Intracellular Protein Synthesis During Standard and Defective Hz-1 Virus Replication

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(Accepted 12 September 1985)

SUMMARY

An isolate of the non-occluded baculovirus Hz-1, containing a high level of defective particles, was recovered after serial passage in Trichoplusia ni (TN-368) tissue culture cells. DNA from defective virions contained deletions of up to 91 kilobase pairs. Defective particles were shown to interfere with the infection or replication of standard virions. Standard virus appeared to be required for replication of defective interfering (DI) particles. Initiation of both standard and DI viral DNA replication occurred at 4 h post-inoculation. Virus-induced protein synthesis was studied by pulse-labelling with \[^{35}S\]methionine. During defective virus replication in the presence of low amounts of standard virus, there was a significant reduction in the synthesis of 14 proteins (mol. wt. 109, 93, 92, 90, 85, 69, 57, 50, 40, 27.5, 23, 17.5, 17 and 14, all \(\times 10^{-3}\)) and an increase in the synthesis of five proteins (mol. wt. 104, 75, 41, 37 and 14.2, all \(\times 10^{-3}\)) as compared to standard virus infections.

INTRODUCTION

The evolution of defective viral genomes and defective interfering (DI) virus particles during serial passage \textit{in vitro} has been reported for many RNA and DNA viruses (Huang & Baltimore, 1970; Huang, 1973; Brockman \textit{et al.}, 1973; Henry \textit{et al.}, 1979; Rixon & Ben-Porat, 1979; Kaerner \textit{et al.}, 1979; Tanaka \textit{et al.}, 1980; Nayak, 1980; Frenkel, 1981; Norkin, 1982; Blumberg & Kolakofsky, 1983; Schröder \textit{et al.}, 1984). These defective particles are involved in the establishment and maintenance of persistent infections by many animal viruses. We have recently investigated a subgroup C baculovirus, the Hz-1 virus, which elicits both productive, lytic infections and persistent infections in lepidopteran cell cultures (Burand 	extit{et al.}, 1983b). The Hz-1 standard virus particles elicit productive infections and, following serial passage \textit{in vitro}, a high proportion of defective particles is produced. These defective particles are required for the establishment of persistent infections (Burand \textit{et al.}, 1983b).

The Hz-1 virus was discovered in a persistently infected Heliothis zea cell culture, IMC-Hz-1 (Granados \textit{et al.}, 1978). Its host range \textit{in vitro} includes Trichoplusia ni (TN-368), Spodoptera frugiperda (IPLB SF-212), Heliothis zea (IPLB-1075), Mamestra brassicae and Porthetria dispar (IPLB-65Z) (Granados \textit{et al.}, 1978; Ralston 	extit{et al.}, 1981; Langridge, 1981; Kelly 	extit{et al.}, 1981). Persistent infections have been obtained in the \textit{T. ni} and \textit{S. frugiperda} cell lines (Burand \textit{et al.}, 1983b; Ralston 	extit{et al.}, 1981). Detailed studies concerning the establishment and maintenance of persistent infections have not been reported. Therefore, the following studies were undertaken to investigate the early events which occur during the establishment of persistent infections of \textit{T. ni} cell cultures (TN-368) and to re-investigate the possibility that the defective particles interfere with the infection or replication of standard virus particles. In order to assess differences in the replicative events leading to productive and persistent infections, we also investigated the initiation of viral DNA replication and alterations in virus-induced protein synthesis following inoculations with standard and defective virus particles.
METHODS

Cells and viruses. Trichoplusia ni (TN-368) cells were cultivated at 26 °C in modified TNMFH medium (Wood, 1980). The plaque-purified Hz-1 virus isolate denoted B1 (standard virus) was obtained from a TN-368 cell line persistently infected with the IMC-Hz-1 virus. The B5 virus isolate was plaque-isolated from the previously reported Hz-1 virus isolate (Burand et al., 1983b), generated by 10 serial undiluted passages of the B1 virus in TN-368 cells.

Plaque assay. Virus isolates were obtained following a modification of the plaque assay procedure of Wood (1977). Virus samples and TN-368 cells at 4 × 10⁶ cells/well were seeded into Falcon multiwell plates and centrifuged at 1000 g for 1 h (centrifugal inoculation). The liquid was then removed and the cells were overlaid with 1.0% (w/v) Sea-Plaque agarose (Marine Colloids, Rockland, Me., U.S.A.). After incubation for 3 days at 26 °C, 0.2 ml trypan blue solution (0.5% w/v) was added to each well. The excess dye was removed after 5 min, and the plates re-incubated at 26 °C for 24 h. The resulting plaque isolates were harvested and replicated in TN-368 cells.

Purification of Hz-1 virus and viral DNA. TN-368 cells were infected with B1 virus at 10 to 20 p.f.u./cell. The virus was purified from the cell culture supernatant at 48 h post-inoculation by ultracentrifugation through 20% (w/w) sucrose in 100 mM-Tris-HCl pH 7.5, 10 mM-EDTA (TE buffer) as described by Burand et al. (1983b). Supercoiled virus DNA was extracted from B1 and purified on CsCl-ethidium bromide (EtBr) gradients (mean CsCl density 1.50 g/ml, EtBr 200 µg/ml) as outlined by Burand et al. (1980).

Labeling of viral DNA. For DNA :DNA blot hybridization experiments, supercoiled B1 viral DNA was labelled in vitro using the procedure of Rigby et al. (1977). DNA labelled using this procedure routinely had a specific activity of 1 × 10⁸ c.p.m./µg. B1 and B5 DNAs were labelled during replication in TN-368 cells with 32P at 100 µCi/ml and purified as outlined by Burand et al. (1983b).

Restriction enzyme analysis of viral DNA. 32P-labelled DNAs were digested with the restriction enzyme EcoRI in Tris-acetate buffer (33 mM-Tris-HCl pH 7-9, 66 mM-potassium acetate, 10 mM-magnesium acetate, 0.5 mM-dithiothreitol) and the resulting fragments separated by electrophoresis in 0.6% agarose gels as described by Smith & Summers (1979). The gels were then dried and subjected to autoradiography as described below.

Detection of interfering particles. For interference assays TN-368 cells were seeded into Falcon multiwell plates at 3 × 10⁵ cells/well. These cells were first centrifuge-inoculated with the B5 (defective virus) at a m.o.i. of 1, washed with Grace’s medium (Wood, 1980), incubated for 1 h, then centrifuge-inoculated with the standard virus (B1) at a m.o.i. of 1 and washed again. Twenty-four h later the resulting virus was titrated by plaque assay and compared with the titre obtained from cells infected with either B1 or B5 virus alone.

DNA :DNA blot hybridization. At various times TN-368 cells infected with either B1 or B5 were collected, washed once with Grace’s medium and resuspended in TE buffer containing 1% (w/v) SDS. After digestion with proteinase K (100 µg/ml) at 37 °C for 4 h, the samples were heated to 65 °C for 1 h and then treated with 100 µg/ml RNase A (pretreated at 95 °C for 10 min) for 1 h. The total DNA in each sample was purified by extracting three times with phenol, once with phenol-chloroform (1:1), and then precipitated with ethanol. The pellets were dried under vacuum and resuspended in TE buffer at 60 °C for 10 min. Slot blots were prepared using a Schleicher & Schüll Minifold II following a modification of the procedure of Kafatos et al. (1979). Samples containing 500 ng DNA were denatured in 0.3 M-NaOH at 65 °C for 30 min, cooled to room temperature, and neutralized by adding 1.0 vol. 2.0 M-ammonium acetate pH 7-0. Pre-wetted nitrocellulose filters were soaked briefly in 1.0 M-ammonium acetate pH 7-0 and placed in the Minifold II filtration apparatus. The DNA was applied using suction filtration, and the filters were heated to 80 °C for 2 h under vacuum. Pretreatment of filters, hybridizations using 34% formamide, and washings were performed as outlined by Wood et al. (1982). Under these conditions there was no detectable hybridization to controls consisting of DNA from TN-368 cells.

[35S]Methionine labelling of intracellular polypeptides. Differences in the intracellular protein synthesis patterns between B1- and B5-infected cells were determined with a 1 h pulse incorporation of [35S]methionine by virus-infected cells as outlined by Wood (1980). At various times the infected cells were starved in Grace’s medium without amino acids for 1 h then labelled for 1 h with 5 gCi [35S]methionine (10 µCi/ml) in the same medium. The cells were then harvested and prepared for SDS-polyacrylamide gel electrophoresis (PAGE).

PAGE. Virus-infected cells were disrupted in dissociation buffer at 100 °C for 3 min as described by Laemmli (1970). Samples containing 10⁵ c.p.m. of incorporated [35S]methionine were electrophoresed in 11% polyacrylamide vertical slab gels (15 × 16 × 0.15 cm) at 6 W/gel (Laemmli, 1970). After electrophoresis the gels were dried under vacuum and autoradiograms prepared.

 Autoradiography. [35S]-labelled proteins and 32P-labelled DNA were detected by autoradiography using Kodak X-Omat RP X-ray film exposed at −80 °C. Autoradiograms were analysed with a Quick Scan R&D densitometer (Helena Laboratories, Beaumont, Tx., U.S.A.) interfaced with an Apple II+ computer. Data analysis for mol. wt. determinations and peak area was performed with Appligraf (Dynamic Solution, Pasadena, Ca., U.S.A.) software.
RESULTS

Characterization of DI particles

Several populations of defective virus isolates were plaque-isolated from B10. All isolates were a mixture of both standard and defective genomes (J. P. Burand & H. A. Wood, unpublished results). The B5 virus isolate, referred to herein as the defective virus, was chosen for these experiments because it most resembled B10 and contained the highest level of defective genomes. The 11 submolar fragments (A, B, C, F, G, H, J, O, P, R and S) detected in EcoRI restriction enzyme profiles of 32P-labelled B5 DNA (Fig. 1) are not present in equal molar amounts. In comparison with B1 DNA, three additional bands were present in EcoRI digests of B5 DNA. Two of these bands co-migrated with the D and N fragments (denoted by the asterisks). The third additional fragment had a size of approximately 16.6 kilobase pairs (denoted by the arrow). These alterations represent a net deletion of about 92 kb.

In interference assays TN-368 cells infected with the standard virus (B1) or DI virus (B5) at a m.o.i. of 1 gave titres of 1.5 x 10^7 and 1.3 x 10^6 p.f.u./cell, respectively. However, inoculations with B5 and then B1 yielded a titre of only 2.2 x 10^6 p.f.u./ml, while inoculation first with B1 then with B5 gave a titre of 4.0 x 10^6 p.f.u./ml. This is a 85% and a 74% reduction in titre, respectively, compared to B1 alone.

Virus-specific DNA replication in both B1- and B5-infected cells was followed by DNA-DNA hybridization (Fig. 2). No hybridization to uninfected control cells was detected. These results indicate that in both cases viral DNA synthesis begins by 4 h and proceeds at approximately the same rate from 4 to 8 h post-infection.

Comparison of B1 and B5 virus-induced intracellular polypeptides

[35S]Methionine pulse-labelling was used to compare the virus-induced protein synthesis of B1- and B5-infected cells. The results of these experiments are shown in Fig. 3 and summarized in Table 1. The rate of synthesis of 11 virus-induced proteins was similar in both B1- and B5-infected cells. However, 21 proteins were different. In B5 there were three proteins (144K, 57K and 25K) which we had not previously identified in Hz-1-infected cells (Burand et al., 1983a). At various times after infection, 14 proteins (109K, 93K, 92K, 90K, 85K, 69K, 57K, 50K, 40K, 27.5K, 23K, 17.5K, 17K and 14K) were synthesized at a lower rate in B5-infected cells than in B1-infected cells. In addition, five proteins (107K, 75K, 41K, 37K and 14.2K) were synthesized in greater amounts in B5-infected cells than in cells infected with B1.

DISCUSSION

Inoculation of TN-368 cells first with a preparation of defective virus particles (B5) and then the standard virus (B1) resulted in a significant decrease in the titre of infectious virus produced as compared to inoculations with only the standard virus. The defective particles therefore interfere with the infection or replication of the standard virus. This interference phenomenon was not observed in our earlier investigations (Burand et al., 1983b), which utilized a focus-forming assay system. We have repeated this work with the same results and are not sure why interference cannot be demonstrated with the focus-forming assay.

Despite repeated attempts to plaque-purify the B5 isolate, several submolar DNA fragments were always present. Differences in the relative intensities of these fragments indicate that the B5 isolate contains a heterogeneous population of DI particles and a small population of standard virus particles. This is consistent with the paradoxical nature of DI particles; they interfere with the replication of standard virus but require standard virus for replication.

A comparison of the EcoRI restriction enzyme profiles of B5 and standard virus DNA demonstrates several alterations in the DI virus genome (Fig. 1). These alterations represent a net deletion of 91 kb in the virus genome. The 16.6 kb fragment (denoted by the arrow) has been cloned and hybridizes with the EcoRI B fragment (27.9 kb) of the standard virus (J. P. Burand & H. A. Wood, unpublished results). We have yet to determine if host DNA sequences have been incorporated into the DI particle genome.
Fig. 1. EcoRI restriction enzyme profile of B1 (standard virus) and B5 (DI virus) DNA. Individual fragments are identified by letters on the left. The arrow indicates the position of an additional 16 kb DNA fragment in B5. Small circles indicate bands present in B5 in decreased concentration, and asterisks indicate bands present in B5 in increased concentration.

Fig. 2. Kinetics of Hz-1 viral DNA replication. Hz-1 B1 (O) and B5 (●) virus-specific DNA replication was measured by DNA :DNA slot blot hybridization. The relative peak area was calculated from densitometry scans of X-ray films exposed to DNA blotted onto nitrocellulose filters hybridized with a 32P-labelled Hz-1 viral DNA probe.

The B5 isolate not only allows us to examine the nature of DI particles of Hz-1 virus but also to compare the initial events of virus replication leading to productive and persistent infections. The rate of viral DNA replication is very similar in DI- and standard virus-infected cells. However, the intracellular protein synthesis patterns in cells infected with these two virus
Fig. 3. Time course comparison of intracellular polypeptides in B1- and B5-infected cells pulse-labelled for 1 h with [35S]methionine, starting at the times (h) indicated. Labelled samples were collected and analysed by PAGE. Only polypeptides which are different in each pair of virus samples are indicated by their apparent mol. wt. (× 10⁻³) shown at the right. Lane M, mol. wt. standards; lane C, uninfected control cells.

Table 1. Comparison of Hz-1 standard and defective virus-specific intracellular protein synthesis

<table>
<thead>
<tr>
<th>Mol. wt. (× 10⁻³)</th>
<th>Detected in DI-infected cells (h post-infection)</th>
<th>Alteration in synthesis*</th>
<th>Structural protein†</th>
<th>Structural glycoprotein†</th>
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<tbody>
<tr>
<td>109</td>
<td>4-10</td>
<td>–</td>
<td>+</td>
<td>+</td>
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<tr>
<td>107</td>
<td>4-10</td>
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<td>93</td>
<td>8-12</td>
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<tr>
<td>92</td>
<td>10-12</td>
<td>–</td>
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<td>90</td>
<td>10-12</td>
<td>–</td>
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<td>85</td>
<td>10-12</td>
<td>–</td>
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<td>75</td>
<td>8-12</td>
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<td>41</td>
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<td>27-5</td>
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<td>23</td>
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<td>17.5</td>
<td>6-12</td>
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<tr>
<td>17</td>
<td>7-12</td>
<td>–</td>
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<tr>
<td>14.2</td>
<td>9-12</td>
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<tr>
<td>14</td>
<td>5-12</td>
<td>–</td>
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* + and – indicate increase or decrease in relative rates of synthesis during DI virus replication compared to the rates of synthesis during standard virus replication.
† Burand et al. (1983a).

preparations contain several interesting differences. Previously, we had identified 37 virus-induced proteins following infection with standard virus, 28 of which were identified as structural proteins (Burand et al., 1983a). Although all 37 virus-induced proteins are synthesized in DI-infected cells, there were alterations in the rate of synthesis of almost half of these proteins. These dissimilarities are not a result of alterations in the proteins themselves but rather due to regulatory differences that occur during standard and DI virus replication. For example, five of the nine major virus structural proteins (93K, 92K, 85K, 69K and 50K) are made at a
reduced rate during DI replication while the synthesis of one structural protein (37K) is increased. It is also interesting that the 109K protein, which is a highly glycosylated intracellular protein (Burand et al., 1985), is present in lower amounts in DI-infected cells, while the 107K protein, which is thought to be a deglycosylation product of 109K (Burand et al., 1983a), is present in increased amounts. The exact function of these particular proteins is not known. However, it is likely that some of the changes reported here are involved in the persistent Hz-1 virus infection.

The pattern of intracellular protein synthesis (Fig. 3) was very similar to that previously reported by Burand et al. (1983a). However, an additional 14-2K protein was detected in this study. This protein was apparently masked by the 14K protein in the previous investigations. The 144K, 57K and 25K proteins detected only in B5-infected ceils may be of host origin or they may be virus-coded proteins synthesized in sufficient quantities to be detected only during DI particle replication.

Since all proteins detected during standard virus replication are found in DI-infected cells and their mol. wt. are unaltered, further investigations are being conducted to ascertain if differences in the relative rates of synthesis are determined by the conservation or deletion of specific coding regions in DI genomes. For example, genes specifically deleted in DI particles might only be transcribed from the standard virus genome, thereby decreasing the rate of translation based on concentration of messenger RNAs. Preliminary mapping studies indicate that deletions in DIs do occur in a single region of the genome (J. P. Burand & H. A. Wood, unpublished results).

The establishment and maintenance of persistent infections involves more than simply changing the synthetic rates of certain viral proteins. Although all the genetic information required for productive, lytic infections is present in the minor population of standard virus, the DI particles alter these events. The results obtained thus far indicate that persistent Hz-1 infections involve a complex interaction between virus and cell as well as between standard and DI virus.

The technical support of Marta Meda and Mardi Verbanic is gratefully acknowledged. This work was supported in part by a grant from the Jessie Smith Noyes Foundation, Inc. to H. A. Wood.

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Defective baculovirus replication


(Received 19 June 1985)