regions. Figure 4 shows the partial physical map derived from the XhoI cosmid library included restriction fragments from S to V, O to Q and W to U, leaving three unconnected regions. The three unconnected regions in the XhoI overlapping cosmid insertions match very well with those three unconnected regions in the HindIII cosmid library (Fig. 3A, B). In an unsuccessful effort to connect these three regions of the genome, more than 2000 HindIII clones and 1000 XhoI clones were screened. As far as the sizes all of the Hz-1 XhoI, HindIII fragments are concerned, the 33 kb XhoI A fragment is the only one beyond the size capacity for pVK102 cosmid to be cloned (Knauf and Neter, 1982; Leisy *et al.*, 1984). Another approach to clone the missing HindIII D, I, and A and XhoI H, G and D fragments into pBluescript plasmid (KSM+) (Strategene

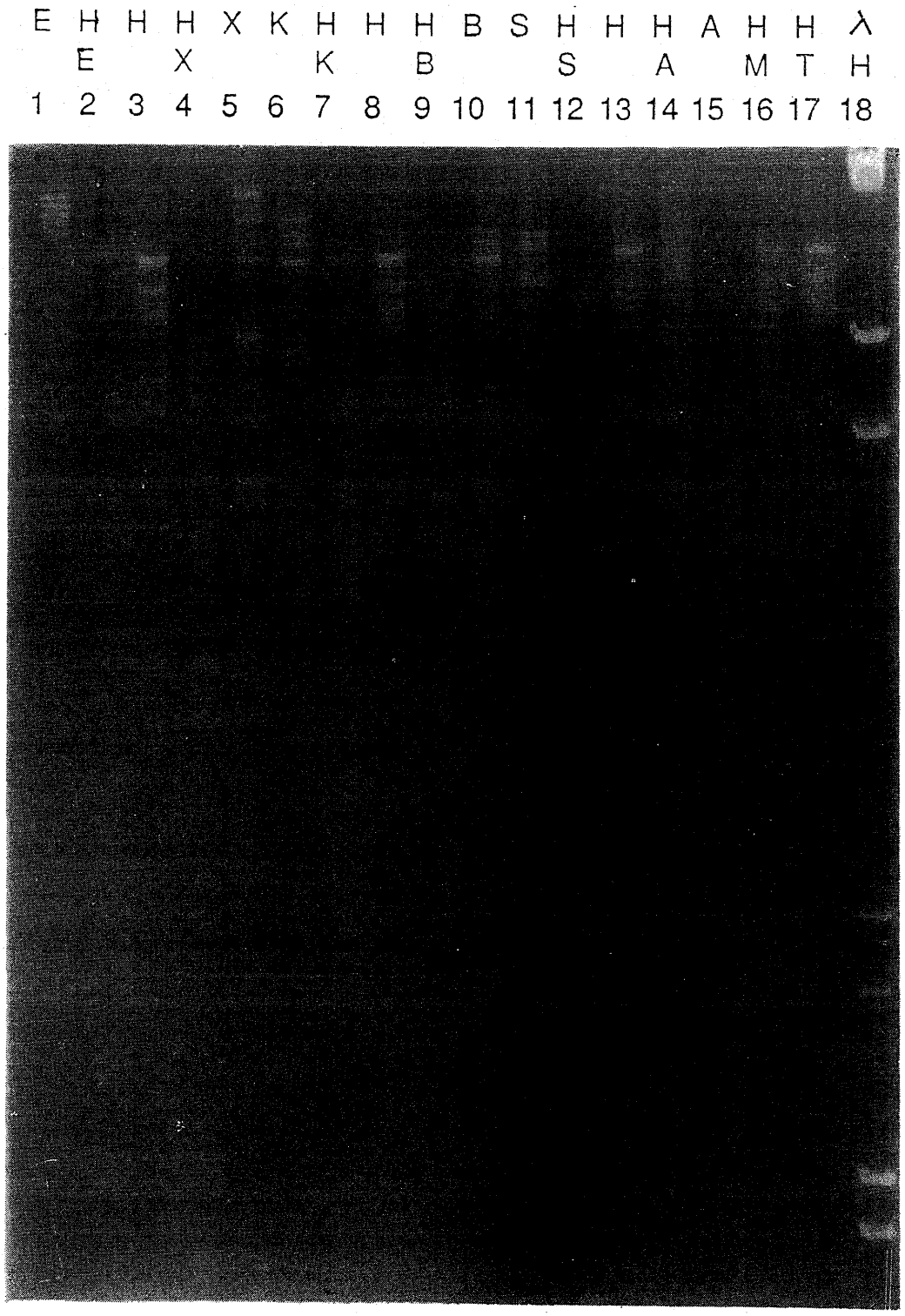
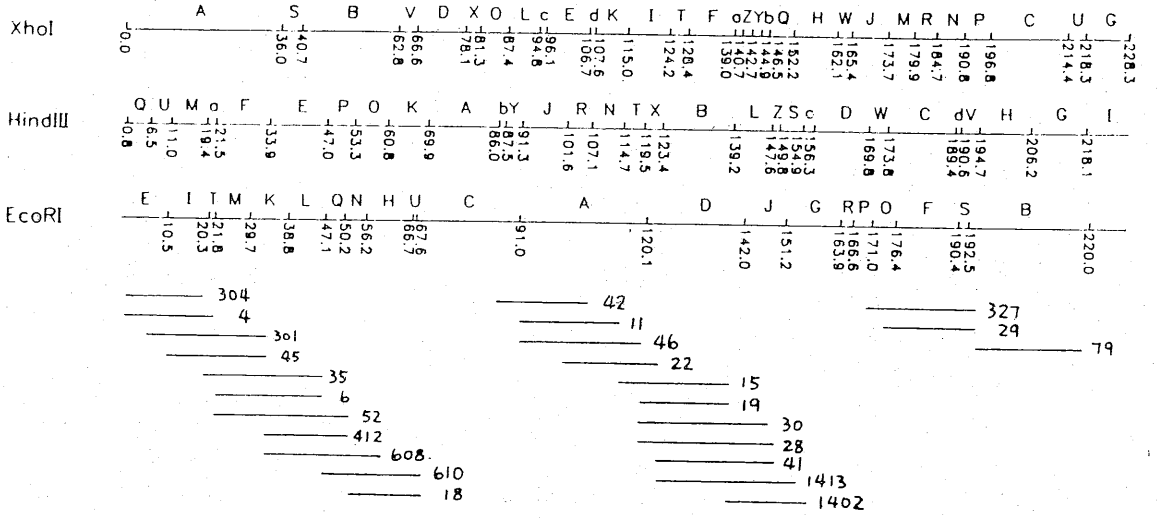


Fig. 2. Hz-1 DNA was single or double digested with EcoRI (E), HindIII (H), XhoI (X), KpnI (K), BamHI (B), SalI (S), XBaI (A), SmaI (M), And SstII (T). These digested fragments were electrophoresed in 0.8% agarose gel, and stained with ethidium bromide. HindIII fragments are included as size markers.

A. Hind III fragments contained in four groups of overlapping pVK102 cosmid clones isolated from Hind III libraries.



B. Xho I fragments contained in three groups of overlapping pVK102 cosmid Clones isolated from Xho I library.

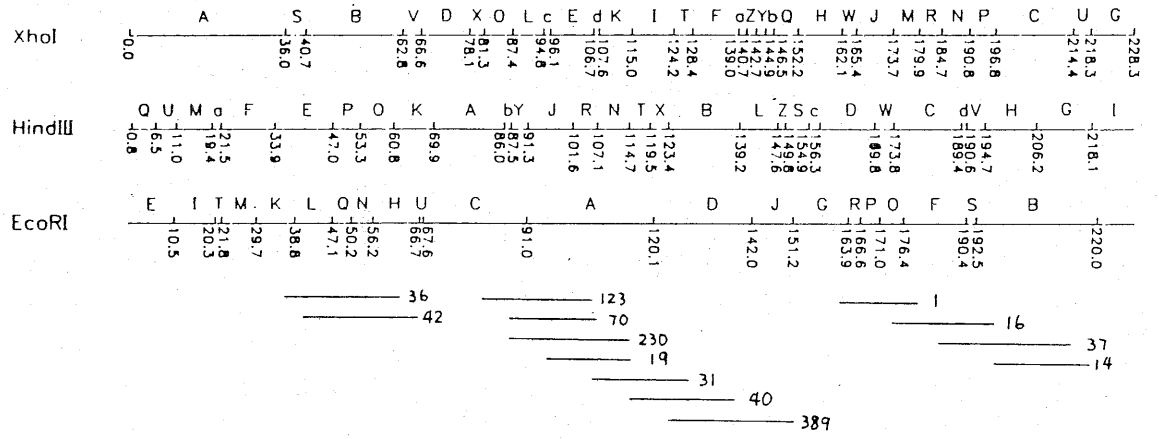


Fig. 3. A. Hz-1 HindIII fragments contained in four groups of overlapped pVK 102 cosmid clones. B. Hz-1 XhoI fragments contained in three groups of overlapped pVK102 cosmid clones.

Co.) were also unsuccesful (unpublished data).

In order to connect the regions mapped by overlapping HindIII and XhoI clones, DNA fragments which mapped at the border of the gaps were labeled and served as probes for hybridization to Southern blots of total viral DNA restricted with HindIII, XhoI and EcoRI. The corresponding HindIII and XhoI physical maps were aligned based on data obtained from single and double restriction digests of the HindIII and XhoI cosmid clones and confirmed by Southern hybridizations

using labeled restriction fragments. An EcoRI physical map was also constructed by the digestion of HindIII cosmid clones singly and doubly with EcoRI and HindIII restriction enzymes. For those fragments or regions missing in the cosmid clones, the EcoRI map was completed by Southern hybridizations using junction fragments. Finally, gel purified EcoRI G, E and C fragments were used as probes to confirm the connections in the three non-overlapping regions of the XhoI cosmid library. Based on these data the Hz-1 genome is a covalently closed, circular DNA as earlier described by Huang *et al.* (1982). Unlike the *Oryctes* (Crawford *et al.*, 1985) and other baculovirus genomes (Cochran *et al.*, 1986), no reiterated sequences were detected in this study. The Hz-1 genome is approximately 100 kb larger than the non-occluded *Oryctes* baculovirus genome and DNA hybridization experiments have indicated that both Hz-1 and *Oryctes* viruses are unrelated (Burand and Wood, unpublished data).

Deletion maps of DI particles

DNA was purified from cloned isolates which contained DI particles and analyzed following digestion with EcoRI. Since, standard virus may serves as helper virus, it has to present in all DI isolates. Deletions in the DNA of DI particles were assessed on the basises of decreased or increased molar ratios and the appearance of additional fragments. Types of DI particle containing isolates are illustrated in Figure 4.

Isolate III2 DI particle has a deletion which encompasses the EcoRI A, C, H and N fragments. The additional DNA fragment which co-migrates with the EcoRI G fragment, hybridizes with EcoRI A fragment (data not shown) and

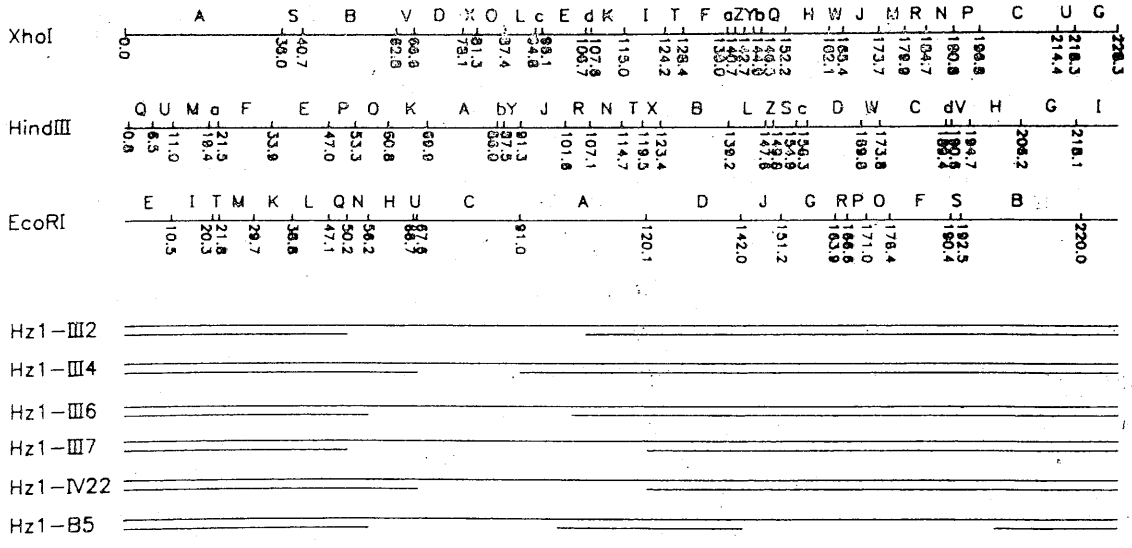


Fig. 4. Illustration of deleted DNA molecules from DI clones. Single line regions show where deletions took place.

therefore arises from a partial deletion in the EcoRI A fragment. Isolate III4 has a deletion in the EcoRI C fragment resulting in the appearance of a new 13 kb fragment. Isolate III6 has a deletion in the EcoRI A, C and H fragments. The new 15 kb fragment arisses from a partial deletion in the EcoRI A fragment. Isolate III7 has a deletion which encompasses the EcoRI A, C, and H fragments. Isolate IV22 has a deletion within EcoRI A and C region. The last one was delived from Burand *et al.* (1986). We intepret their electrophoresed pattern of Hz-1 DI DNA and found that there are two deletions in the DI viral genome. The first one took place at the region spanning EcoRI H, U, C, and A region. The second one covers EcoRI J, G, R, P, O, F, S, and part of B fragments.

MAPPING VIRAL SPECIFIC TRANSCRIPT

Since the possibility exists that some Hz-1 viral mRNAs do not carry poly (A) track (Tun-Chuan and Weaver, 1982, Lubbert and Doerfler 1984), total RNAs were isolated 2, 4, 6, 8, 10 and 12 hrs after standard viral infection. Total RNAs were also isolated from two persistently infected cell lines SFCI and TNP3. They were electrophoreses using agarose gels and blotted into nylon filters. DNAs containing 98% of Hz-1 viral genome from either cosmid clones or gel purified viral fregments were used as probes.

Within 2 h after viral infection, a total of 34 viral specific transcripts were detectable. It was notable that there were three transcripts expressed very strongly almost immediately after viral infection and only trace amount remain ing detectable 12 h after infection. Except a 3.0 kb RNA transcript which was hybridized by probe H-35, all RNAs being transcribed within 2 h after infection were expressed transiently (Table 1). Since there were four distinct classes of transcripts gave rise 2 h, 4 h, 6 h and 8 h after viral infection, they probably correspond to the immediate early, delayed early, late and very late gene products of the viral genome.

In general, Hz-1 viral transcripts are well dispersed. However regions covered by the cosmid H-610, H-42, H-22, H-41, H-1402 and H-79 inserts hybridized predominantly to both earlier or later transcripts. The other region which is covered by the cosmid H-4 insert and the viral HindIII fragment I hybridized predominantly to the early gene products (2 h after viral infection). Since cosmid H-4 insert adjacent to HindIII fragment I, the whole region from HindIII fragment I to H-4 fragment may be assigned as the immediate early gene region. The next region from cosmid H-4 insert is cosmid H-35 insert, which contains HindIII fragments a, F and E, also hybridized predominantly to early gene products (at least 6 transcripts) and only hybridized to 4 RNAs from later gene classes. Probably

Table 1
Hz-1 transcripts hybridized by Cosmid viral or gel purified viral fregment

Probes	Size of RNAs	2 h	4 h	6 h	8 h	10 h	12 h	SFP2	TNP3
H-4 QUMa	6.8 kb	+							
	5.6 kb	###	##	##	+	##			
	5.2 kb	##	##	+					
	4.4 kb	##	##	##	+	+			
	3.7 kb	+							
	3.2 kb	##	##	##	+	+			
	1.7 kb	+	+						
H-35 aFE	9.9 kb	+	+	+	+	+			
	7.2 kb	+	##	##					
	5.8 kb		##	##	##	##	##		
	5.2 kb		+	+	+	+	+		
	4.2 kb	+	+	+	##	##	##		
	3.8 kb	+	+	+	###	###	##		
	3.0 kb	###	###	###	###	###	###	+	##
	2.3 kb	##							
	1.6 kb	##	##	##	##	##	##		
	1.3 kb		###	###	####	####	####		
	1.0 kb		+	##	##	##	##		
H-610 POK	7.2 kb				+	+			
	4.2 kb		+	+	+	+			
	3.9 kb	+	+	+	##	##	+		
	2.7 kb		+	##	##	+	+		
	1.6 kb	+	+	+	+		+		
H-A A	7.2 kb			+	+	+			
	6.3 kb	##	##	##					
	5.9 kb			+	+	+			
	5.1 kb			+	+	+	+		
	4.6 kb	##	##						
	4.1 kb	+	###	###	##	##	+		
	3.9 kb			+	+	+			
	3.2 kb	+	+	##	##	##	+		
	2.8 kb	+	+	+					
	2.5 kb			##	+	+			
	2.3 kb			+	+	+	+		
	1.7 kb	+	+	+	+	+	+		
	1.4 kb		+	###	###	###	###		
	1.1 kb		+	##	##	##	##		
	0.7 kb			+	+	+	+		
H-42 bYJR	7.0 kb			+	+	+	+		
	6.6 kb			+	+	+			
	5.8 kb		+	##	##	##	##		
	5.0 kb		+						
	4.7 kb			###	###	###	###	###	
	4.0 kb		+	+	+	+	+		
	3.0 kb		##	###	###	###	###		
	1.8 kb		+	+	+	+	+		
	1.6 kb		+	+					
	1.5 kb		##	##	##	##	##		

Table 1 (continued)

Probes	Size of RNAs	2 h	4 h	6 h	8 h	10 h	12 h	SFP2	TNP3
H-22 RNTX	5.1 kb			+	+	+	+		
	4.3 kb		+	+					
	4.2 kb		+	+	+	++	+		
	3.2 kb		+	++	+++	+++	++		
	1.9 kb		+	++	++	++	++		
	1.4 kb			+	+	+			
	1.2 kb			+	+	+	+		
H-41 BLZ	5.8 kb			+	+				
	5.2 kb			+	+	+			
	4.6 kb		+	+	+	+			
	4.4 kb	+	+	+	+				
	4.2 kb		+	+	+	+			
	3.5 kb		+	+	+	++	+		
	3.2 kb	+	+	+	+	+	+		
	2.6 kb			+	++	+++	++		
	1.9 kb		+	+	+	+	+		
	1.8 kb			+	++	+	+		
	1.5 kb		+	++	+++	++	+		
	1.2 kb		+	++	+++	++	++		
	1.0 kb			+	+	+	+		
H-1402 LZSc	5.1 kb		+	++	+	+			
	4.5 kb			+	+	+			
	2.8 kb		+	+	+				
	2.4 kb				+++	++	++		
	1.5 kb			+	+	+	+		
	1.2 kb			+	+	+	+		
	0.9 kb				++	++	++		
H-D D	9.9 kb	+	+	+					
	6.6 kb	++	++	++	+	+	+		
	5.1 kb	++	++	++					
	5.0 kb	+++	+++	+++	+++	+++	+		
	4.1 kb	+	+	+	++	++	+		
	3.5 kb	+	+	+	+	+	+		
	3.0 kb	+	+	+	+	+	+		
	2.6 kb		+	+	+	+	+		
	1.8 kb	+	+	+	+	+	+		
	1.7 kb		+	++	++	+	+		
	1.4 kb		+	++	++	+	+		
X-16 MRNP	7.1 kb				++	++	++		
	5.0 kb				+	+	+		
	4.3 kb				+	+	+		
	3.4 kb			++	+++	+++	+++		
	2.8 kb				+	+	+		
	1.9 kb			+	+	+	+		
	1.8 kb			+	++	++	+		
H-79 HG	7.2 kb				+	+	+		
	4.8 kb				+	+	+		
	3.6 kb				+++	++	++		
	3.1 kb				++	+	+		
	2.0 kb		+	++	+++	+++	+++		
	1.7 kb				+	+	+		
	1.2 kb				+	+	+		
H-I I	6.2 kb	+++	+++	+++	++	++	+		
	1.7 kb	+	+	+	+	+			

the previously described putative early gene region extends into this region (Table 1).

Burand *et al.* (1983a) examined the synthesis of Hz-1 virus specific intracellular and structural proteins following the sequence of their appearance. They found that the replication of Hz-1 virus could be divided into three stages: (1) The early stage which includes events occurring up to 4 h postinfection, (2) The intermediate stage including events occurring between 4 and 8 h postinfection, and (3) The late stage which includes all virus-specific events occurring after 8 h postinfection. Although without more experiments, it is not easy to correlate the appearance of viral proteins and mRNAs, in general, these data agreed with ours very well.

It was shown that Hz-1 persistently infected insect cell lines produce infectious viral particles and refrectory to the homologous viral infection. When their total RNA were purified and viral specific RNA compared with those from viral productive infected cell lines, results were very surprising. Regardless the existence of more than one hundred viral specific RNAs under productive infection,

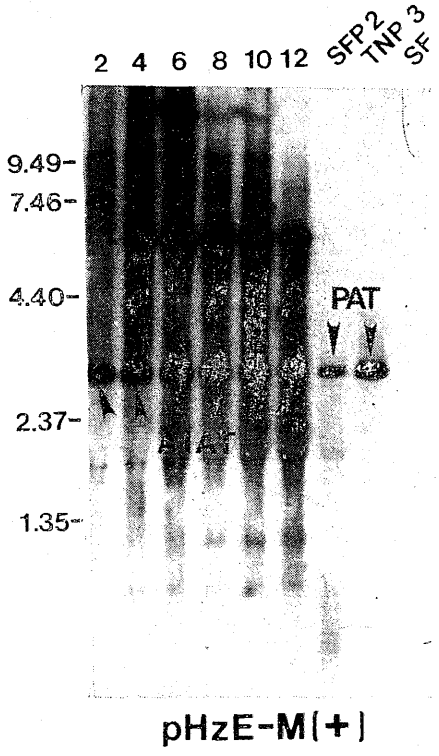


Fig. 5. Northern analysis using strand specific RNA probes from EcoRI M fragment. Plus-RNA probe (pHzE-M(+)) was transcribed from plasmid pHzE-M by using T3 polymerase and hybridized to a constitutively expressed 3.0 kb RNA species (AIAT) during acute infection and to an only dectable RNA species (PAT) with the same size during persistent infection.

there was only one viral specific RNA with size 3.0 kb could be detected from either early or late persistently infected cells. Also, regardless of cell types, both persistently infected TN or SF cell lines gave rise the same 3.0 kb transcript which were hybridized by the same probe from cosmid H-35 insert. The size of the unique 3.0 kb transcript detected from persistently infected cell lines obviously identical to a 3.0 kbcon stitutively expressed "early" transcript detected from productive infection. We therefore refer the 3.0 kb transcript detected during persistent infections as persistency associate transcript (PAT) and the other 3.0 kb transcript detected during productive infection as acute infection associated transcript (AIAT). During the course of productive infection, this was a constitutively expressed RNA species (Table 1 and Fig. 5). Total viral DNA was also used to survey the possible missing viral specific transcripts during persistent infection. Still, the 3.0 kb RNA was the only detectable trancript (data not shown).

It is of interesting to find that PAT was the only detectable viral specific RNA in both early (1 month) and late (3 years) persistently infected cell lines. More interestingly, this 3.0 kb PAT, as judged from their size, probe hybridization, and stranded determination, it may be the same as AIAT. AIAT was the only "early" gene product which remains at a constent level throughout 2 to 12 h after viral infection. There are at least two explanations for the continuously exist of the PAT. First, it may be a constitutively expressed transcript. Second, it may be very stable. However, since PAT existed in a three years old persistently infected cell line, TNP3, RNA stability alone was obviouly insufficient to explain.

RNA STRAND DETERMINATION

In order to study the identity of both 3.0 kb transcript from either productive or persistent infections, the use of strand specific probes is necessary. However, cosmid H-35 contains a relatively big insert. Therefore, before doing strand specific RNA probe hybridization, it is necessary to narrow down the region(s) which may hybridize to both AIAT and PAT. As a first step, we found HindIII fragment F contained in cosmid H-35 was the only region in this cosmid hybridizing to both 3.0 kb transcripts (data nat shown). This HindIII F fragment was mapped to contain a HindIII-EcoRI 0.3 kb, a EcoRI-EcoRI 7.9 kb and a EcoRI-HindIII 4.2 kb DNA fragment in order. The first two fragments together (HindIII-EcoRI 0.3 kb plus EcoRI-EcoRI 7.9 kb) hybridized to both 3.0 kb transcripts but not the third one. This fragment (HindIII-EcoRI 8.2 kb) was then inserted into a pBluescript vector (KSM+, Strategene Co.) and plus or minus strand of ^{32}p labeled RNA probes were transcribed *in vitro* using T 3 or T 7 bacteria phage RNA polymerases respectively. The results of strand specific probe hybridizations are

interesting. We found that by using plus strand of RNA probe, both AIAT and PAT were hybridized (Fig. 5). AIAT was obviously transcribed very early after viral infection and constitutively expressed subsequently. It is also interesting to note that when using minus strand of RNA probe to do northern hybridization, a transiently expressed 3.0 kb RNA (TE 3.0 kb RNA) species was hybridized. TE 3.0 RNA was only detectable at 6 h, reached a peak at 8 h, and decay at 12 h during viral productive infection. This RNA has exactly the same molecular weight with the PAT or AIAT under agarose gel resolution.

Specific mRNA can be functionally inactivated *in vivo* by antisense RNA has well been documented (Strickland *et al.*, 1988, Rosenberg *et al.*, 1985, Izant, T. G. and Weintraub, H., 1984, and Simons R. W. and Kleckner, N. 1983). For the injection of antisense Krüppel RNA, Rosenberg *et al.* (1983) reported a minimal of 50 fold excess the number of sense RNA was needed to result in a phenotypic response. For an endogenous bacteria transposon Tn10 anti-sense IS 10 RNA, Simons and Kleckner (1983) estimated a 20 fold excess against sense RNA was existed. In our strand-specific hybridization experiments we found the TE 3.0 kb sense RNA was expressed transiently. When it reached to a peak at 8 h after viral infection, an estimation of 10 fold excess of PAT exists at that particular moment. For other infection stages, i. e. 6, 10 and 12 h after infection, a 15-40 fold excess were estimated. Such excesses of PAT is practically sufficient to inhibit the activity of TE 3.0 kb RNA if antisense mechanism exists.

CONCLUSION

Hz-1 virus was originally identified from a persistently infected cell line IMC-Hz-1 cell (Granados *et al.* 1978). Later, several groups of insect virologists found that this virus could establish persistent infection easily in a number of lepidopteran cell lines, including TN-368 and SF-21 cells (Ralson *et al.*, 1981 and Burand *et al.*, 1983b). It is obviously that this virus is programmed to shift the infection from productive infection cycle into persistent infection if the host cell is somehow not to be killed after initial infection. PAT is probably an early gene product, which is expressed relatively high very early after viral infection and then constitutively expressed throughout the infection cycle. If it is the gene product for suppressing productive infection and / or maintaining persistent infection, certainly the virus is "ready" to do so at any moment.

Alternatively, PAT may somehow switch the phase into and maintains persistent infection through its protein product just similar as lamda phage lysogeny been established by CII and CIII gene products (Johnson *et al.*, 1979). In order to investigate the possibility that it produce protein product, we labeled two newly established SF (SFC1 and SFC2) and the TNP3 persistently infected cell lines with

^{35}S-methionine. Preliminary result showed, for SF cells, one extra protein could be detected from each of the SF persistently infected cells. The molecular weights of these extra proteins were different, however, one of them was way too big to be encoded by the 3.0 kb PAT. For TN cell, regardless the relative amount of PAT contained in TNP 3 cell was more than those in SFC 2 cell as judged by northern hybridization (Fig. 5), there was no extra protein detected from TNP 3 cell.

Stevens *et al.* (1987) and Spivack and Fraser (1987) reported previously that a latent associated RNA complementary to a herpes virus early gene ICP-O is prominent in latently infected neurons. They proposed this RNA to be an anti-sense RNA which may regulate the expression of ICP-O gene. Such result may provide intringuing hint for the study of the molecular mechanism of Hz-1 viral persistent infection in the host insect cells. How persistent infection were induced or maintained in the cells is still largely unknown. The work on Hz-1 virus persistent infection will help to shed a light on such important though mysterious phenomenon.

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