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# Isolation of a Fully Infectious Variant of Parvovirus H-1 Supplanting the Standard Strain in Human Cells

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**A variant H-1 virus, designated H-1 dr virus, was isolated from stock of the standard H-1 virus strain propagated in the newborn human kidney cell line NB-E. Molecular cloning and sequence analysis revealed an in-frame deletion at map positions 39 to 41. This deletion affects the open reading frames encoding the nonstructural proteins NS-1 and NS-2 and the untranslated leader sequence of the R3 transcripts encoding the capsid proteins. In addition, H-1 dr virus harbors a 58-nucleotide duplication inboard from the right-hand terminal palindrome. Internal deletions and terminal reiterations are hallmarks of H-1 virus type I variants that typically are defective interfering particles. Indeed, H-1 dr virus was found to progressively supplant the standard strain in serially coinfected NB-E cell cultures. However, H-1 dr virus differed from previously described type I variants in its full infectivity, as was apparent from its ability to give yields of replication and progeny virus production that were similar to those of the standard virus strain in NB-E cells. Hence, the interference of H-1 dr virus in the propagation of standard H-1 virus in coinfected cells was not accompanied by a drop in the titer of infectious virus. Moreover, H-1 dr virus proved to induce the same pathogenic effects in newborn hamsters as the standard virus strain did.**

Parvoviruses are a large family of viruses which infect animal species from insects to humans. Parvovirus H-1 belongs to the subgroup of autonomously replicating parvoviruses of vertebrates (for reviews, see references 1 and 4). H-1 virus contains a linear, single-stranded genome of about 5 kb, which comprises two overlapping transcription units (19) (Fig. 1C). The early promoter, P4, directs the synthesis of a transcript whose spliced derivatives, R1 and R2, encode the nonstructural proteins NS-1 and NS-2, respectively. NS-1 and NS-2 are phosphoproteins which share their 85 N-terminal amino acids but differ in their C-terminal portions as a result of the splicing process. The late promoter, P38, regulates the production of the viral capsid proteins VP-1 and VP-2, which are encoded by alternatively spliced R3 transcripts.

The growth of animal viruses in eukaryotic cells frequently results in the production of variant particles with altered genomes. Parvoviruses are no exception to this rule (2). Variant genomes of minute virus of mice, a close relative of H-1 virus, belong to two distinct classes. Type I DNA molecules bear internal deletions and may have terminal reiterations, while type II variant genomes consist exclusively of 5′-terminal sequences in a stem-loop configuration (for a review, see reference 6). All of the H-1 virus variants that have been characterized can be classified as type I since they are deleted internally but retain both genomic termini (15, 16). The majority of changes in H-1 virus variant genomes are due to additions between map units (m.u.) 91 and 95.7 and deletions in the regions 80 to 91 m.u. and/or 32 to 44 m.u. (15–17). Additions are caused by multiple reiterations of a 55-nucleotide (nt) element that is close to the origin of replication for viral strand synthesis. Internal deletions are located in the region of the late P38 promoter, making these variant viruses deficient in capsid protein production and dependent on wild-type helper virus for propagation. Among the H-1 variant viruses analyzed so far, only H-1-DI-1 virus is viable, although it grows poorly and produces very small plaques (15). The only known alteration in the genome of H-1-DI-1 virus is the duplication of a 58-nt repeated sequence inboard from the right-hand terminal palindrome (2, 15). All H-1 virus variant particles, including H-1-DI-1, have proved to have interfering properties, i.e., to cause a drop in infectious virus production in cells coinfected with the standard strain (15).

**Isolation of a nondefective H-1 virus variant.** In the course of routine plaque purification of wild-type H-1 virus produced in the simian virus 40-transformed newborn human kidney cell line NB-E, virus genomes originating from 10 plaques were studied by restriction enzyme digestion and subsequent Southern blotting analysis. While H-1 virus DNA from three plaques had the restriction pattern expected from the genome of the standard virus strain (hereafter designated H-1 st [19]), the seven other DNA preparations were characterized by internal *Eco*RI-*Hin*dIII and 5′-terminal *Hin*dIII fragments that were about 100 nucleotide pairs shorter and longer, respectively, than the corresponding H-1 st virus fragments (Fig. 1A). Since all variants were indistinguishable in this analysis, one stock, designated H-1 dr virus, was used for further investigation.

The restriction pattern alterations exhibited by H-1 dr virus DNA are reminiscent of the previously reported genomic variations of type I defective interfering H-1 virus particles, which

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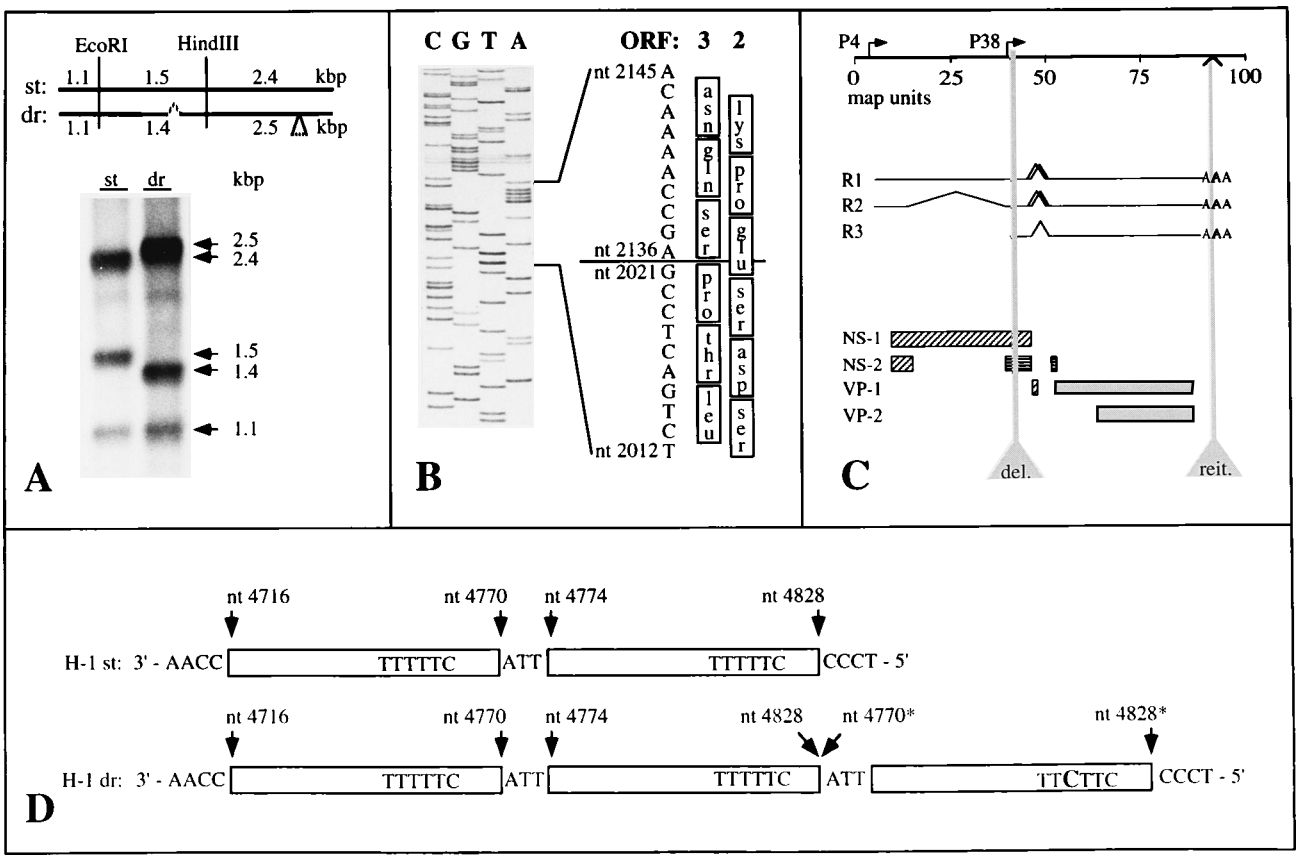


FIG. 1. Comparison of variant (dr) and standard (st) H-1 virus DNAs. (A) Restriction enzyme mapping of genomic alterations. Replicative-form DNA was extracted from H-1 st virus- and H-1 dr virus-infected NB-E cells, digested with *Eco*RI and *Hin*dIII, and analyzed by Southern blotting. The positions of restriction sites previously determined for standard virus DNA are indicated in the upper scheme. Restriction fragment lengths (in kilobase pairs) are indicated. (B) Identification of an internal deletion within H-1 dr virus DNA. Analysis of H-1 dr virus and H-1 st virus molecular clones by the dideoxy sequencing method revealed a deletion spanning nt 2022 to 2135 in the variant virus DNA. The H-1 dr virus DNA sequence across this deletion is shown, with reference to the standard virus DNA sequence for nucleotide numbering. The deletion is in frame, disrupts no codon for NS-1 (open reading frame [ORF] 3), and re-creates the original glutamic acid codon in ORF 2 that encodes part of NS-2. (C) Location of the genomic deletion (del.) and reiteration (reit.) on the H-1 dr virus expression map. The diagram represents the genome (upper line) with its promoter regions (P4 and P38), the aligned transcripts (R1, R2, and R3), and the ORFs (ORF 1, grey boxes; ORF 2, horizontally hatched boxes; ORF 3, crosshatched boxes) encoding nonstructural (NS-1 and NS-2) and capsid (VP-1 and VP-2) proteins. (D) Schematic representation of the tandem duplication toward the right-hand terminus of the H-1 dr virus genome. The virus (minus) strands of H-1 st and H-1 dr virus DNAs are shown; open box, the 55-nt repeated motif. The H-1 dr virus genome contains an extra copy of the 55-nt element and ATT spacer, as indicated by nucleotide numbers with asterisks. A point mutation in the right-hand repeat is printed in boldface type. The origin of the insertion is unclear but may involve slipped mispairing between directly repeated sequences during DNA replication. The 55-nt element contains the 5′-CTTTTT-3′ motif, which may favor rearrangements through duplex DNA unwinding and/or nicking around that position (6).

are characterized by internal deletions and/or 5′-terminal reiterations (2, 15, 16). None of the H-1 virus variants described so far is infectious, except for H-1-DI-1 virus, which is viable but grows poorly and produces very small plaques. In contrast, the presently isolated H-1 dr virus variant formed plaques of normal size, compared with those of the standard virus. Subsequent analysis confirmed that H-1 dr virus can be distinguished from other type I H-1 virus variants by its full infectivity. (i) The H-1 dr virus stock went through a second round of plaque purification. Forty individual plaques were picked up and found to be free of the standard virus (within the sensitivity limits of Southern blotting analysis). If a very low level of contaminating standard virus was still responsible for the infectivity of the H-1 dr virus stock, the particle-to-infectivity ratio should have been 1 or several orders of magnitude higher for H-1 dr virus preparations than for H-1 st virus preparations, given the larger excess of variant genomes in the former. The particle-to-infectivity ratios of H-1 dr virus and H-1 st virus were actually similar (around $10^{-5}$ hemagglutination units per PFU), indicating that H-1 dr virus grows autonomously and is as infectious as the standard virus strain. (ii) While the standard H-1 virus could be propagated in both human NB-E and rat FR3T3 fibroblasts, the H-1 dr virus variant proved to be noninfectious for the latter cells (see below). This difference in host range argues against the possibility that the infectivity of the H-1 dr virus stock, as detected in NB-E cells, is due to contaminating standard virus.

In order to analyze the H-1 dr virus DNA alterations at the nucleotide sequence level, monomer-length replicative forms were extracted from infected NB-E cells and inserted into the *Eco*RV site of the pBluescript KS vector. This gave rise to a molecular clone (pH1dr) spanning most of the H-1 dr virus genome (equivalent to nt 2 through 4949 of standard H-1 virus DNA). Given the above-mentioned changes in the restriction pattern, subsequent sequence analysis has focused more particularly on two regions: (i) the whole transcription unit encoding the nonstructural proteins, which encompasses the internal restriction fragment of decreased size, and (ii) the right-hand portion of the genome, which is included in the 5′-

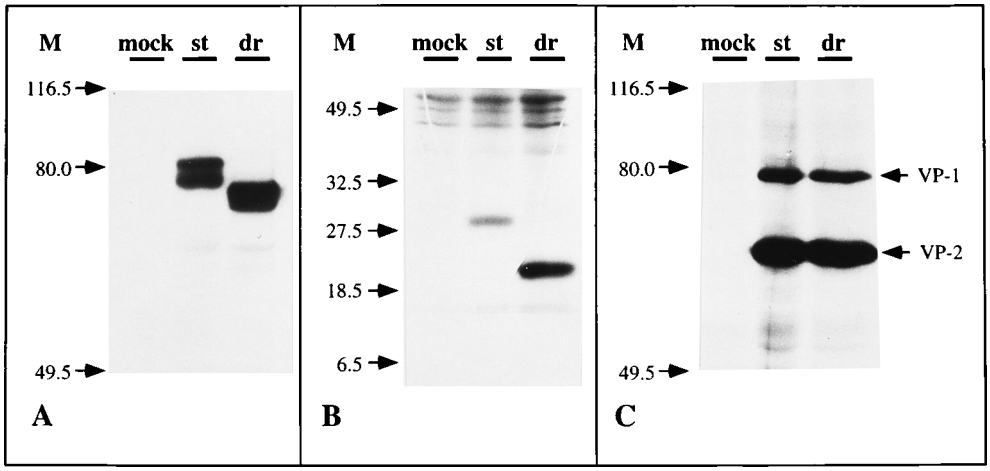


FIG. 2. Production of viral proteins in NB-E cells infected with H-1 st and H-1 dr viruses. After virus inoculation (3 PFU per cell), cultures were incubated for 2 h in the presence of [$^{35}$S]methionine and processed for sodium dodecyl sulfate-polyacrylamide gel electrophoresis and autoradiography after immunoprecipitation with antisera specific for NS-1 (SP8) (A), NS-2 (SP4) (B), and VP-1 and VP-2 (PV-1 [3]) (C). Samples were matched for total labelled protein content before immunoprecipitation. M, molecular mass markers (in kilodaltons). Mock, mock infected.

terminal fragment of increased size and is known to be often rearranged in type I H-1 virus variants.

**Sequencing of the H-1 st and H-1 dr virus nonstructural transcription units.** Three changes were detected in the open reading frame encoding the nonstructural proteins of H-1 st virus, in comparison with the published DNA sequence of standard H-1 virus. These discrepancies are likely to be errors or interstrain variations that affect the published sequence. (i) An A-to-C transversion at nt 305 is of no consequence for the corresponding amino acid of NS-1 and NS-2 proteins (threonine 14), (ii) a C-to-A transversion at nt 634 converts the expected proline at amino acid 124 of NS-1 into a glutamine, and (iii) a G-to-A transition at nt 1101 results in a change at amino acid 280 of NS-1 from glutamic acid to lysine. It is worth noting that a lysine is conserved at this position in the NS-1 proteins of many autonomous parvoviruses. Sequencing of a reference molecular clone of H-1 virus, pSR19 (see below), confirmed that these differences are rectifications to be brought to the standard H-1 virus DNA sequence rather than characteristic features of the H-1 dr virus variant.

In addition, the H-1 dr virus genome was found to contain a point mutation and an in-frame deletion within the nonstructural open reading frames, both of which distinguish the variant genome sequence from the corrected standard virus DNA sequence. (i) The point mutation is silent and consists of a C-to-T transition at nt 767, keeping an alanine residue at the corresponding position (amino acid 168) of NS-1. (ii) As illustrated in Fig. 1B and C, the deletion removes 114 nt (nt 2022 to 2135; m.u. 39.06 to 41.25) from a region that is part of the nonstructural-coding sequences of transcripts R1 and R2 and the untranslated leader sequence of transcript R3. The splice junctions and promoter P38 are not affected. The deletion is in frame and removes amino acids 587 to 624 and 96 to 133 from NS-1 and NS-2, respectively, leaving downstream residues intact. The joining of nt 2021 to nt 2136 does not generate a new codon since it does not disrupt open reading frame 3 (used for NS-1) and re-creates the original glutamic acid codon in open reading frame 2 (used for NS-2).

**Sequencing of the right-hand termini of the H-1 st and H-1 dr virus genomes.** Sequencing of several clones of wild-type H-1 virus replicative-form DNA containing portions of the right-hand hairpin sequences, with dITP instead of dGTP in the sequencing reactions, avoided a strong tendency for the generation of compression bands because of the GC-rich composition of the center of the palindrome and therefore revealed further corrections to be brought to the original standard H-1 virus DNA sequence:

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correct:  5033-GCTAAGCTCGAACCAACCGGACCGGCTAA
original: 5033-GCTAAGCTCGAA-CAA---GA-CGGCTAA
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This corrected sequence shows only two of the original five mismatches formed in both sequence orientations (flip and flop) of the stem of the right-hand hairpin. This new sequence was used to correct a deletion in a genomic wild-type H-1 virus clone with synthetic oligonucleotides to generate the infectious reference H-1 virus clone pSR19.

In addition, the H-1 dr virus genome was found to contain an insertion, compared with standard H-1 virus DNA. The H-1 st virus genome contains two copies of a 55-nt element, which are located between map positions 91.3 and 95.7 (nt 4716 to 4770 and 4774 to 4828) and are separated by the trinucleotide 3′-ATT-5′. Analysis of this region revealed the presence of three copies of this 55-nt element within the H-1 dr virus genome as a result of the tandem duplication of a 58-nt sequence, including the ATT spacer (Fig. 1D). The right-hand 55-nt repeat was found to harbor an A-to-G transition at position 4814* (according to the nucleotide numbering shown in Fig. 1D). It is worth noting that the region involved in this duplication lies outside the coding sequences but close to the right-hand origin of replication.

**Analysis of H-1 dr virus-encoded nonstructural proteins.** Because of its location within the coding sequences of the nonstructural transcription unit (Fig. 1C) and its in-frame character, the H-1 dr virus DNA deletion should result in the production of shortened NS-1 and NS-2 proteins, lacking an internal set of amino acids. This was verified by comparing the sizes of nonstructural proteins that had been immunoprecipitated from NB-E cells previously infected with H-1 st and H-1 dr viruses.

To this end, antisera specific for either NS-1 or NS-2 polypeptides were prepared as follows. *Xho*II-*Stu*I (nt 2066 to 2397) and *Sau*3A-*Stu*I (nt 2201 to 2397) fragments from the right-hand side of the nonstructural transcription unit of

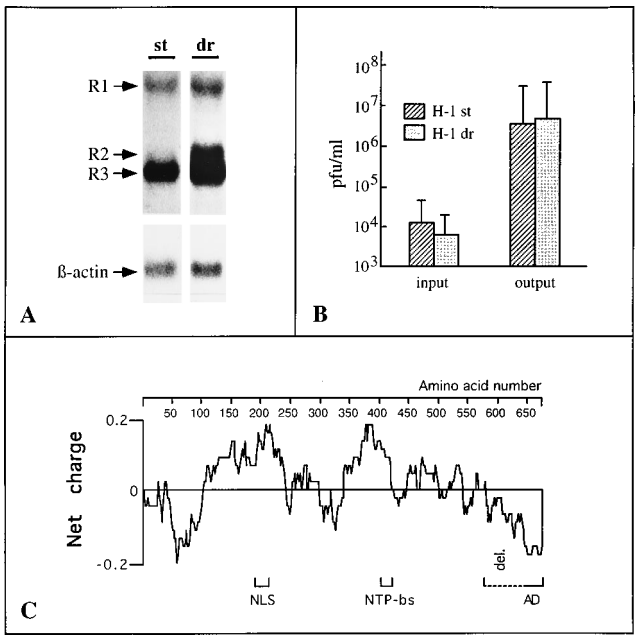


FIG. 3. Growth parameters of H-1 st and H-1 dr viruses. (A) NB-E cell cultures infected at a multiplicity of infection of 3 PFU per cell were incubated for 18 h and processed for Northern blotting analysis, as previously described (3). R1, R2, and R3 are the viral mRNAs depicted in Fig. 1C. The blot was rehybridized with a β-actin probe to control the total amount of RNA applied to each lane. (B) The production of infectious particles was measured by plaque titration of viruses recovered from inoculated NB-E cell cultures 4 days (output) or 2 h (input) after infection. Titers are given in PFU per ml of virus suspension. (C) The net charge (curve) and functional domains (brackets) of the NS-1 product throughout the protein are shown. The charge distribution was calculated by using DNASTAR software (Lasergene Protean program). The H-1 virus genomic deletion (del.) is indicated by a shaded box. NLS, nuclear localization signal (14); NTP-bs, nucleoside triphosphate binding site (9, 11); AD, transcriptional activation domain (9, 10).

minute virus of mice were inserted between the *Bam*HI and *Hin*dIII sites of the pLC25 vector (21). This gave rise to temperature-inducible chimeric genes encoding 98 amino acids of the MS2 polymerase fused to polypeptides that are specific for the C-terminal portions of NS-1 and NS-2, respectively. These epitopes were 78.1 and 79.5% identical to the corresponding portions of the NS-1 and NS-2 proteins of H-1 st virus. MS2–NS-1 and MS2–NS-2 fusion polypeptides (21 and 13 kDa, respectively) were isolated and used to immunize rabbits as previously described (7). Antisera obtained in this way were able to recognize specifically the NS-1 (SP8 antiserum) and NS-2 (SP4 antiserum) proteins from both minute virus of mice and H-1 virus (data not shown).

SP4 and SP8 antisera, directed against nonstructural sequences located to the C-terminal side of the deletion, were used to immunoprecipitate NS-1 and NS-2 proteins, respectively, from H-1 st virus- and H-1 dr virus-infected NB-E cells. As shown in Fig. 2A and B, both antisera recognized internally deleted proteins, confirming that the deletion was in frame and that downstream sequences were correctly translated. An apparent molecular mass reduction of 6 to 8 kDa was observed for the NS-1 (Fig. 2A) and NS-2 (Fig. 2B) products of H-1 dr virus, compared with those of H-1 st virus, in fair agreement with the difference predicted from the genomic deletion. Unmodified and phosphorylated forms of NS-1 were clearly detected in extracts of cells infected with either virus (Fig. 2A). In contrast to nonstructural polypeptides, the VP-1 and VP-2 proteins encoded by H-1 st virus and H-1 dr virus were found to comigrate (Fig. 2C), as expected from the location of the deletion outside the structural transcription unit (Fig. 1C).

**Measurement of H-1 dr virus growth.** The life cycles of H-1 dr and H-1 st virus particles after infection of NB-E cells were compared. No significant differences between variant and standard viruses were detected with regard to the ability to synthesize replicative forms and progeny single-stranded DNA, as measured by Southern blotting analysis (data not shown), or to produce capsid and nonstructural proteins (Fig. 2). The only consistent discrepancy was a greater abundance of the R2 transcript in H-1 dr virus-infected cells than in H-1 st virus-infected cells. This was apparent on Northern (RNA) blots (Fig. 3A), which were densitometrically scanned and showed a fivefold-higher R2/R1 ratio for the virus variant than for the standard virus. Both R1 and R2 transcripts are programmed by the P4 promoter; they differ from each other in the removal of a large intron from the latter. A precedent for an R2/R1 imbalance can be found in a recent report; it showed that nonsense mutations in the second NS-2-encoding exon inhibit splicing of the R1 transcript (13). This is not the case for the H-1 dr virus variant which harbors an in-frame deletion and accumulates a greater proportion of the spliced transcript, pointing to other effects of the H-1 dr virus structure, e.g., on RNA transport or stability. As mentioned above, H-1 st and H-1 dr virus preparations exhibited similar particle-to-infectivity ratios, indicating that both of them were able to give rise to a burst of infectious viruses. This burst was quantitated by titrating progeny particles that had been recovered from NB-E cells previously exposed to the same inoculum of either virus. As shown in Fig. 3B, the H-1 dr virus variant was found to give a yield of productive infection similar to that of the standard strain.

Altogether, these data indicate that the genetic alterations sustained by the H-1 dr virus variant do not impede its growth in human NB-E cells. The location of the H-1 dr virus deletion outside the promoter and capsid protein-encoding regions is a unique feature that allows this new virus variant to be viable. Nevertheless, the H-1 dr virus deletion removes part of the NS-1- and NS-2-encoding sequences, making its innocuousness somewhat surprising.

(i) The H-1 virus life cycle in NB-E cells is controlled, in particular, by the NS-1 protein, which was found to be essential for both viral DNA replication and expression (12). As shown above, the internal deletion within the H-1 dr virus genome results in the production of a shortened NS-1 protein, lacking a stretch of 38 amino acids toward its C-terminal part (Fig. 1B and C and 2A). It can be concluded from these results that the missing peptide is not required for the fulfillment of NS-1 multiple activities in NB-E cells. One NS-1 function is *trans* activation of the parvovirus P38 promoter that directs the structural transcription unit (8, 18). Moreover, NS-1 was found to *trans* inhibit a number of heterologous promoters, including the long terminal repeat of the Rous sarcoma virus (10, 20). In order to compare the *trans*-regulating activities of the NS-1 proteins encoded by variant and standard virus strains, NB-E cells were first transfected with a reporter (chloramphenicol acetyltransferase) gene programmed by either P38 or the long terminal repeat and then infected (or not) with H-1 st or H-1 dr virus. These experiments confirmed the previously reported up- and down-modulation of P38 and the long terminal repeat, respectively, in the presence of NS-1 but did not show any significant difference between the levels of *trans* regulation achieved by standard and variant proteins (data not shown). Therefore, these artificial substrates also failed to reveal functional impairment of the H-1 dr virus-encoded NS-1 product. NS-1 is a multifunctional protein that consists of distinct do-

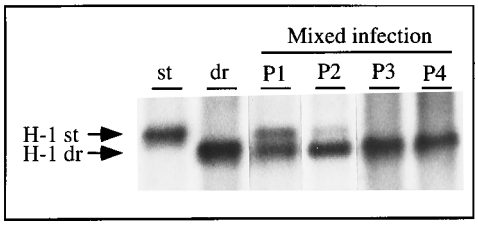


FIG. 4. Capacity of H-1 dr virus to supplant the standard virus strain. Plaque-purified standard and H-1 viruses were mixed and inoculated at a 1:1 ratio into NB-E cell cultures (multiplicity of infection, $10^{-3}$ PFU per cell). At the time of culture lysis, released virus particles were recovered and further passaged serially in NB-E cells. Viral replicative-form DNA was extracted from cells infected either with isolated H-1 st virus or H-1 dr virus (lanes st and dr, respectively) or with successive passages of mixed inoculum (lanes P1 through P4). DNA was digested with *Eco*RI and *Hin*dIII restriction enzymes and analyzed by Southern blotting (Fig. 1A). Only the region of the blot that corresponds to the internal restriction fragment harboring the deletion is shown. Although it constituted a significant fraction of total viral DNA after the initial coinfection (P1), the standard virus genome was progressively supplanted and no longer detectable after the third passage (P3).

mains (9–11, 14), some of which have been mapped as indicated in Fig. 3C. Acidic residues concentrate in the amino- and carboxy-terminal parts of the polypeptide and are thought to be responsible for the ability of the latter end to constitute a transcriptional activator when it is bound to appropriate promoters (10). Although it maps within the region shown to contain this activation domain, the H-1 dr virus deletion lies upstream of the most acidic amino acid stretch (Fig. 3C). The location of the H-1 dr virus-specific deletion outside known functional domains of NS-1 may account for the fact that the shortened protein remained operational.

(ii) The NS-2 protein is required for efficient productive infection of Wistar rats and certain rat cell lines with H-1 virus (12). In contrast, the NS-2 product does not appear to be a necessity for growth of H-1 virus particles in Syrian hamsters or the human NB-E cell line (12). Therefore, successful propagation of H-1 dr virus in NB-E cells does not allow any conclusion to be drawn as to the role of the deleted peptide in NS-2 functioning. It is worth mentioning in this respect that the H-1 dr virus hardly grew in Fisher rat FR3T3 fibroblasts, which were most proficient in replication of the standard H-1 virus strain. Altogether, these observations raise the possibility that the H-1 dr virus deletion results in the production of defective NS-2 proteins and indicate that cells from the natural host species, i.e., rats, should be used to prepare H-1 st virus stocks without amplifying the type of virus variant described in this paper.

**Assessment of H-1 dr virus pathological effects.** Like H-1 st virus, the H-1 virus variant induced the formation of clear plaques in permissive NB-E cell cultures, indicating that growth of both strains led to the lysis of infected cells. As mentioned above, the standard and variant viruses were indistinguishable with respect to their particle-to-infectivity ratios. Since infectivity was measured by plaque assay, this feature implied that a similar fraction of NB-E cells was killed by a given inoculum of either virus. This was ascertained by matching infected cultures with the number of cells sustaining virus replication, as determined by means of an in situ hybridization assay (5). Furthermore, the viral cytopathic effect was assessed by measuring the reduction in the capacities of infected cells for trypan blue exclusion and colony formation. These methods confirmed the similar toxicities of equivalent inocula of H-1 st and H-1 dr viruses for NB-E cells (data not shown).

The cytotoxicity of H-1 virus in cell cultures is the in vitro counterpart of the pathogenic properties of this agent in vivo. The pathological features of H-1 virus in rats and hamsters are well documented (4, 12, 22). In particular, H-1 virus inoculation into newborn hamsters was reported to result in mortality or induction of the so-called osteolytic syndrome (dwarfism and/or mongoloid-like appearance) among survivors. This prompted us to investigate whether infection of newborn hamsters with the H-1 dr virus variant gave rise to similar pathological signs. In this respect, too, H-1 dr virus acted like the standard virus strain. Of 10 newborn hamsters (each inoculated with 10 infectious units of variant virus), 4 died and 3 of the survivors were dwarfs, including 1 which acquired mongoloid-like features.

**Interference of H-1 dr virus in the multiplication of standard H-1 virus.** The high proportion of H-1 dr virus particles in the virus stocks prepared in this laboratory suggests that this variant virus supplants the reference viral strain in mixed infections of NB-E cells. Since H-1 dr virus does not appear to have a growth advantage over H-1 st virus in singly infected cells, the possibility that this variant virus interferes with multiplication of the standard virus strain in coinfected cells arises. This was verified by performing reconstitution experiments in which plaque-purified H-1 st and H-1 dr viruses were mixed at a 1:1 ratio and serially passaged in NB-E cells. Virus bursts were subsequently analyzed by Southern blotting. While similar amounts of H-1 st and H-1 dr virus DNA were found after the first passage, the standard virus component was inhibited over time and was no longer detected after the third passage (Fig. 4). This finding argues for heterotypic interference by the variant virus with standard H-1 virus in coinfected cells. Precedents for such interference between parvoviral agents have previously been reported (for reviews, see references 2 and 6). In particular, standard H-1 virus was found to inhibit propagation of the closely related parvovirus H-3 (16). It is worth noting that the standard H-1 virus genome contains two copies of the previously mentioned 55-nt repeat element near the right-hand origin of replication, while H-3 virus DNA has only one copy of this motif. Furthermore, the H-1-DI-1 virus variant (see above), whose only known alteration is triplication of the 55-nt element, interferes with the growth of standard H-1 virus (16). Hence, it is tempting to speculate that the 55-nt motif reiteration number determines the capacity of this virus to interfere with the replication of related agents. If that is the case, the interfering properties of H-1 dr virus may be ascribed to the increased reiteration of this region, i.e., the insertion of an additional copy of the 55-nt sequence, compared with the standard virus genome. Interestingly, this sole insertion appears to be associated with a striking reduction in the growth capacity of H-1-DI-1 virus (15). In contrast, the H-1 dr virus variant was found to be fully competent in replication. These observations may tentatively be reconciled by assuming that the deletion compensates for reduced packaging efficiency due to the insertion-associated increase in size. The latter possibility is supported by the finding that H-1-DI-1 virus generated wild-type levels of replicative-form DNA and capsid proteins (18a), although it produced smaller plaques, suggesting that the growth disadvantage of H-1-DI-1 virus is at the level of encapsidation.

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