JOURNAL OF VIROLOGY, Mar. 1984, p. 970–979
0022-538X/84/030970-10$02.00/0

Vol. 49, No. 3

Deletions in the Genomes of Pseudorabies Virus Vaccine Strains and Existence of Four Isomers of the Genomes

BELA LOMNICZI,† MAYME LEE BLANKENSHIP, AND TAMAR BEN-PORAT*
Department of Microbiology, Vanderbilt University School of Medicine, Nashville, Tennessee 37232

Received 30 June 1983/Accepted 17 October 1983

As part of a study designed to identify the genes responsible for the virulence of pseudorabies virus, we have mapped the genomes of two independently derived vaccine strains (Bartha and Norden) by restriction enzyme analysis. The structures of these genomes have been compared with that of the genome of a laboratory strain previously mapped, of restriction fragments which had been cloned. The genome of the Bartha strain was found to be very similar to that of other pseudorabies virus strains, except that a deletion of approximately 2.7×10^6 daltons was found in the unique short (U_S) region. This deletion was also observed in the genome of the Norden vaccine strain but was not observed in the genomes of any other pseudorabies virus strains that have been studied (more than 20). The genome of the Norden strain differs from that of other pseudorabies virus strains in several other respects as well. The most important difference is that in contrast to all other pseudorabies virus strains analyzed to date, which contain a type 2 herpesvirus DNA molecule (in which the U_S region only inverts itself relative to the unique long [U_L] region), the genome of the Norden strain is a type 3 molecule in which both the U_S and the U_L regions of the genome invert themselves, giving rise to four isomeric forms of the genome. The ability of the U_L region to invert itself is probably related to the fact that a sequence normally present in all other pseudorabies virus strains at the end of the U_L only is found also in inverted form at the junction of the U_L and the internal inverted repeat in the Norden strain.

The genomes of the herpesviruses consist of linear, double-stranded, noncircularly permuted molecules with molecular weights ranging from 80×10^6 to 150×10^6 among the members of the group. On the basis of the presence of direct, as well as inverted, repeat sequences first observed within the genomes of herpes simplex virus (20), the genomes of the herpesviruses have been divided into three classes (10). Pseudorabies virus (PRV) (Suid herpesvirus 1) contains a class 2 DNA molecule, consisting of two segments of unique sequences, the unique long (U_L) and the unique short (U_S), the U_S sequence being bracketed by inverted repeated sequences (IRS). As a consequence, the U_S sequence inverts itself relative to the U_L sequence and two isomeric forms of the genome exist. Even though the restriction patterns of the DNA of the various PRV strains isolated in Europe and the United States which have been examined vary somewhat (5, 8, 9, 14, 15), all PRV strains examined to date contain a class 2 DNA molecule.

As part of a study designed to identify the genes responsible for the virulence of PRV, we have examined several aspects of the biology and molecular biology of strains of PRV which differ in their degree of virulence. In this paper, we report the results of experiments in which the genomes of two vaccine strains (Norden and Bartha) were mapped by restriction enzyme analysis.

Two salient findings emerged from these experiments. (i) Both the Bartha and the Norden vaccine strains (two independently isolated attenuated strains of PRV) have deletions in the U_S between 0.855 and 0.882 map units. This deletion appears to be a characteristic of the attenuated strains; it was not found in more than 20 other strains which we have analyzed and which had been isolated in the United States and Europe. (ii) The Norden vaccine strain contains a genome that is a class 3 DNA molecule; i.e., in contrast to other strains of PRV in which only the U_S inverts itself relative to the U_L, in the Norden strain the U_L sequence also inverts itself relative to the U_S sequence, so that four isomeric forms of the genome exist.

* Corresponding author.
† Present address: Veterinary Medical Research Institute, 1581 Budapest, Hungary.

MATERIALS AND METHODS

Virus strains. PRV(Ka) is a strain which has been carried in our laboratory for 25 years; its origin is uncertain (11). The Norden and Bartha vaccine strains and the attenuated Bucharest strain were received from P. S. Paul. The origins of these strains have been described (15).

Medium. Eagle synthetic medium (7) plus 3% dialyzed bovine serum was used.

Enzymes and chemicals. Restriction endonucleases were purchased from Bethesda Research Laboratories, proteinase K was from Worthington Diagnostics, and nuclease-free pronase was from Calbiochem-Behring. [α-^{32}P]dCTP was purchased from ICN.

Purification of virions. A continuous line of pig kidney cells was infected (multiplicity, 0.5 PFU per cell) and incubated for 48 h in Eagle synthetic medium plus 3% dialyzed bovine serum at 37°C. Virions were purified as described previously (4).

Extraction of DNA. Extraction of DNA was carried out as follows. Sodium sarkosinate (final concentration, 2%) was added to the samples, which were heated (60°C for 15 min) and digested with nuclease-free pronase (1 mg/ml) for 2 h. The DNA was then extracted four times with phenol-chloroform-isoamyl alcohol (50:48:2) and dialyzed against buffer (0.01 M Tris, 0.001 M EDTA, pH 7.6).

Restriction enzyme digestion and gel electrophoresis of DNA fragments. Digestion and agarose gel electrophoresis of virus DNA were carried out as described by Rixon and Ben-Porat (19). Filter strips to which restriction fragments of PRV DNA were fixed were prepared by the method of Southern (21).

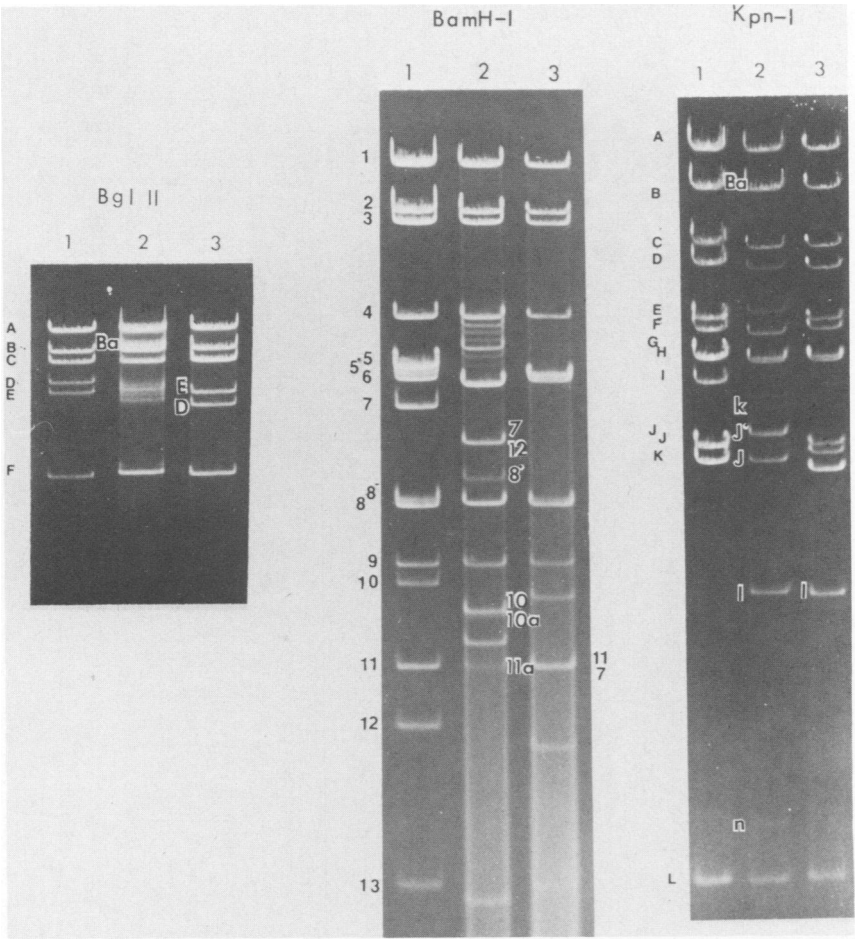

FIG. 1. Restriction enzyme patterns of the DNA of the PRV(Ka) strain (lane 1), the Norden strain (lane 2), and the Bartha strain (lane 3).

Nick translation of cloned PRV DNA restriction fragments. PRV DNA restriction fragments, cloned in pBR325 as described previously (12), were nick translated by the method of Rigby et al. (18).

Electron microscopy of DNA. Electron microscopy was performed as described previously (5).

RESULTS

Figure 1 shows the restriction patterns obtained by digestion with several enzymes of the DNA of PRV(Ka), the Bartha vaccine strain, and the Norden vaccine strain. As has been pointed out previously by others, there are several differences in the restriction patterns of the DNA of the vaccine virus strains and of that of wild-type virus (8, 9, 15).

The most interesting observation to emerge from the inspection and scanning of the gels is that submolar fragments are produced by digesting the DNA of the Norden strain; these are not found when wild-type strains are digested with the same enzymes. Some of these submolar fragments appear as ladder-like bands (even though the DNA in the virions was analyzed after three plaque purifications and a single amplification thereafter); others are present in 0.5 or 1.5 M amounts (Table 1).

To identify the nature of the differences between the restriction fragment patterns of the two vaccine strains and of the wild-type PRV(Ka) strain, the genomes of the two vaccine strains were mapped by the Southern technique (21), using nick-translated cloned fragments of PRV(Ka) (encompassing the whole genome) as probes. Such an analysis allows one to identify the origin of the various bands generated by digestion of the DNA of the vaccine strains with restriction enzymes and allows the construction of restriction maps of the genomes of these strains.

The autoradiograms, obtained with *Bam*HI and *Kpn*I digests, which illustrate the most important points obtained in these types of experiment are illustrated in Fig. 2, 3, 4, and 5. On the basis of these results (which were corroborated by results obtained with digests of *Bgl*II, *Pst*I, and *Sal*I), we conclude the following: (i) the genomes of both the Norden and Bartha strains have a deletion in the U_S which maps between 0.855 and 0.882 map units; (ii) the Norden strain contains a class 3 DNA molecule, probably the consequence of a translocation of a sequence of nucleotides normally present at the end of the U_S to a region adjacent to the internal IRS. Details concerning the construction of the physical maps of the DNA of the vaccine strains, as well as details justifying the conclusions we have drawn, are described below.

The Bartha strain. The patterns generated by various restriction enzymes from the genome of the Bartha vaccine strain do not differ greatly from those seen when the genomes of other PRV strains are similarly analyzed. Although variations in the migration patterns of some bands were observed (especially in some hypervariable fragments

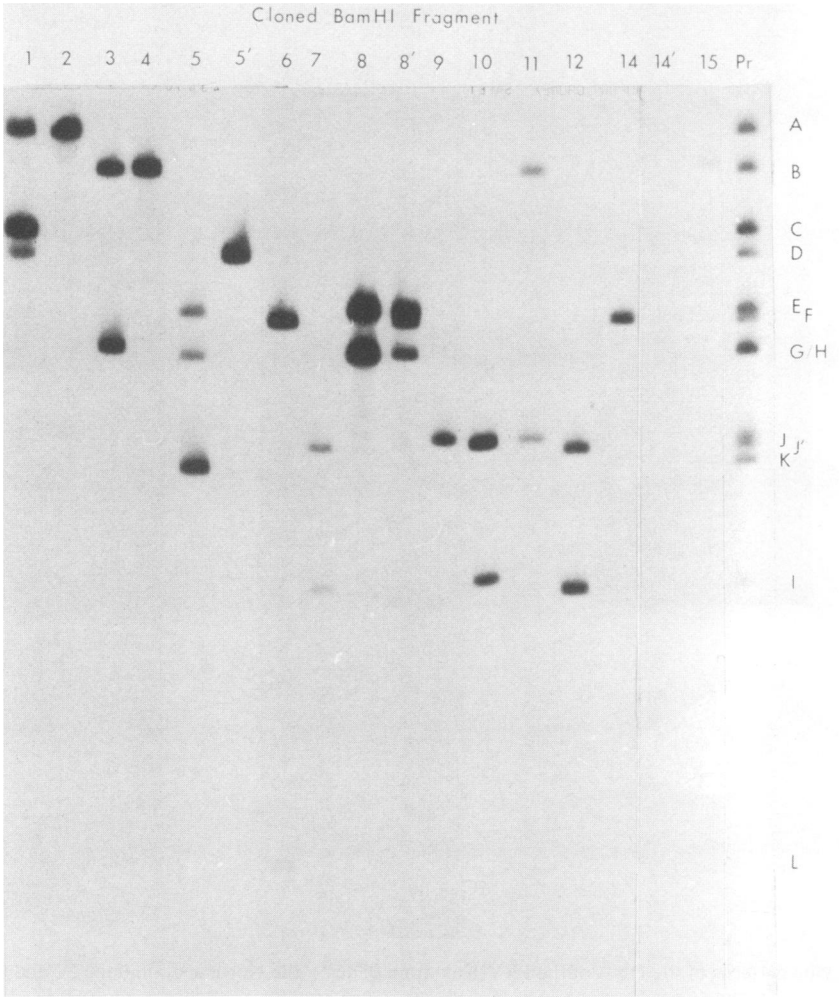

FIG. 2. Hybridization of *Kpn*I restriction fragments generated from the Bartha strain genome to nick-translated, cloned *Bam*HI restriction fragments of PRV(Ka) DNA. The hybridization patterns of the *Bam*HI fragments to *Kpn*I digests of PRV(Ka) and the Bartha strain are very similar (see map in Fig. 6) except that *Kpn*I fragment I, which hybridizes to *Bam*HI fragments 7 and 12 (and also to the IRS of fragment 10), is smaller by 2.7×10^6 daltons.

which often vary in size when the genomes of different isolates are analyzed [3, 9]), most of the fragments generated had migration characteristics similar to those generated from the PRV(Ka) strain (Fig. 1). Figures 2 and 3 show the hybridization of *Bam*HI and *Kpn*I restriction fragments generated from the Bartha strain DNA to cloned *Bam*HI restriction fragments of the DNA of the PRV(Ka) strain.

The major difference between the Bartha and PRV(Ka) strains, as well as several other field isolates that have been studied in this respect (3; T. Ben-Porat, unpublished data), is that the Bartha strain genome has a deletion in the U_S sequence which is approximately 2.7×10^6 daltons in size. This can be deduced from the sizes of *Bam*HI fragment 7 and *Kpn*I fragment I (map in Fig. 6; Fig. 2 and 3; Table 1). The sizes of the fragments generated from the appropriate region of the genome by digestion of the Bartha strain and PRV(Ka) strain genomes with other enzymes are summarized in Table 2. From these data the deletion in the Bartha strain can be estimated to be approximately 2.7×10^6 daltons and can be localized between 0.855 and 0.882 map units. The differences in the estimation of the size of the deletions obtained with the various enzymes probably represents errors in the determination of the molecular weights of the fragments. No extensive differences in the size of fragments originating from other regions of the genome (nor any type of rearrangement) were observed between the Bartha and the PRV(Ka) strains.

The Norden strain. Figures 4 and 5 show the hybridization of the *Bam*HI cloned fragments of PRV(Ka) DNA to *Bam*HI and *Kpn*I digests of the Norden strain genome. Despite the fact that the Bartha and Norden vaccine strains have been derived independently from different parental PRV strains, they both have the same deletions. This can be deduced, for example, from the fact that *Kpn*I fragment I in both strains is only 2.1×10^6 daltons in size (see Fig. 1, 2, 4, and Table 1) as compared to 4.8×10^6 daltons in the PRV(Ka) strain (and 4.6×10^6 to 4.8×10^6 daltons in all other PRV strains examined [data not shown]). The fragment generated from the Norden DNA by *Bam*HI digestion, which hybridizes to *Bam*HI fragment 7 (Fig. 5), is smaller than *Bam*HI fragment 7 generated from wild-type DNA, but larger than that generated from the DNA of the Bartha strain. The fragment

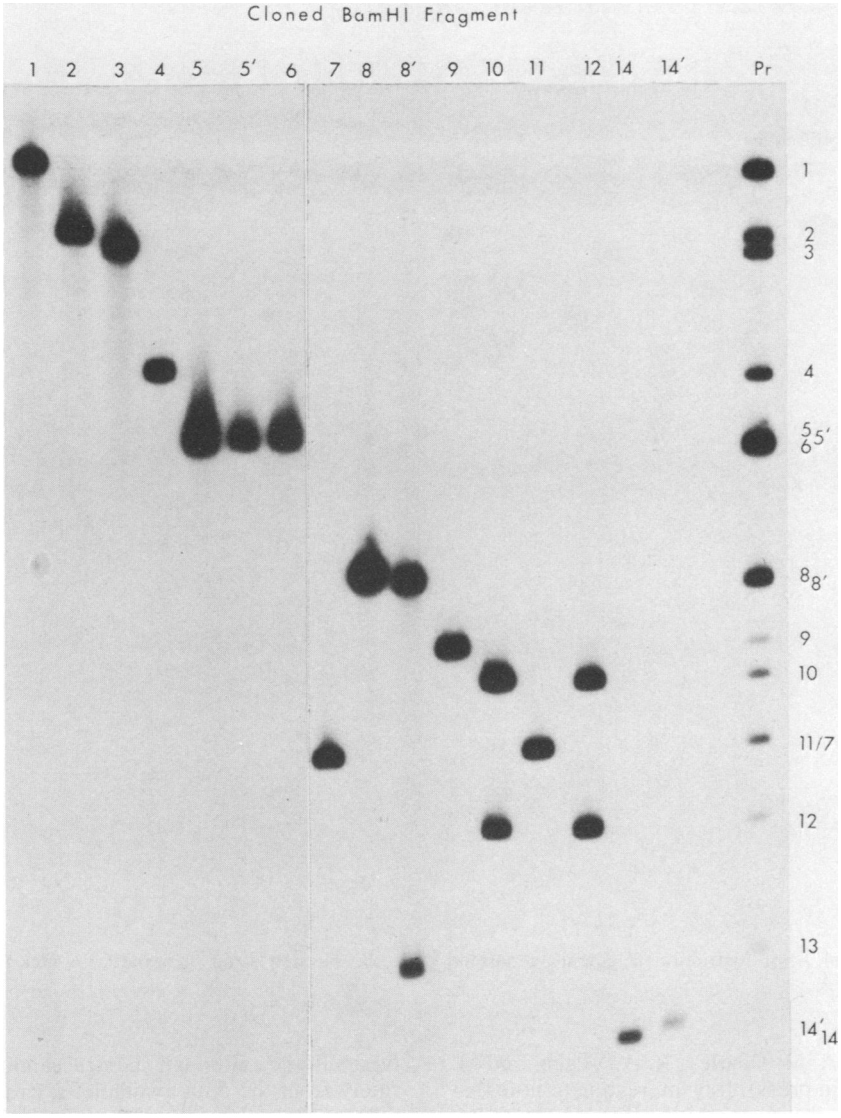

FIG. 3. Hybridization of *Bam*HI restriction fragments generated from the Bartha strain genome with nick-translated, cloned *Bam*HI restriction fragments of PRV(Ka) DNA. *Bam*HI fragment 8′ comprises the junction between the IRS and the U_S; it hybridizes to itself as well as to fragment 13, which is generated from the TRS (see map in Fig. 6). Both fragments 10 and 12 are junction fragments between the IRS and the U_S; they cross-hybridize because of the presence of repeat sequences (see map in Fig. 6).

generated from the Norden strain is, however, a fusion fragment resulting from the loss of the *Bam*HI cleavage site between fragments 7 and 12 which is normally present on wild-type genomes (it hybridizes to the *Bam*HI fragment 7, 10, and 12 probes). An analysis of the Norden genome with other restriction enzymes similar to that performed with the Bartha strain (see Table 2) indicated that a deletion in the same region of the U_S and of the same size (2.7×10^6 daltons) is present in the Norden and Bartha strains.

Two other notable differences between the hybridization patterns of the Norden and Bartha strains, as well as the PRV(Ka) strain, have been detected.

(i) Despite the fact that the Norden strain had been thrice plaque purified and amplified only once before analysis of its DNA, a great degree of heterogeneity of the fragments generated from the repeats was observed (Fig. 1). The heterogeneity of the fragments originating from this region of the repeat was observed in the hybridization pattern with cloned *Bam*HI fragment 5 to *Bam*HI fragment 5 (Fig. 5) and *Kpn*I fragment K (Fig. 4). The heterogeneity is also clearly distinguishable in the *Bgl*II digest (Fig. 1), but not in the hybridization patterns of the *Bgl*II fragments (Fig. 7).

A heterogeneity in the same region of the repeats has also been observed in the genomes of other populations of PRV virions. Thus, a population of virions of a modified Bartha vaccine strain also exhibits this characteristic (unpublished data). Furthermore, a population of virions [TK^-(101)], which had been rendered thymidine deficient by growing the virions in increasing concentrations of 1-β-D-arabinofuranosylthymidine also became enriched for DNA with a similar characteristic. In the latter case, the region of heterogeneity was analyzed in detail and was found to be due to a reduplication of some sequences within that region of the genome and subsequent out-of-phase intermolecular recom-

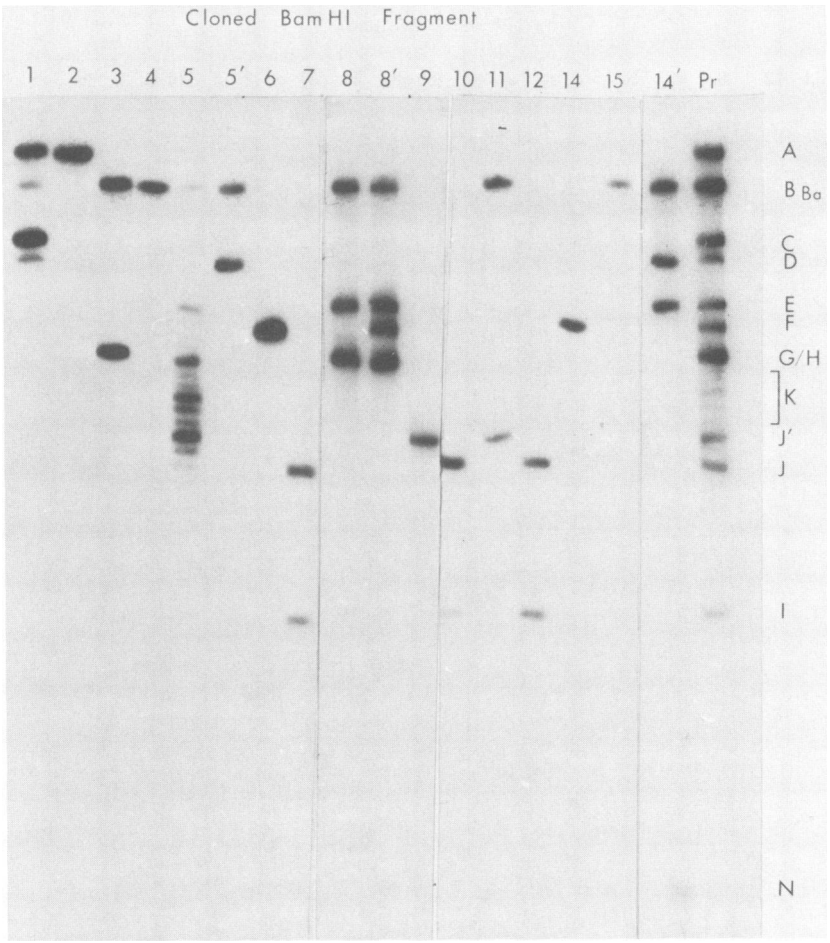

FIG. 4. Hybridization of *Kpn*I restriction fragments generated from the Norden strain genome with nick-translated cloned *Bam*HI fragments of PRV(Ka) DNA.

bination (T. Ben-Porat, A. M. Deatley, R. A. Veach, and M. Blankenship, Virology, in press). It is interesting to note that all three populations of viruses exhibiting this characteristic [Bartha, Norden, and TK^-(101)] are avirulent in swine (the natural host) and are either avirulent or have reduced virulence in a variety of laboratory animals (1, 13; unpublished data).

(ii) An interesting finding was that cloned *Bam*HI fragment 14′, which originates from the end of the U_L sequence of the standard PRV genome and which hybridizes to only one end fragment (for example, *Bam*HI fragment 14′ or *Kpn*I fragment D; see map in Fig. 6), generated by digestion of all other PRV strains, hybridizes to four fragments generated from the Norden strain (see Fig. 4 and 5). Similarly, *Bam*HI fragment 8′, which hybridizes normally to two *Bam*HI fragments (see Fig. 2 and 3) consisting of the junction fragment between the U_L and the IRS, as well as the end fragment generated from the terminal IRS, also anneals to four *Bam*HI fragments generated from the Norden strain. Furthermore, some of these fragments are present in the digests in 0.5 M quantities (Table 1).

Analysis of the data showed that a sequence of nucleotides (which is included in *Bam*HI fragment 14′) normally present in the genomes of other PRV strains at the end of the U_L sequence only is also present in the Norden strain in the region adjacent to the internal IRS. This sequence is relatively small and, although its size cannot be determined accurately from the data available, it can be estimated from the differences in the sizes of the junction fragments between the IRS and the U_L regions generated from the Norden strain and other PRV strains [PRV(Ka) and Bartha strains, for example]. Thus, the *Kpn*I junction fragment (E) is 0.3×10^6 daltons larger in the Norden than in the PRV(Ka) and Bartha strains. Similarly, the *Bam*HI junction fragment (fragment 8′) is also 0.3×10^6 daltons larger in the Norden than in the PRV(Ka) and Bartha strains (Tables 1 and 3). Since the junction fragments (*Kpn*I-E and *Bam*HI-8′) generated from the Norden strain hybridized to sequences originating from *Bam*HI fragment 14′, we assume that in the Norden strain 0.3×10^6 daltons of DNA from the end of the U_L are present also near the junction of the U_L and the internal IRS.

Examination of denatured self-annealed DNA of the Norden strain revealed the presence of dumbell structures (Fig. 8) similar to those observed when herpes simplex virus DNA was similarly analyzed (20), illustrating that in the Norden strain both the U_S and the U_L are bracketed by IRS.

The hybridization patterns and the molarity of the restriction fragments generated from the Norden strain (Fig. 4 and 5; Table 1) indicate also that the U_L region (which is normally found in other PRV strains in one orientation only relative to the U_S) inverts itself in the Norden strain. Thus, digestion of the DNA of the Norden strain with either *Kpn*I

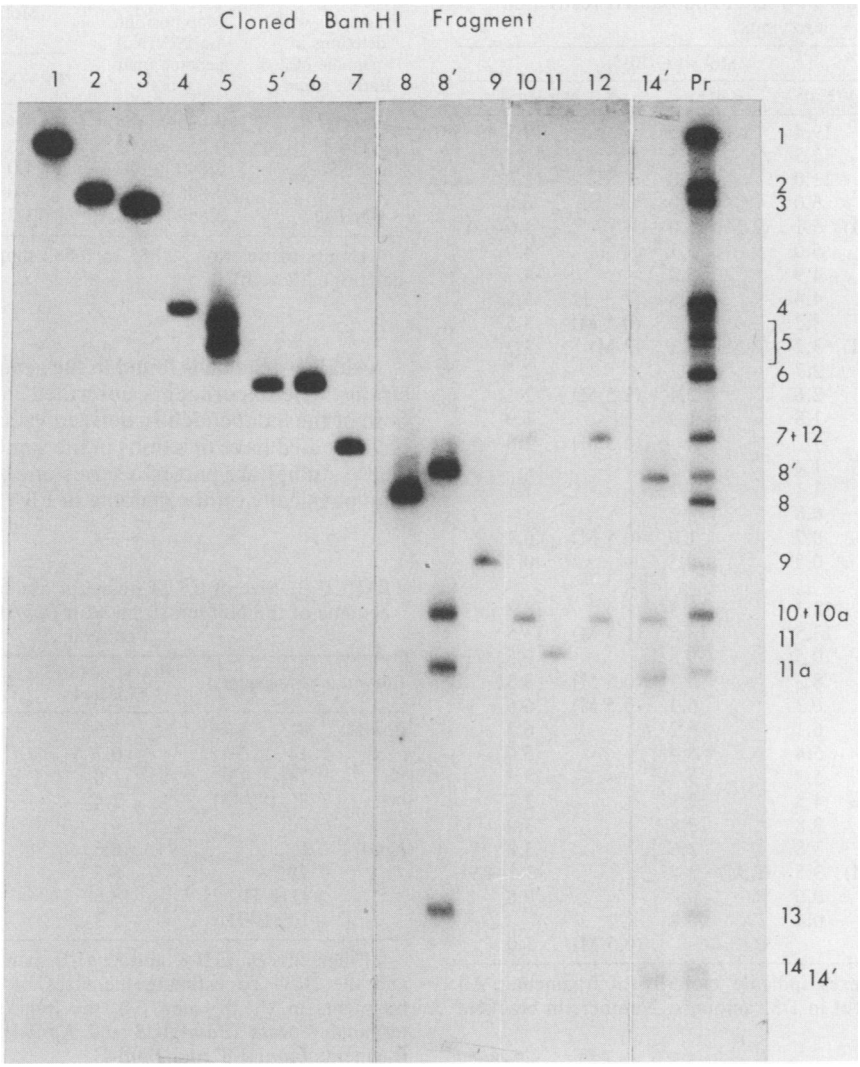

FIG. 5. Hybridization of *Bam*HI restriction fragments generated from the Norden strain genome with nick-translated, cloned *Bam*HI fragments of PRV(Ka) DNA.

or *Bam*HI generates four 0.5 M fragments, as expected when a molecule of DNA composed of two unique regions bracketed by repeats and which invert themselves are digested with an enzyme that cleaves sequences within one set of repeats (in this case the IRS bracketing the U_S) but not the other. Table 3 summarizes the sizes of the 0.5 M fragments generated by digestion with *Kpn*I and *Bam*HI that one would expect if the U_L inverted itself, as well as the sizes of the 0.5 M fragments that were observed. A rather good correlation was found. The minor differences between the expected and the experimentally determined molecular weights of the 0.5 M fragments probably represent errors in the determination of these molecular weights.

Figure 9 summarizes the restriction maps for *Kpn*I and *Bam*HI of the genome of the Norden strain. The genome was similarly mapped after digestion with *Pst*I, *Bgl*II, and *Sal*I. The results were similar; i.e., a sequence of the DNA present in all other PRV strains at the end of the U_L only is present in the genome of the Norden strain near the junction of the U_L and the internal IRS also. Furthermore, the U_L is found in two orientations relative to the U_S.

Analysis of the genomes of all PRV strains, including the Norden strain, with *Bgl*II restriction enzyme (an enzyme that does not cleave within the IRS bracketing the U_S) shows that the U_S region inverts itself relative to the U_L. Thus, whereas all other PRV strains contain genomes which are present in two isomeric forms, the genome of the Norden strain exists in four isomeric forms. This is illustrated by the results in Fig. 7 which show the hybridization pattern of *Bgl*II digests of the Norden and PRV(Ka) strains to *Bam*HI fragments 14′ and 8′, as well as in Fig. 10, which illustrates the restriction map for *Bgl*II of the genome of the Norden strain.

DISCUSSION

The salient findings in this paper are as follows: (i) both the Bartha and Norden vaccine strains have a similar deletion in their genomes; and (ii) in contrast to all PRV genomes examined to date which are class 2 herpesvirus DNA molecules and which are found in two isomeric forms, the Norden strain genome is a class 3 molecule and is found in four isomeric forms.

TABLE 1. Estimation of molecular weights of fragments generated by digestion of the DNA of PRV(Ka), Bartha, and Norden vaccine strains with *Kpn*I and *Bam*HI restriction enzymes[a]

Enzyme	Fragment	Mol wt ($\times 10^{-6}$)		
		PRV(Ka)	Bartha	Norden
*Bam*HI	1	19.4	19.5	19.5
	2	12.5	12.5	12.5
	3	11.0	11.0	11.0
	4	6.0	6.0	6.0
	5	(2 M) 5.1	(2 M) 5.0	~4.6–6.0[b]
	5′	5.0	5.1	4.9
	6	4.9	5.0	4.9
	7	4.4	1.8	[7 + 12] 3.8
	8′	3.2	3.2	(0.5 M) 3.5
	8	(2 M) 3.2	(2 M) 3.1	(2 M) 3.2
	9	2.7	2.7	2.7
	10, 10a	2.6	2.4	(1.5 M) 2.3
	11	1.8	1.9	1.9
	11a			(0.5 M) 1.8
	12	1.4	1.3	
	13	1.1	1.1	1.1
	14	0.8	0.7	
	14′	0.7	1.0	(0.5 M) 0.7
	15	0.5	0.5	0.5
*Kpn*I	A	18.3	18.3	18.3
	B, Ba	13.5	13.5	(1.5 M) 13.5
	C	9.5	9.5	9.5
	D	8.3	8.3	(0.5 M) 8.3
	E	6.3	6.3	(0.5 M) 6.6
	F	6.1	6.2	6.2
	G	5.4	5.4	5.4
	H	5.3	5.3	5.3
	I	4.8	2.1	2.1
	J	3.8	3.8	3.9
	J′	3.8	3.6	3.8
	K	(2 M) 3.5	(2 M) 3.4	~3.5–4.9[b]
	L	0.6	0.6	0.6
	M	0.4	0.8	
	N			(0.5 M) 1.0

[a] Numbers in parentheses indicate molarity of fragments. All other fragments are present in 1 M amounts. Numbers in brackets indicate fusion fragment.
[b] Heterogeneous.

TABLE 2. Estimation of the map position and size of the deletion in the genome of the Bartha strain[a]

Fragments with deletions in genome of Bartha strain	Map position on PRV(Ka) genome (map units)	Mol wt ($\times 10^{-6}$) of fragments		Size of deletion ($\times 10^{-6}$)
		PRV(Ka)	Bartha	
*Bam*HI-7	0.845–0.888	4.4	1.8	2.6
*Kpn*I-I	0.855–0.904	4.8	2.1	2.7
*Sal*I-5	0.840–0.887	4.0	1.5	2.5
*Pst*I-1	0.805–0.884	8.0	4.9	3.1
*Bgl*II-D	0.852–1.000	13.7	11.0	2.7

[a] Limits of deletion, 0.855 to 0.882 map units. Probable size of deletion, 2.7×10^{-6}.

A similar deletion is found in the genomes of the two vaccine strains. The experiments described in this paper show that both of the independently derived vaccine strains of PRV we have studied have deletions in the same sequences in the U_S. These studies are part of a series of experiments designed to map physically on the genome of PRV the genes responsible

TABLE 3. Size of 0.5 M fragment obtained by digestion of the genome of the Norden strain with *Bam*HI and *Kpn*I restriction enzymes[a]

Enzyme	Fragment(s)	Mol wt ($\times 10^{-6}$) expected	Fragment	Mol wt ($\times 10^{-6}$) found
*Bam*HI	8′	3.5	8′	3.5
	14′	0.7	14′	0.7
	14′ + 13	1.8	11a	1.8
	$8'_2$ (8′–13)	2.4	10a	2.3
*Kpn*I	E	6.6	E	6.6
	D	8.3	D	8.3
	D + H	13.6	Ba	13.5
	E_2 (E–H)	1.3	N	1.0

[a] Fragments *Bam*HI-8′ and *Kpn*I-E span the joint between the U_L and the IRS. To estimate the sizes of the sequences in these fragments in U_L ($8'_2$ and E_2), the fragments generated from the terminal repeats (*Bam*HI-13 and *Kpn*I-H) were subtracted from fragments *Bam*HI-8′ and *Kpn*I-E.

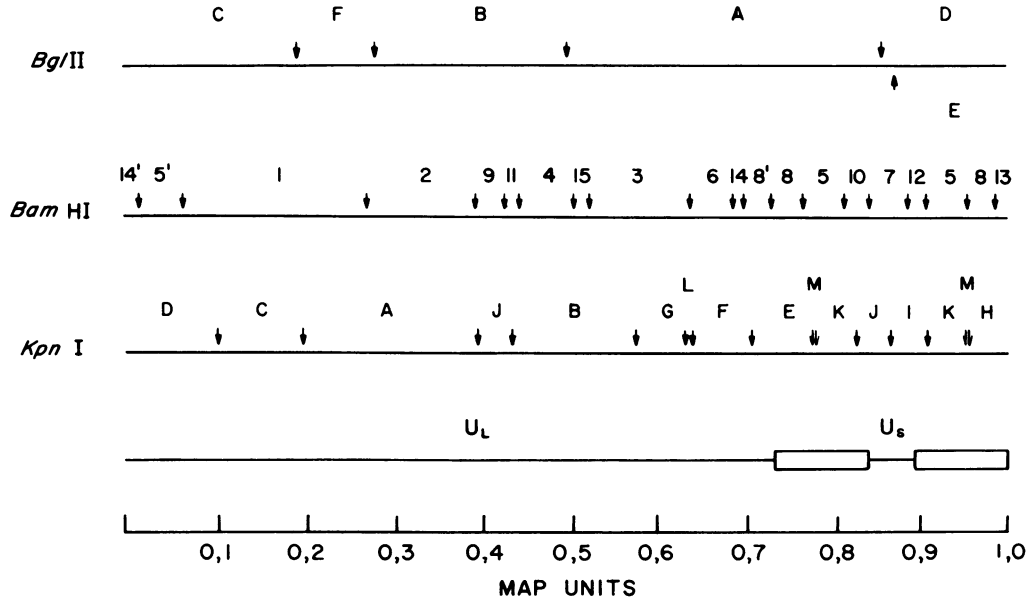

FIG. 6. Restriction maps of the genome of the PRV(Ka) strain. Rectangles represent the IRS regions. Arrows indicate points of cleavage.

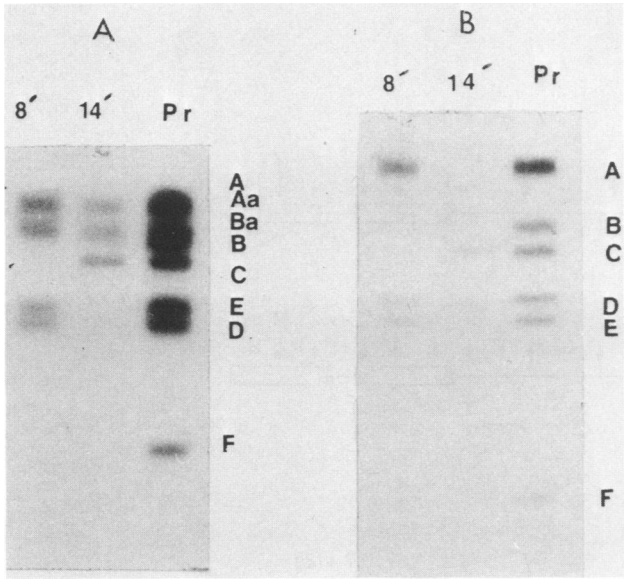

FIG. 7. Hybridization of *Bgl*II restriction fragments generated from the Norden and PRV(Ka) strain genomes. Fragment 14′ hybridizes to PRV(Ka) fragment C only (see Fig. 6 for map) and to bands A, Ba, and C of the Norden strain. Band A in the Norden strain consists of four 0.25 M joint fragments which comigrate because they are of approximately the same size (29×10^6 to 33×10^6 daltons). Fragment Ba (molecular weight, 21×10^6) is generated as a result of the inversion of the U_L (see Fig. 10).

for the virulence of the virus, as well as to determine the functions of these genes. Preliminary results indicate that virulence of PRV is controlled by several genes and that genes located in the region of the genome that is deleted from the vaccine strains may be implicated in virulence.

The deletion in the U_S region of the genome of the Bartha and Norden strains does not appear to affect significantly the ability of these viruses to replicate in cell culture. Although several mRNA species normally originating from the region of the genome that is deleted in these viruses are, as expected, not formed in cells infected with these virus strains, similar amounts of virus are produced (S. Watanabe,

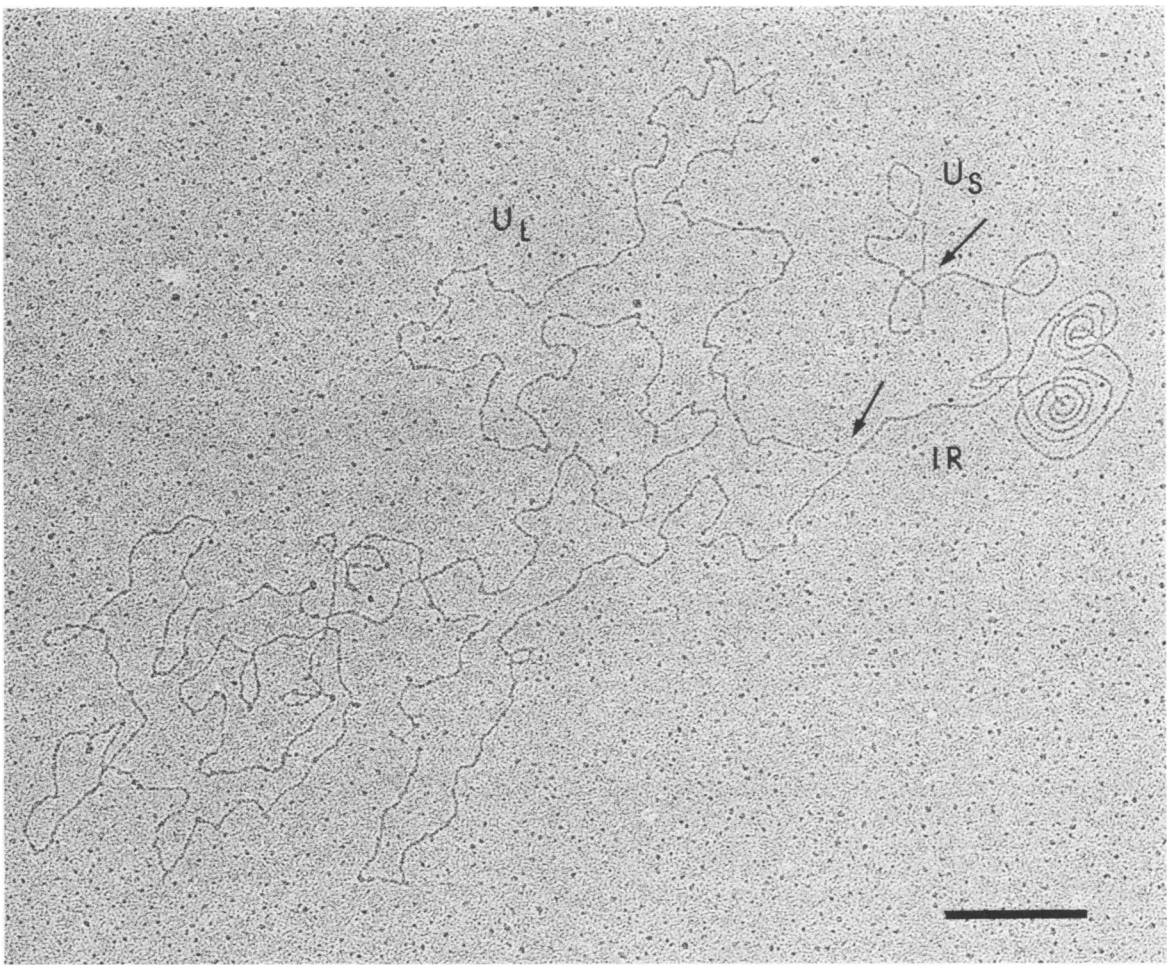

FIG. 8. Electron micrograph of a self-annealed, denatured DNA molecule of the Norden strain. Bar, 0.5 μm. In contrast to self-annealed DNA of wild-type PRV, in which only the U_S forms a single-stranded loop (5), in the Norden strain both the U_S and the U_L form single-stranded loops. Ten self-annealed molecules were measured. The results obtained were similar to those previously obtained with wild-type PRV (Ben-Porat et al., submitted) with the following exceptions. (i) The size of the double-stranded region of DNA (IR) differed significantly between different molecules and was always 20 to 40% larger than that in wild-type DNA, confirming the heterogeneity and increased size of the repeat regions detected by analysis of the DNA with restriction enzyme (see text). (ii) As expected, the size of the U_S in the Norden strain was about 40% smaller than that in the wild type, reflecting the deletions that were observed in that strain.

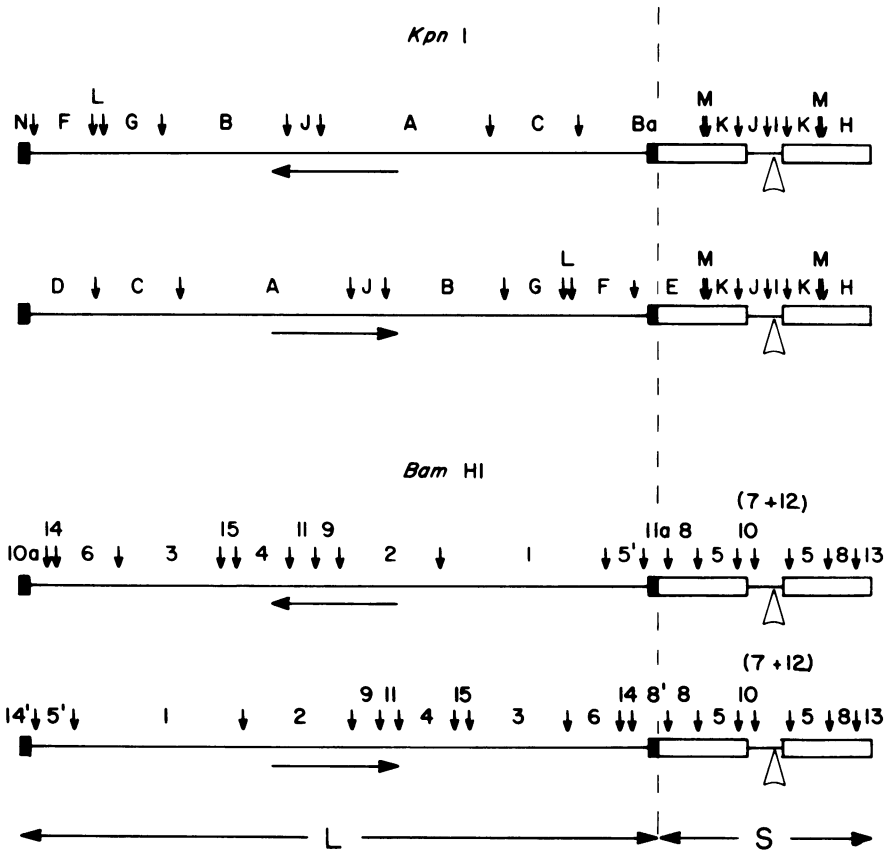

FIG. 9. *Kpn*I and *Bam*HI restriction maps of the genome of the Norden strain illustrating the inversion of the U_L. Open rectangles represent the inverted repeats bracketing the U_S; closed rectangles represent the inverted repeats bracketing the U_L.

unpublished data). Thus, the lack of expression of this region of the genome does not affect virus replication in cell culture.

The Norden PRV vaccine strain contains a class 3 herpesvirus DNA molecule. Herpes simplex virus DNA has been classified as a class 3 herpesvirus DNA molecule; it contains two sets of repeats, each bracketing a segment of DNA with a unique sequence of nucleotides. These two unique sequences invert themselves with respect to one another, so that four isomeric forms of the herpes simplex virus genome exist. PRV DNA, on the other hand, has been classified as a class 2 molecule. It also contains a U_L and a U_S sequence, but only the U_S sequence is bracketed by inverted repeats and only two isomeric forms of the DNA exist (2).

It has been postulated that despite the apparent dissimilarities, when displayed in linear form, between the structures of class 2 and 3 herpesvirus DNA molecules, the molecules appear similar when they are displayed in the circular form and the two type genomes differ only in the position of the sites at which the circular or concatemeric form of the DNA is cleaved before encapsidation (6). A comparison of the sequence homology between herpes simplex virus type 1 and PRV failed, however, to reveal any evidence that such a translocation of the cleavage-encapsidation site had occurred (6). The evolutionary changes that have allowed the transition from class 2 to class 3 DNA molecule (or vice versa) are thus not known at present.

Our results show that the Norden vaccine strain of PRV contains a genome that can be classified as a class 3 herpesvirus DNA molecule. Both the U_S (as detected by cleavage with *Bgl*II) and the U_L sequences (Table 3; and Fig. 9) invert themselves with respect to one another, and four isomeric forms of the genome are found.

A sequence of nucleotides (estimated to be 0.3×10^6 daltons in size), normally present in all other PRV strains at the end of the U_L only is present in the genome of the Norden strain in inverted form (see Fig. 9 and 10) next to the internal IRS also. It is likely that, as a consequence of the

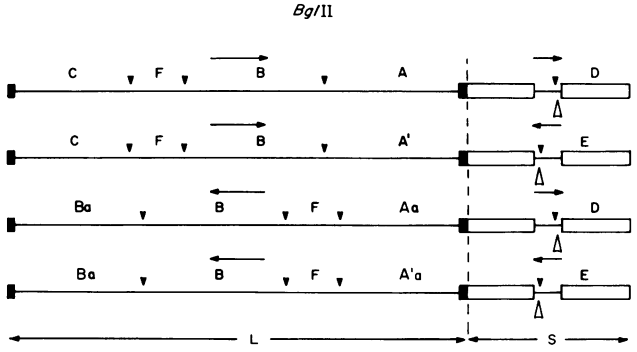

FIG. 10. *Bgl*II restriction map of the genome of the Norden strain illustrating the inversions of the U_L and U_S.

presence of inverted repeats bracketing the U_L in the Norden strain, the U_L inverts itself.

The Norden vaccine strain is a derivative of the Bucharest strain which, in turn, was derived from a virulent strain that had been attenuated by more than 800 passages in embryonated eggs and in chicken embryo fibroblasts (1). The original, parental PRV strain (isolated in Romania) is unavailable at present in the United States and may be difficult to obtain. We have analyzed the Bucharest strain and found that it also contains a type 3 DNA molecule (unpublished data). It is likely, however, that the original, parental strain is similar to all other field isolates of PRV and that a modification of the parental genome from a type 2 to a type 3 herpesvirus DNA molecule had occurred during passage of the virions in cell culture. Whatever the case may be, the availability of the Norden (and Bucharest) strain provides an interesting tool to study further the requirements for the conversion of a type 2 to a type 3 class herpesvirus DNA molecule.

The Norden and Bartha strains are similar in that both have deletions of approximately 2.7×10^6 daltons in the U_S sequence of their genome and some messages specified by sequences in the U_S region of wild-type strains are not detectable in cells infected with either vaccine strain (S. Watanabe, unpublished data). Both are less virulent than the wild type when injected intraperitoneally into mice or intracerebrally into baby chicks (1, 13). Both also have similar growth characteristics in vitro. Thus, despite the fact that the Bartha strain contains a type 2 and the Norden strain contains a type 3 DNA molecule, no significant differences between the biological characteristics of these viruses have been detected to date.

ACKNOWLEDGMENTS

This investigation was supported by Public Health Service grant AI-10947 from the National Institutes of Health. B.L. was supported by the scientific exchange program of the U.S. National Academy of Sciences and the Hungarian Academy of Sciences.

LITERATURE CITED

1. **Baskerville, A., J. B. McFerran, and C. Dow.** 1973. Aujeszky's disease in pigs. Vet. Bull. **43**:465–480.
2. **Ben-Porat, T.** 1982. Organization of herpesvirus DNA, p. 142–172. *In* A. S. Kaplan (ed.), Organization and replication of viral DNA. CRC Press, Inc., Boca Raton, Fl.
3. **Ben-Porat, T., A. M. Deatly, B. C. Easterday, D. Galloway, A. S. Kaplan, and S. McGregor.** 1983. Latency of pseudorabies virus. Curr. Top. Vet. Med. Anim. Sci.
4. **Ben-Porat, T., J. M. DeMarchi, and A. S. Kaplan.** 1974. Characterization of defective interfering particles present in a population of pseudorabies virions. Virology **60**:29–37.
5. **Ben-Porat, T., F. J. Rixon, and M. Blankenship.** 1979. Analysis of the structure of the genome of pseudorabies virus. Virology **95**:285–294.
6. **Ben-Porat, T., R. A. Veach, and S. Ihara.** 1983. Localization of the regions of homology between the genomes of herpes simplex virus, type 1, and pseudorabies virus. Virology **127**:194–204.
7. **Eagle, H.** 1959. Amino acid metabolism in mammalian cell cultures. Science **130**:432–437.
8. **Geck, P., Jr., E. Nagy, and B. Lomniczi.** 1982. Differentiation between Aujeszky's disease virus strains of different virulence by restriction enzyme analysis of the DNA. Magy. Allatorv. Lapja **37**:651–656.
9. **Gielkens, A. L. J., and A. J. M. Berns.** 1982. Differentiation of Aujeszky's disease virus strains by restriction endonuclease analysis of the viral DNAs. Curr. Top. Vet. Med. Anim. Sci. **17**:3–13.
10. **Honess, R. W., and D. H. Watson.** 1977. Unity and diversity in the herpesviruses. J. Gen. Virol. **37**:15–37.
11. **Kaplan, A. S., and A. E. Vatter.** 1959. A comparison of herpes simplex and pseudorabies viruses. Virology **7**:394–407.
12. **Ladin, B. F., S. Ihara, H. Hampl, and T. Ben-Porat.** 1982. Pathway of assembly of herpesvirus capsids: an analysis using DNA^+ temperature-sensitive mutants of pseudorabies virus. Virology **116**:544–561.
13. **Lomniczi, B.** 1974. Biological properties of Aujeszky's disease (pseudorabies) virus strains with special regard to interferon production and interferon sensitivity. Arch. Gesamte Virusforsch. **44**:205–214.
14. **Ludwig, H., B. Heppner, and S. Hermann.** 1982. The genomes of different field isolates of Aujeszky's disease virus. Curr. Top. Vet. Med. Anim. Sci. **17**:15–22.
15. **Paul, P. S., W. L. Mengeling, and E. C. Pirtle.** 1982. Differentiation of pseudorabies (Aujeszky's disease) virus strains by restriction endonuclease analysis. Arch. Virol. **73**:193–198.
16. **Platt, K. B., C. J. Maré, and P. N. Hinz.** 1979. Differentiation of vaccine strains and field isolates of pseudorabies (Aujeszky's disease) virus: thermal sensitivity and rabbit virulence markers. Arch. Virol. **60**:13–23.
17. **Platt, K. B., C. J. Maré, and P. N. Hinz.** 1980. Differentiation of vaccine strains and field isolates of pseudorabies (Aujeszky's disease) virus: trypsin sensitivity and mouse virulence markers. Arch. Virol. **63**:107–114.
18. **Rigby, P. W. J., M. Dieckmann, C. Rhodes, and P. Berg.** 1977. Labeling deoxyribonucleic acid to high specific activity *in vitro* by nick translation with DNA polymerase. J. Mol. Biol. **113**:237–251.
19. **Rixon, F. J., and T. Ben-Porat.** 1979. Structural evolution of the DNA of pseudorabies defective viral particles. Virology **97**:151–163.
20. **Sheldrick, P., and N. Berthelot.** 1974. Inverted repetitions in the chromosome of herpes simplex virus. Cold Spring Harbor Symp. Quant. Biol. **39**:667–678.
21. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98**:503–517.