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Role of Essential Glycoproteins gII and gp50 in Transneuronal Transfer of Pseudorabies Virus from the Hypoglossal Nerves of Mice

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The propagation of pseudorabies virus (PrV) mutants deficient in essential glycoproteins gp50 and gII was studied after inoculation of transcomplemented gp50$^-$ and gII$^-$ PrV into the motor hypoglossal (XII) nerves of mice. In this model, viral spread from the infected XII motoneurons involves specific transneuronal transfer to connected cells and local, nonspecific transfer. For comparison, a PrV mutant lacking the nonessential nonstructural glycoprotein gX was included. Although the efficiencies of first-cycle replication were similar for the three viruses, only gX$^-$ and gp50$^-$ progeny mutants could spread from XII motoneurons via transneuronal and local transfer. The extents of transfer of gX$^-$ and gp50$^-$ PrV were comparable. The results show that the absence of gp50 does not alter the pattern of transneuronal or local spread of PrV, whereas gII is essential for both processes.

The alphaherpesvirus pseudorabies virus (PrV) or suid herpesvirus 1 is the causative agent of Aujeszky's disease, a severe illness of pigs which involves prominent neurological and respiratory symptoms (32). Beside pigs, many other mammal species are also susceptible to PrV infection. PrV shares several features with another neurotropic alphaherpesvirus, herpes simplex virus type 1 (HSV-1) (15). The neuroinvasiveness of PrV and HSV-1 is mediated by virus uptake at peripheral endings of primary sensory, motor, sympathetic, and parasympathetic neurons (for a review, see reference 12). The propagation patterns of PrV and HSV-1 in vivo are largely consistent with transneuronal transfer between chains of connected neurons (3, 5, 12, 24, 25, 29–31).

Several findings indicate that penetration of these viruses into target cells is mediated by surface glycoproteins on the viral envelope. For PrV, six structural glycoproteins and one nonstructural glycoprotein that all exhibit homologies to HSV-1 glycoproteins have been characterized (for a review, see reference 15). Whereas gI (gE), gIII (gC), gp63 (gI), and gX (gG) are nonessential for a complete replicative cycle in cell culture, glycoproteins gII (gB), gp50 (gD), and gH (gH) are essential. Virus particles lacking any one of the three essential glycoproteins are noninfectious because of a defect in penetration and therefore have to be propagated on transcomplementing genetically engineered cell lines. Interestingly, whereas gII, like its homolog gB (HSV-1), is also essential for direct viral cell-to-cell spread in culture, gp50, despite being essential for penetration, is not required for cell-to-cell spread in cell culture (19, 22). Recent results after intranasal, subcutaneous, or intraperitoneal inoculation of phenotypically complemented gp50 and gII deletion mutants (6, 20) indicate that gp50 is also not required for the expression of a neurovirulent phenotype in mice. In those studies, the neurovirulent phenotype was correlated with the presence of virus in the brain at the time of death. Although they demonstrated that viral mutants lacking gp50 can reach central neurons, those studies could not provide information on the precise routes of propagation of gp50$^-$ mutants, since the kinetics and the extent of distribution of the mutants in the brain were not examined. Eventual differences in the propagation pattern of gp50$^-$ mutants relative to wild-type PrV were also not studied.

The aim of the present study was to analyze in detail the transneuronal transfer of isogenic PrV mutants deficient in glycoproteins gX, gp50, and gII in a well-characterized neuronal circuit, i.e., infection of the motor hypoglossal (XII) nerves of mice and subsequent transneuronal transfer to connected (second-order) neurons (30) (Fig. 1). The experiments made use of three isogenic deletion mutants in genes encoding glycoproteins gX (gX$^-$), gp50 (gp50$^-$), and gII (gII$^-$) (17, 22, 23), constructed from the Kaplan strain of PrV (8). Each virus mutant contained a β-galactosidase expression cassette inserted into the respective glycoprotein gene to facilitate the detection of infected cells in the animal (17). Since deletion of the gX gene does not cause any apparent changes in viral growth or neurovirulence (9, 10, 16, 17, 27), the gX$^-$ PrV β-galactosidase insertion mutant was included to reflect the behavior of wild-type PrV and was utilized for comparison of transneuronal transfer of the gp50$^-$ and gII$^-$ mutants. The gX$^-$ stock was propagated in normal (African green monkey kidney Vero) cells, whereas the gp50$^-$ and gII$^-$ mutants were grown in genetically engineered transcomplementing cells which constitutively express gp50 (MT50-3 [22]) or gII (MT3 [23]), leading to phenotypic complementation of the genetic defect. Cells and supernatants were harvested 48 h postinfection, cells were sonicated for 30 s, and all supernatants were concentrated as described previously (21). For the gII$^-$ PrV stocks, the frequency of revertant virus rescued by the resident glycoprotein gene during culture in transcomplementing cells was determined by plaque assay on noncomplementing cells. Since the complemented gp50$^-$ PrV mutant is able to form plaques in normal cells, analysis for revertants in the gp50$^-$ PrV stocks required one additional passage in noncomplementing cells. Two different stocks with different revertant

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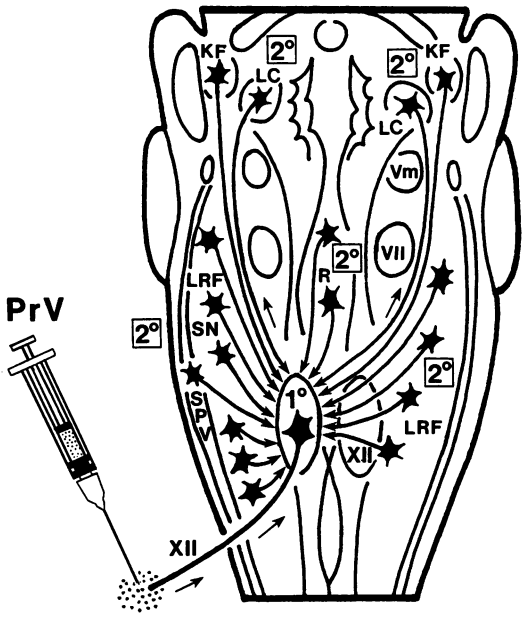

FIG. 1. Summary of retrograde transneuronal transfer of PrV after unilateral inoculation into the XII nerve. After the first cycle of viral replication in XII MN (1°), progeny virus can be transferred from XII MN to several groups of connected second-order neurons (2°) in the brain stem: lateral reticular formation (LRF), solitary tract nucleus (SN), spinal trigeminal nucleus (SPV), raphe nuclei and adjoining ventral reticular formation (R), nucleus coeruleus and subcoeruleus (LC), and nucleus of Kölliker-Fuse (KF). Other abbreviations: Vm, trigeminal motor nucleus; VII, facial nucleus. (Redrawn and adapted with permission from Fig. 4 of reference 12.)

frequencies were used for both gp50$^-$ and gII$^-$ mutants (see Tables 2 and 3).

The neuroinvasiveness of the gX$^-$, gII$^-$, and gp50$^-$ deletion mutants was studied by means of unilateral inoculation of 0.25 μl of virus into the medial branch of the XII nerves of female Swiss mice (age, 8 to 10 weeks) anesthetized with equithesine (4% chloral hydrate, 16% pentobarbital). The virus was injected into the nerve by means of glass micropipettes; the nerve was ligated immediately after injection to avoid peripheral leakage of the inoculum (see other details in reference 30). In this model, initial viral penetration involves mainly the axons of XII motoneurons (MN) (first-order neurons) (Fig. 1). After retrograde transport and replication in motoneuronal cell bodies, the virus can propagate via retrograde transneuronal (specific) transfer to several groups of connected (second-order) premotor interneurons in the brain stem (30) (Fig. 1). At long survival times, the virus can also spread via local (nonspecific) transfer from motoneuronal cell bodies and axons to adjoining glial cells and neurons (3, 12, 29, 30). The use of this test system makes it possible to quantify the extent of viral retrograde transport (to XII MN) and subsequent spread via retrograde transneuronal transfer (to premotor interneurons) and local transfer as done previously for HSV-1 (30) and PrV (3) and to compare the three PrV deletion mutants for neuroinvasiveness. Since viral replication is unlikely to occur at the point of inoculation, XII MN should be infected with transcomplemented (i.e., phenotypically equivalent to wild-type) gII$^-$ and gp50$^-$ viruses, whereas progeny virus in MN should comprise noncomplemented deletion mutants (plus progeny of revertant virus present in the inoculum). Thus, determining whether progeny mutants can be transferred from XII MN to connected neurons in this model in vivo is equivalent to studying two successive replication cycles of complemented mutants in noncomplementing cells in culture.

TABLE 1. Propagation of gX$^-$ PrV mutants from the XII nerve[a]

Animal no.	Time postinoculation (h)	No. of XII MN (first order)[b]	No./transneuronal transfer (second order)[b,c]	Local transfer[d]
734	48	200	3,000	+
735	48	300	3,000	+
766	53	411	4,000	+
767	53	288	4,000	+

[a] Titer, 1.7×10^9 PFU/ml; titer in inoculum (0.25 μl), 4.2×10^5 PFU.
[b] Approximate counts (cell counts made on one of two parallel series throughout the brain stem).
[c] Transneuronal transfer from XII MN to connected premotor interneurons (localization, see Table 4).
[d] Local tranfer from XII axons to adjoining cells.

All inoculations made use of viral stocks with high titers (Tables 1 to 3) to maximize the extent of viral transneuronal transfer that is dependent on the titer of the inoculum (29, 30). The experimental parameters (amounts injected, survival times, and detection method) were kept constant in all experimental groups to enable direct comparison of the behavior of gX$^-$, gp50$^-$, and gII$^-$ PrV (Tables 1 to 3). Since the results in gX$^-$ PrV-infected mice (see below and Table 1) showed that at 48 to 53 h postinoculation progeny virus had already undergone extensive spread from MN via transneuronal and local transfer, these long survival times were chosen to study the extent of spread of gp50$^-$ and gII$^-$ PrV (48 to 59 h postinoculation for gp50$^-$ and 48 to 63 h postinoculation for gII$^-$ PrV; see Tables 2 and 3). In three controls, the contribution of revertant virus to the propagation of gII$^-$ and gp50$^-$ PrV was estimated by inoculation into the XII nerve of 0.25 μl of medium containing approximately 40 PFU of gX$^-$ PrV (similar to the number of revertants per inoculum). The mice inoculated with the gp50$^-$ and gX$^-$ PrV mutants showed neurological symptoms of PrV infection

TABLE 2. Propagation of the gp50$^-$ PrV mutants from the XII nerve[a]

Stock and animal no.	Time postinoculation (h)	No. of XII MN (first order)[b]	No./transneuronal transfer (second order)[b,c]	Local transfer[d]
Stock A				
743	48	400 (A)	4,000 (A)	+
744	48	400 (A)	4,000 (A)	+
745	52	400 (A)	6,000 (A)	+
747	59	400 (A)	5,000 (A)	+
Stock B				
751	52	39	5	−
752	52	160	3,000 (A)	+
753	52	205	3,000 (A)	+

[a] gp50$^-$ stock A: titer, 1.2×10^9 PFU/ml; revertant frequency, 4×10^{-3}; titer in inoculum (0.25 μl), 3×10^5 PFU (75 revertants). gp50$^-$ stock B: titer, 5.6×10^8 PFU/ml; revertant frequency, 2.8×10^{-5}; titer in inoculum (0.25 μl), 1.4×10^5 PFU (0.5 revertants).
[b] (A), approximate counts (cell counts made on one of two parallel series throughout the brain stem).
[c] Transneuronal transfer from XII MN to connected premotor interneurons (localization, see Table 4).
[d] Local transfer from XII axons to adjoining cells.

TABLE 3. Propagation of the gII$^-$ PrV mutants from the XII nerve[a]

Stock and animal no.	Time post-inoculation (h)	No. of XII MN (first order)[b]	Transneuronal transfer (second order)[b,c]	Local transfer[d]
Stock A				
738	48	196	3	–
739	48	252	3	–
740	52	292	5	–
741	52	329	315	–
742	52	179	6	–
748	62	140	14	–
749	62	100	10	–
750	62	36	6	–
Stock B		–		
754	52	270	2	–
755	52	113	0	–
756	52	339	0	–
769	63	180	0	–
770	63	85	0	–
771	63	145	0	–
772	63	221	5	–
773	63	254	0	–

[a] gII$^-$ stock A: titer, 3.2×10^9 PFU/ml; revertant frequency, 1.6×10^{-4}; titer in inoculum (0.25 μl), 7.5×10^5 PFU (47 revertants). gII$^-$ stock B: titer, 1.3×10^{10} PFU/ml; revertant frequency, 1.2×10^{-5}; titer in inoculum (0.25 μl), 3.2×10^6 PFU (27 revertants).

[b] Cell counts were made on one of two parallel series throughout the brain stem.

[c] Transneuronal transfer from XII MN to connected premotor interneurons.

[d] Local transfer from XII axons to adjoining cells.

leading to death from 52 to 59 h postinoculation, whereas no symptoms were observed in either the gII$^-$ PRV-infected animals or the controls. At the chosen survival times, the mice were euthanized and subjected to transcardial perfusion (flow rate, 10 ml/min) with phosphate-buffered saline (PBS) (150 mM NaCl, 7.4 mM Na_2HPO_4, 2.4 mM KH_2PO_4) (20 ml), 4% paraformaldehyde in PBS (150 ml), and 20% sucrose in PBS (60 ml). Brains were dissected, kept in 20% sucrose–PBS for 24 h at 4°C for cryoprotection, frozen at −70°C, and cut in transverse serial sections that were collected in two parallel series on slides. For detection of the marker enzyme β-galactosidase expressed in cells infected with the different viral mutants, one series of sections was incubated for 4 h in a substrate solution containing 330 μg of 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside per ml, 5 mM $K_4Fe(CN)_6$, 5 mM $K_3Fe(CN)_6$, 2 mM $MgCl_2$, and 0.1% Triton X-100 in PBS. After counterstaining with neutral red (0.01%), the sections were mounted with DPX (BDH, Pole, England) or Entellan (Merck, Darmstadt, Germany). Some parallel sections were stained with cresyl violet (Nissl's staining).

The results showed that the first cycle of replication of gX$^-$ and phenotypically complemented gp50$^-$ and gII$^-$ PrV in the XII nucleus was similar. For all three mutants, the infection generally involved a large number of XII MN located in the regions of the XII nucleus that supply the injected medial branch of the XII nerve (11) (Fig. 2A, B, and D). The number of MN infected with gX$^-$ and phenotypically complemented gp50$^-$ PrV appeared to be similar to that observed after infection with phenotypically complemented gII$^-$ PrV, although precise counts of infected cells in many cases of gX$^-$ and gp50$^-$ PrV infection were difficult to obtain because specific MN labelling was blurred by diffuse background labelling in the XII nucleus (Fig. 2A and D). Most of the MN labelled in the cases of gII$^-$ and gp50$^-$ PrV infection must have been infected with transcomplemented mutants and not with the few revertants in the inoculum, since infected MN were either absent or present in very small numbers (two to seven) in the controls that received approximately 40 PFU of gX$^-$ PrV, an amount of virus similar to the number of revertants in the inoculum of the phenotypically complemented gp50$^-$ and gII$^-$ mutants. For all three mutants, the situation in the XII nucleus was that of motoneuronal degeneration caused by long-standing infection (3, 30), with intense β-galactosidase labelling of motoneuronal cell bodies, dendrites, and axons; a considerable increase in cell size (up to four times) (Fig. 2A, B, and D); loss of Nissl's staining with cresyl violet (Fig. 2C); and the presence of a local infiltrate of polymorphonucleates that could be identified in Nissl-stained sections on the basis of their characteristic morphology. In the gX$^-$ and gp50$^-$ PrV-infected animals, labelling of motoneuronal cell bodies was accompanied by labelling of adjoining glial cells, which reflects local (nonspecific) transfer of progeny virus and is known to occur at long survival times after the onset of motoneuronal infection (3, 30). This did not occur in most of the gII$^-$ PrV-infected animals or in the controls, probably explaining why MN could be better visualized in these cases.

Although the first cycles of replication appeared to be similar for all three viruses used, significant differences were observed with regard to the extent of transneuronal transfer of progeny virus from XII MN to connected second-order neurons at similar times. Thus, extensive viral transfer occurred in the animals infected with either gX$^-$ (Table 1) or gp50$^-$ (Table 2) PrV. With the exception of one animal, the pattern of transneuronal transfer of gp50$^-$ PrV was similar to that seen at comparable survival times in gX$^-$ PrV-infected animals. This involved the infection of several thousands of neurons in many brain stem regions, i.e., medullary and pontine lateral reticular formation bilaterally, nucleus of the solitary tract mainly ipsilaterally, dorsal part of the ipsilateral spinal trigeminal complex, caudal raphe nuclei and adjoining ventral reticular formation, locus coeruleus, and nucleus of Kölliker-Fuse bilaterally (see Table 4, Fig. 1, and Fig. 2E and F). Labelling in all these regions, which has also been obtained after inoculation of wild-type strains of HSV-1 into the XII nerve (30), is consistent with retrograde transneuronal transfer from XII MN to connected premotor (second-order) neurons (1, 2, 7, 14, 26, 28) (Fig. 1). Some variations occurred in both the gX$^-$ and gp50$^-$ PrV-infected animals with regard to the density of neuronal labelling and its extent in some of these cell groups (rostral part of the lateral reticular formation, locus coeruleus, nucleus of Kölliker-Fuse) (Table 4). This may reflect the fact that viral transneuronal transfer from XII MN to these cell groups is less efficient than transfer to the other groups of second-order neurons. This is based on the finding that transneuronal labelling of the former cell groups is delayed relative to that of the latter cell groups during retrograde transneuronal transfer of HSV-1 from XII MN (30). In all gX$^-$ and gp50$^-$ PrV-infected animals, infection of some glial cells and neurons also occurred along the intramedullary trajectory of XII axons (Fig. 2D), reflecting local viral transfer from transporting XII axons to adjoining cells, the second (nonspecific) mode of cell-to-cell spread of HSV-1 and PrV that is caused by long-standing infection (3, 12, 30) and is obviously also not dependent on the presence of gp50. In all gX$^-$ and gp50$^-$ PrV-infected animals, infected neurons were also found in the Gasserian ganglion, almost exclusively ipsilaterally. In

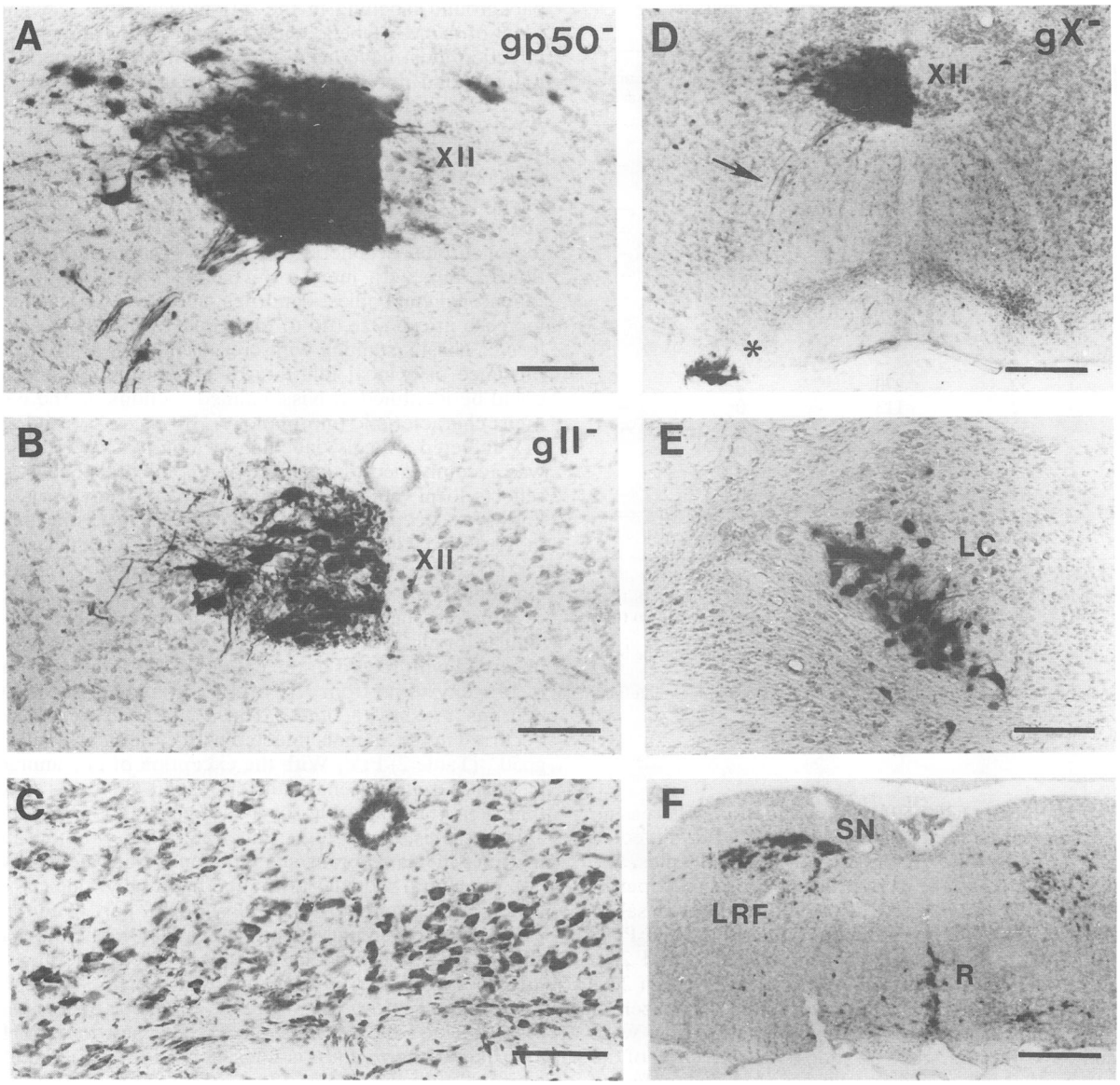

FIG. 2. (A, B, D, and F) β-Galactosidase labelling of infected neurons in the brain stem after inoculation of gX⁻ PrV or complemented gp50⁻ or gII⁻ PrV deletion mutants into the left XII nerve. (C) Cresyl violet (Nissl's) staining of XII nucleus (gII⁻ PrV). (A to D) Primary infection of XII MN (left XII) with gp50⁻ PrV (A), gII⁻ PrV (B and C), or gX⁻ PrV (D). In all cases, infected MN are numerous, appear much enlarged (left XII nucleus in panels A, B, and D; compare with noninfected right XII nucleus), and show loss of Nissl's staining in sections stained with cresyl violet (gII⁻ PrV-infected left XII nucleus; panel C). Motoneuronal labelling with gp50⁻ and gX⁻ PrV is blurred by labelling of adjoining glial cells (see panels A and D); this does not occur with gII⁻ PrV (see panel B). Note in panel D (gX⁻ PrV infection) the occurrence of local (nonspecific) viral transfer from XII axons (arrow) to adjoining cells along the axon trajectory and especially at the XII root entry zone (asterisk). (E and F) Second-order neurons infected by retrograde transneuronal (specific) transfer of gp50⁻ progeny mutants from XII MN. (E) Ipsilateral nucleus coeruleus (LC). (F) Raphe and adjoining ventral reticular formation (R), solitary tract nucleus (SN), and lateral reticular formation (LRF). Bars: 120 μm (A, B, and E), 90 μm (C), 300 μm (D), and 500 μm (F).

most cases with survival times equal to or longer than 52 h, neuronal infection also occurred in additional cell groups, i.e., ipsilateral motor nucleus of the vagus and nucleus ambiguus.

The results with the gII⁻ mutant were strikingly different from those with the gX⁻ and gp50⁻ mutants (Table 3). Although the efficiency of first-cycle replication, i.e., the number of infected XII MN, was similar to that obtained in gX⁻ and gp50⁻ PrV-infected animals, all animals infected with gII⁻ PrV showed only a very small amount of transneuronal transfer. Even after inoculation of the gII⁻ PrV stock with the highest revertant frequency (Table 3, stock A), transneuronal transfer comprised at the most 3 to 14 infected second-order neurons in total per brain series, which were mostly restricted to only one of the groups of second-order neurons, i.e., raphe and adjoining ventral reticular formation bilaterally. In one gII⁻ PrV-infected animal of this group (no. 741; Table 3, stock A), the neurons

TABLE 4. Extent of transneuronal transfer of gp50$^-$ and gX$^-$ PrV from XII MN to premotor interneurons[a]

Premotor interneurons[b]	Transfer of gX$^-$ PrV in animal at indicated h postinoculation				Transfer of gp50$^-$ PrV in animal at indicated h postinoculation					
					Stock A				Stock B	
	734 (48 h)	735 (48 h)	766 (53 h)	767 (53 h)	743 (48 h)	744 (48 h)	745 (52 h)	747 (59 h)	752 (52 h)	753 (52 h)
LRF ipsi										
a	+	+	++	++	++	++	+++	++	+	+
b	++	++	++	++	++	++	+++	+++	+	+
c	+/−	+/−	++	++	+	+	++	++	+	+/−
d	+	+/−	+	+	+	+	+	+	+/−	−
LRF contra										
a	+/−	+/−	+	+	+	+	++	+	+/−	+/−
b	+	+	++	++	++	++	+++	++	+	+
c	−	−	+	++	+/−	+	++	++	+	−
d	−	+/−	+	+	+/−	+	+	+	−	−
SN										
Ipsi										
Caudal	+	+	+	++	+	++	++	++	+	+
Rostral	+/−	−	++	++	+	++	+++	+++	++	++
Contra, caudal	+/−	+/−	+/−	+/−	−	+	+	+	+	+
SPV ipsi	+	+	+	+	+	+	++	+	+	+
R-VRF bilateral	++	++	++	++	+++	+++	+++	+++	++	++
LC										
Ipsi	+	+/−	+	+	+	+	++	++	−	−
Contra	+	−	+	+	+	+	++	++	−	−
KF										
Ipsi	−	−	+	+	+	+	++	++	−	−
Contra	−	+/−	+/−	+	+	+	++	++	−	−

[a] Localization of second-order neurons labelled by transneuronal transfer from the XII nerve in gX$^-$ and gp50$^-$ PrV-infected animals. Symbols: −, no labelling; +/−, labelling restricted to only a few cells; +, ++, and +++, pronounced labelling (the number of crosses indicating the number of infected cells.

[b] Abbreviations: LRF, lateral reticular formation, levels a to d (from caudal to rostral: a, LRF at the level of the XII nucleus; b, LRF from level of the rostral end of XII nucleus to the rostral end of VII [facial] nucleus; c, LRF adjoining the descending roots of VII nerve; d, LRF at the level of the trigeminal motor nucleus); SN, nucleus of the solitary tract; SPV, spinal trigeminal nucleus (labelling restricted to the dorsal third of the nucleus); R-VRF, caudal raphe nuclei (raphe obscurus, pallidus, and magnus) and adjoining ventral reticular formation; LC, locus coeruleus; KF, nucleus of Kölliker-Fuse.

labelled by transneuronal transfer were more numerous (315) and more widely distributed, but still much fewer neurons were labelled compared with the many thousands labelled in gp50$^-$ and gX$^-$ PrV-infected animals (Tables 1 and 2). None of the gII$^-$ PrV-infected animals showed local transfer from XII intramedullary axons (Table 3), in contrast with the occurrence of local transfer in the gp50$^-$ and gX$^-$ PrV-infected animals at similar survival times. In most animals, infection of some neurons (25 to 65 per series) also occurred in the ipsilateral Gasserian ganglion, which is consistent with uptake from the few trigeminal fibers present in the XII nerve (18). After inoculation of the gII$^-$ stock with a lower frequency of revertants, the results were even more striking, showing the absence of transneuronal and local transfer in virtually all cases (Table 3, stock B). The Gasserian ganglion was not infected. Transneuronal and local transfer never occurred in the controls.

The extensive viral spread by transneuronal and local transfer of the gX$^-$ PrV mutant observed in the present study correlates with reports that the deletion of gX does not seem to alter the propagation pattern of PrV in vivo (6, 13). Our comparison of the behavior of gX$^-$ and gp50$^-$ PrV at similar survival times and after injection of similar amounts showed that the extent of transneuronal transfer after inoculation of mice with phenotypically complemented gp50$^-$ PrV was similar to or even greater than that in gX$^-$ PrV-infected animals (Table 4). It appears most unlikely that this extensive transneuronal transfer of gp50$^-$ PrV could be mediated by the few revertants present in the inoculum. In fact, the observation that virus spread was virtually absent in the gII$^-$ PrV-infected animals despite the presence of a similar number of revertants in the respective inocula of gII$^-$ and gp50$^-$ PrV (Tables 2 and 3) suggests that the contribution of revertant virus to the pattern of spread of the mutants was minimal. In addition, no transfer occurred in the controls infected with a number of PFU of gX$^-$ PrV similar to the number of revertants in the mutant virus stocks. Thus, the striking differences in the patterns of propagation in gp50$^-$ and gII$^-$ PrV-infected animals clearly reflected differences in the neurotropic properties of the mutants.

In conclusion, the present study provides the first detailed analysis of the transneuronal propagation of gX$^-$, gp50$^-$, and gII$^-$ PrV deletion mutants in a well-defined hierarchical system of neuronal connections in vivo, involving primary uptake by XII MN and retrograde transneuronal transfer to connected interneurons. The findings clearly demonstrate that the essential glycoprotein gp50 of PrV is not necessary for efficient transneuronal (specific) transfer of virions from first- to second-order neurons. Local (nonspecific) transfer of virus from infected neurons to adjoining cells similarly does not require the presence of gp50. The absence of gp50 does not even seem to reduce the extents of both transneuronal and local viral spread. In contrast, glycoprotein gII is necessary for both transneuronal and local transfer of PrV. These findings are in agreement with recent reports (6, 20) but extend them to a detailed analysis of specific transneuronal transfer. They add to recent observations that the deletion of another surface glycoprotein (gI) can alter the propagation pattern of PrV (4). They also show that the behavior of gp50$^-$ and gII$^-$ PrV with regard to transneuronal transfer in vivo mimics that seen for direct viral cell-to-cell spread in cultured cells in vitro (19, 22). It is therefore likely

that the mechanisms for direct viral cell-to-cell spread in cultured cells closely resemble those mediating neuroinvasion in animals. Further studies of the requirement for viral spread in vitro should therefore also shed more light on the prerequisites for transneuronal spread in vivo.

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