

Deletions in Vaccine Strains of Pseudorabies Virus and Their Effect on Synthesis of Glycoprotein gp63

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The pseudorabies virus vaccine strains Norden and Bartha each have been reported to have deletions in the small unique component of the genome (B. Lomniczi, M. L. Blankenship, and T. Ben-Porat, *J. Virol.* 49:970-979, 1984). The deletion in Norden was shown to delete the entire coding region for gI but not any of the coding sequences for gp63. However, gp63 in Norden-infected cells was only 36 kilodaltons, and a 44-kilodalton form of gp63 was released into the medium. In Bartha, the deletion removed the coding region for all but 89 amino acids of gp63, and no gp63 was detected in either Bartha-infected cells or medium.

Pseudorabies virus (PRV) is a herpesvirus that is of major economic concern to the swine industry. Because of the economic impact, there has been considerable interest in the development of vaccines against PRV, and live and killed vaccines are commercially available. Many of the most

United States is derived from the attenuated BUK strain of PRV (16). An independent attenuated PRV strain derived very early was the Bartha K strain (1), which is the basis for the Duvaxyn vaccine sold in Europe (18).

Lomniczi et al. (7) have shown that there are profound

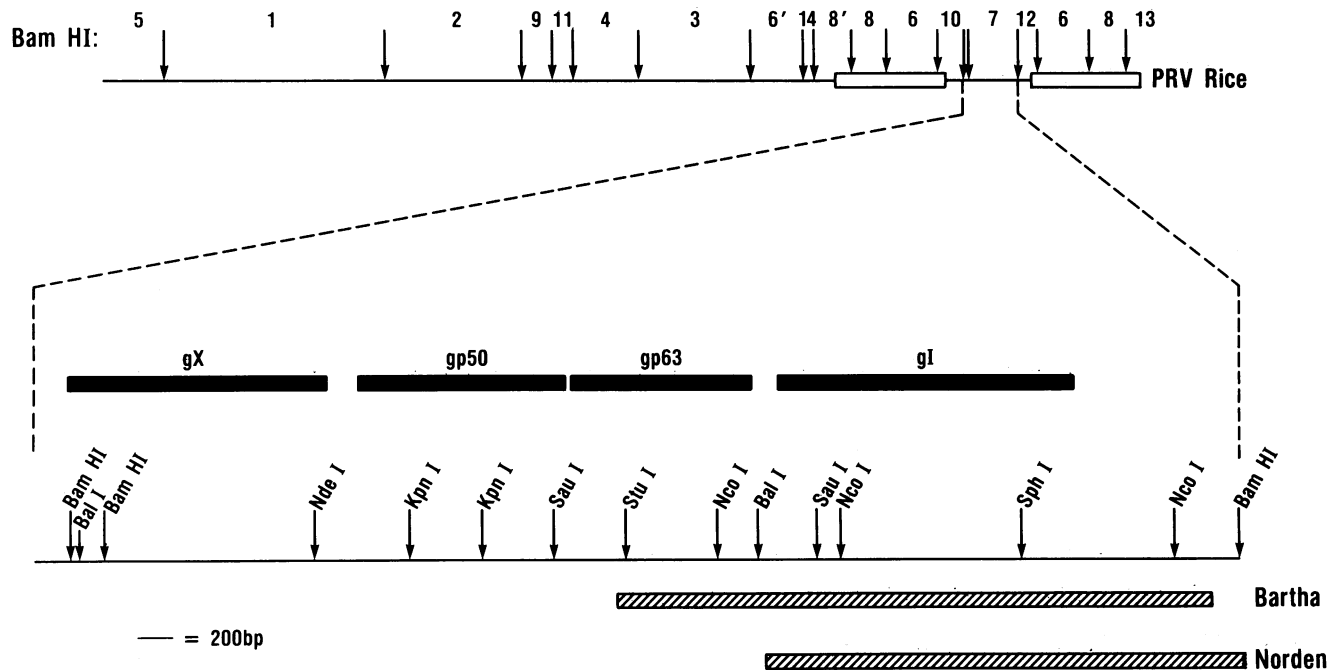


FIG. 1. Restriction enzyme cleavage sites in the cluster of glycoprotein genes in the small unique part of the genome. At the top, the BamHI cleavage sites in PRV Rice are shown (15), with the inverted repeats of the genome indicated by boxes. The expanded map shows the position of the four glycoprotein genes relative to various restriction enzyme cleavage sites (13-15). The hatched bars at the bottom mark the region of the expanded map that is deleted in the two vaccine strains. The deletion in Norden extends into BamHI-12 for an undetermined distance. bp, Base pairs.

important live PRV vaccines marketed today are derived from the early work on attenuated strains done in Eastern Europe, where PRV was first a serious economic problem (reviewed in reference 2). The Norden vaccine sold in the

molecular differences in the genomes of vaccine and wild-type strains of PRV. The Norden genome has four isomers (7), as opposed to the two isomers of the wild-type genome. Both Norden and Bartha have deletions in the small unique components of their genomes, and these deletions are at least partially responsible for the reduced virulence (4, 8). Mettenleiter et al. have shown that the deletions in Norden

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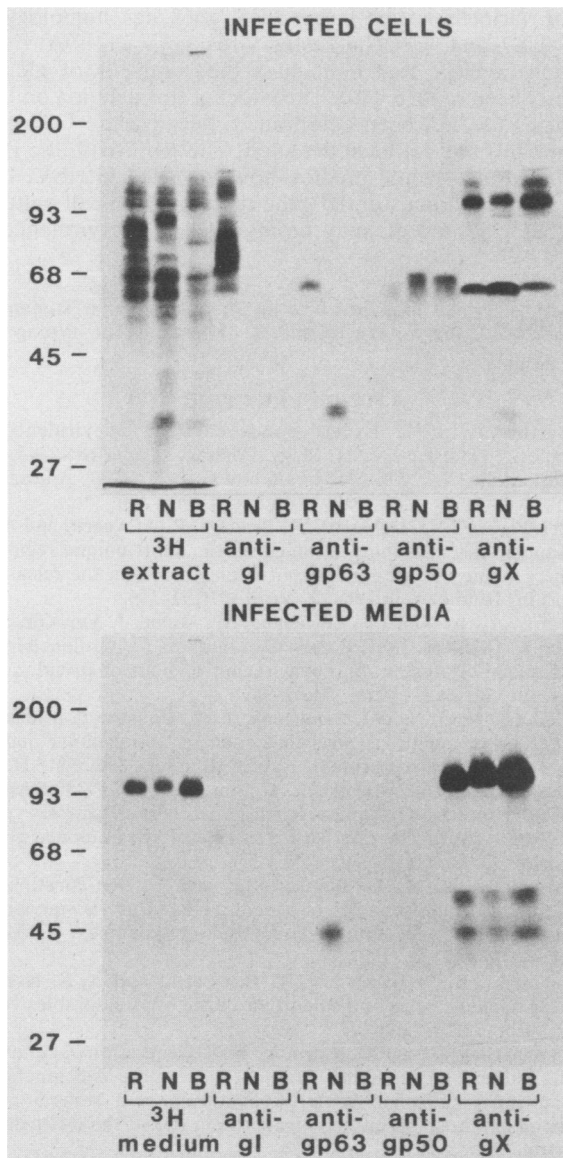


FIG. 2. Immunoprecipitation of [³H]glucosamine-labeled extracts and media from infected cells. Vero cells were infected with PRV Rice (R), Norden (N), or Bartha (B). Labeling and immunoprecipitation analysis with the indicated antisera were done as described in the text. Masses of proteins are shown in kilodaltons.

and Bartha result in the failure of these viruses to produce glycoprotein gI (12). The small unique component of the PRV genome contains a cluster of four glycoprotein genes (Fig. 1): gX (15), gp50 (13, 19), gp63 (14), and gI (11). In this study, we examined the endpoints of the deletions in the small unique components of the genomes of Norden and Bartha and whether the deletions affected the synthesis of any glycoproteins in addition to gI.

PRV Norden (PR-Vac) was purchased from Norden Laboratories, Lincoln, Neb., and PRV Bartha was obtained from M. Wathen of The Upjohn Co., Kalamazoo, Mich. Both were plaque purified twice before use. These viruses and the wild-type PRV Rice strain were propagated on Vero cells, and DNA was prepared by sodium iodide density gradient centrifugation as previously described (15). Labeling of viral glycoproteins with [³H]glucosamine and analysis by immunoprecipitation were done as previously described (13, 15). The antisera used for immunoprecipitations were raised against proteins synthesized in *Escherichia coli* in the case of gX (15), gp63, and gI (14). The antibody used to analyze gp50 expression was monoclonal antibody 3A-4 (13).

Figure 2 shows the immunoprecipitation analysis of the four glycoproteins encoded by the small unique component of the PRV Rice genome in comparison with those of Norden and Bartha. All three viruses were found to produce gX, in contrast to the results reported by Todd and McFerran that Bartha fails to produce gX (17). All three viruses produced gp50. As reported by Mettenleiter et al., Norden and Bartha failed to produce gI (12). No gp63 synthesis was detected in the Bartha-infected cells. In the case of Norden-infected cells, however, a 36-kilodalton form of gp63 was found. The medium of the Norden-infected cells contained a 44-kilodalton form of gp63. We hypothesized that the 36-kilodalton intracellular form of Norden gp63 was a precursor to the 44-kilodalton extracellular form. Support for this idea came from the observations that the intracellular form was endo-β-N-acetylglucosaminidase H sensitive, indicating that the N-linked oligosaccharides were in the high-mannose form; and that the extracellular form was endo-β-N-acetylglucosaminidase H resistant, indicating a more extensive processing of the N-linked oligosaccharides (data not shown).

To examine the exact endpoints of the deletions that had these effects on gp63 synthesis in the vaccine strains, we sequenced the endpoints of the deletions. The *Bam*HI fragments containing the deletions from each vaccine strain were cloned into pUC19 (20). In the case of Bartha, the 3.1-kilobase *Bam*HI 7 fragment (7) was cloned. In the case of Norden, the 6.0-kilobase *Bam*HI 7/12 fragment (7) was cloned. The *Bam*HI 7/12 fragment in Norden is the result of

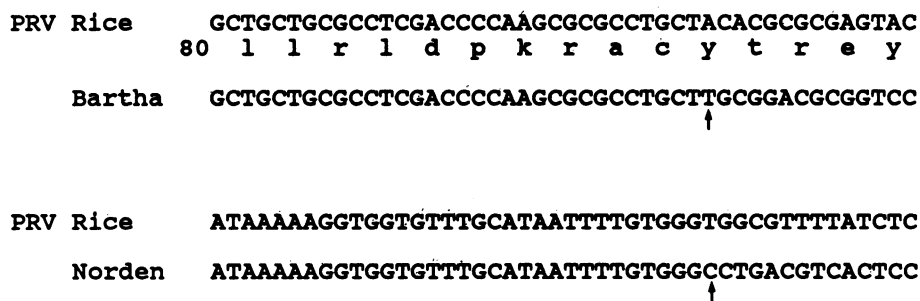


FIG. 3. DNA sequences of deletion endpoints in PRV Norden and Bartha. The sequence of the Bartha endpoint is aligned with the region of PRV Rice in the gp63 gene; the sequence of the Norden endpoint is aligned with the region of PRV Rice between the gp63 and gI genes (14). Arrows indicate the points of divergence of the Rice and vaccine strain sequences. In the comparison with Bartha, the translation of the gp63 gene is indicated by the one-letter amino acid abbreviation under the second base of each Rice codon. The amino acid sequence begins with amino acid 80 of gp63.

the deletion of the *Bam*HI cleavage site that separates *Bam*HI-7 and *Bam*HI-12 in wild-type strains of PRV. The deletions were approximately mapped by using the restriction enzyme cleavage sites shown in Fig. 1. The Norden deletion endpoint was sequenced by end labeling the *Bal*I cleavage site near the gp63 gene. The Bartha deletion endpoint was sequenced by end labeling the *Sau*I cleavage site in the gp50 gene and the *Hind*III cleavage site in pUC19. Sequencing was done by the method of Maxam and Gilbert (10). The results of these sequencing experiments are shown in Fig. 3.

The deletion in Bartha removes most of the gp63 gene, with the deletion endpoint being 55 base pairs upstream from the *Stu*I cleavage site. DNA coding for only 89 amino acids of gp63, which normally is 350 amino acids long (14), is present. Since the anti-gp63 antiserum used for the immunoprecipitation analysis was raised against the fusion protein produced by the λ gt11 clone λ 37, which contains DNA downstream from the *Stu*I cleavage site (14), the antiserum recognized only the gp63 amino acids encoded downstream from the *Stu*I cleavage site. This leaves open the possibility that a small fragment of gp63 that could not be detected by the anti- λ 37 antiserum is produced by Bartha.

The Norden deletion endpoint was found to be between the gp63 and gI genes, i.e., 78 base pairs downstream from the termination codon of the gp63 gene and 75 base pairs upstream from the initiation codon of the gI gene. Thus, the entire coding region for gp63 is present. The simplest explanation for the smaller size of gp63 in Norden-infected cells and its secretion was that the deletion removed coding sequences for the transmembrane and cytoplasmic domains of gp63, which, when done artificially, resulted in the secretion of influenza hemagglutinin (5) and herpes simplex virus (HSV) gD (6). Since the entire gp63 gene of Norden has not been sequenced, it remains a possibility that a nonsense mutation has the same function. Also, it is possible that a point mutation makes Norden gp63 sensitive to a protease. If a nonsense mutation does not truncate the primary translation product, Norden gp63 could be similar to PRV gX. The glycoprotein gX sequence appears to have transmembrane and cytoplasmic domains (15) yet is released into the medium of infected cells. Processing experiments indicate that the release of gX into the medium may be the result of a protease action (L. M. Bennett, J. G. Timmons, D. R. Thomsen, and L. E. Post, Virology, in press).

The other endpoint of the Bartha deletion was 160 base pairs away from the *Bam*HI cleavage site between *Bam*HI-7 and *Bam*HI-12, removing the entire coding region for gI. Nothing is known about the function of the DNA sequences at this deletion endpoint. Likewise, the other endpoint of the Norden deletion was within *Bam*HI-12, in which no genes have yet been characterized.

One of the differences between both Norden and Bartha vaccine strains and wild-type PRV is in gp63. In Bartha, no gp63 was detectable with the λ 37 antiserum. This probably indicates that gp63 is a nonessential gene for the replication of PRV in cultures, although a small piece of gp63 not detected by the antiserum used may be produced by Bartha. Norden contains the entire coding region for gp63 but produces an altered form of the protein. Ben-Porat et al. (3) reported that some function present in Norden but absent from Bartha is important for the release of PRV from some cell types. The exact location of the endpoints of the deletions in the vaccine strains raises the possibility that this function may be provided by gp63.

PRV gI has protein sequence homology to gE of HSV and

gpI of varicella-zoster virus; PRV gp63 has homology to HSV US7 and varicella-zoster virus gpIV (14). A viable deletion in HSV that eliminates the synthesis of gE has recently been reported (9). The effect of this deletion on US7 synthesis has not been determined, because the US7 gene product has not yet been detected. The work with the PRV vaccine strains would predict, however, that in HSV, US7 may also be a nonessential gene for growth in cell cultures and that US7 and gE may be involved in the virulence of HSV.

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