JOURNAL OF VIROLOGY, Sept. 1995, p. 5829–5832 Vol. 69, No. 9
0022-538X/95/$04.00+0

The Capsid Determinant of Fibrotropism for the MVMp Strain of Minute Virus of Mice Functions via VP2 and Not VP1

IAN H. MAXWELL,[1]* AUSTIN L. SPITZER,[1] FRANÇOISE MAXWELL,[1] AND DAVID J. PINTEL[2]

University of Colorado Cancer Center and Health Sciences Center, Denver, Colorado 80262,[1] and Department of Molecular Microbiology and Immunology, School of Medicine, University of Missouri—Columbia, Columbia, Missouri 65212[2]

Received 21 February 1995/Accepted 22 May 1995

The minute virus of mice, prototype strain MVMp, productively infects cultured murine fibroblasts but not T cells. The immunosuppressive strain, MVMi, shows the converse tropism. These reciprocal tropisms are mediated by the viral capsids, in which their determinants have been mapped to a few specific amino acids in the primary sequence shared by VP1 and VP2. Which of these proteins is relevant in presenting these determinants during infection is not known. We have approached this question using a recombinant parvovirus system in which a LuIII-derived transducing genome, containing the luciferase reporter in place of viral coding sequences, can be packaged by capsid proteins from separate helper sources. We generated transducing virions by using helper constructs expressing either VP1 or VP2, containing the MVMp or MVMi tropic determinant region, in various combinations. The virions were used to infect human NB324K cells and murine A9 fibroblasts. Transduction of the human cells (permissive for both MVMp and MVMi) required both VP1 and VP2 and was successful with all combinations of these proteins. In contrast, significant transducing activity for A9 cells was detected only with recombinant virions containing VP2 of MVMp, while the use of either source of VP1 had little effect. We conclude that VP2 from MVMp is necessary to enable infection of murine A9 fibroblasts.

Two isolates of the autonomous parvovirus minute virus of mice, designated MVMp and MVMi, productively infect murine fibroblasts and T-lymphoid cells, respectively, but are restricted in their reciprocal host cells (9, 11). Early studies showed that virions of the two strains competed for binding to either cell type, arguing against restriction at the level of a cell surface receptor (10). The observation that single-cycle virus production was efficient in murine fibroblasts transfected with infectious plasmid clones of either MVMp or MVMi suggested that permissiveness or restriction was mediated by the virion particles (5). Studies with intertypic recombinants localized the primary determinants of this tropism to a small region of the capsid coding sequence (1, 6), and it was shown that mutating two specific codons of MVMi to those of MVMp was sufficient to confer fibrotropism (2).

We have previously constructed recombinants of the parvovirus LuIII with essentially complete replacement of the viral coding sequences by a luciferase reporter gene (8). Transducing virions can be generated by transient cotransfection of the recombinant genome, together with a replication-defective helper construct supplying viral nonstructural and capsid proteins (8). These functions can be provided by helpers derived from LuIII or from related parvoviruses (7). In particular, we showed that packaging of the recombinant with capsid proteins supplied by an MVMp helper, but not by an MVMi helper, conferred the ability to transduce murine A9 fibroblasts (7). By packaging a totally heterologous genome, these results confirmed that the fibrotropic determinant was specified by the MVMp capsid and excluded the possible additional involvement of its encoding DNA.

* Corresponding author. Mailing address: Medical Oncology B171, University of Colorado Health Sciences Center, 4200 E. 9th Ave., Denver, CO 80262. Phone: (303) 270-4760. Fax: (303) 270-8825. Electronic mail address: Maxwell_I@defiance.hsc.colorado.edu

Capsids of the rodent family of parvoviruses are composed of the proteins VP1 (≈85 kDa), VP2 (≈66 kDa), and VP3 (≈63 kDa). VP1 constitutes ≈15% of the mass of the capsid, and this protein includes the entire sequence of VP2, in addition to a unique N-terminal region of ≈140 amino acids (3). VP1 and VP2 are translated from alternatively spliced transcripts from the P38 promoter. In contrast, VP3 is derived by posttranslational cleavage, near the N terminus, of some molecules of VP2 following DNA encapsidation (3, 12). The roles of VP1 and VP2 in virus assembly and infection have been investigated by constructing mutant genomes encoding either protein (13). Expression of VP2 was found to be sufficient to generate capsids containing packaged DNA, but these particles were noninfectious (13, 14). It could therefore be inferred that incorporation of VP1 into capsids containing VP2 was essential for infectivity. The expression of VP1 alone did not generate virus particles (13).

Since the capsid sequence that determines minute virus of mice cell tropism is present in both VP1 and VP2, the function of this determinant could be mediated by either one of these proteins. Knowledge of whether VP1 or VP2 was involved in this function would contribute to understanding of their relative roles in establishing infection. Here, we have used the recombinant LuIII system to investigate this question by using helpers supplying either MVMp- or MVMi-related VP1 and VP2 proteins in various combinations.

The structures of the transducing genome, GLuLUCΔSV (8), and of the helper constructs are shown in Fig. 1. Recombinant viruses were generated, as described elsewhere (8), by transient cotransfection of NB324K cells, by electroporation, with pGLuLUCΔSV together with one or more of the helper plasmids. For the experiment reported in Fig. 2, each electroporation used the indicated amounts of DNA in 0.1 ml of cell suspension. Subsequent experiments (Table 1) used 0.2 ml of cell suspension with 5 μg of pGLuLUCΔSV, 1 μg of VP1

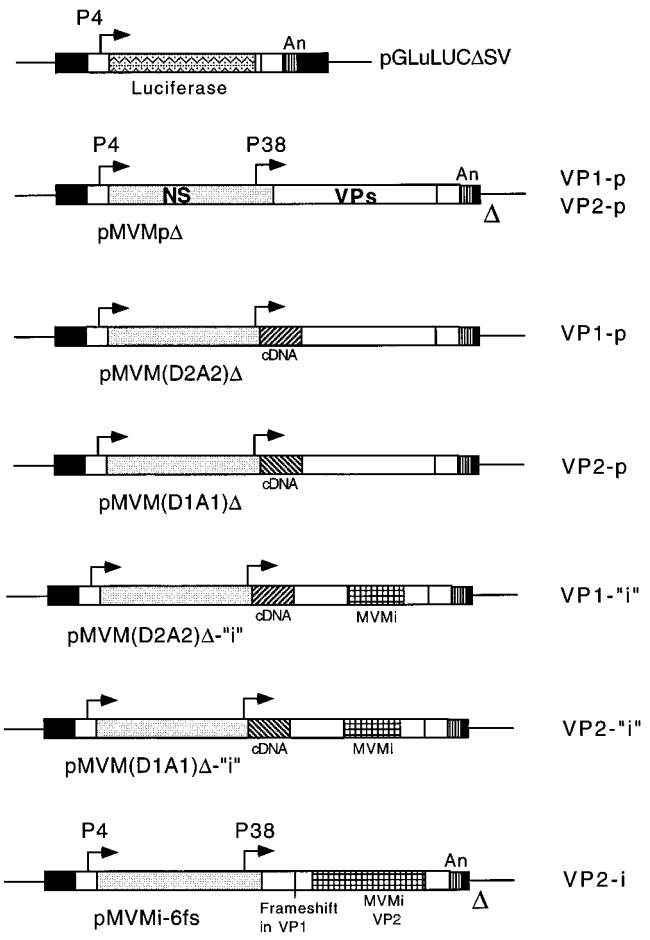

FIG. 1. Plasmids used in cotransfection to generate recombinant virus. The transducing genome, GLuLUCΔSV, is shown in the top row; all other diagrams are of helper constructs, with the products that they express indicated at the right. VP1-p, VP2-p, and VP2-i denote the authentic VP1 or VP2 proteins of MVMp or MVMi. VP1-"i" and VP2-"i" denote proteins containing a segment derived from MVMi, in an MVMp background; the segment includes the coding region for the primary determinant of lymphotropism (1, 2, 6). Plasmids are represented in linear form, although the covalently closed circular forms were used for transfection. pGLuLUCΔSV (8) contains, in pUC18, the left and right terminal inverted repeat sequences of LuIII and the firefly luciferase reporter (4), flanked by the P4 promoter and downstream sequences of LuIII, including the polyadenylation signal. pMVMpΔ (7) is an MVMp genomic clone, defective for replication because of a deletion involving the right terminus; a similar deletion is present in all the helper constructs shown. The construction of pMVM(D1A1) and pMVM(D2A2) by substitution of cDNA spliced in the donor 1-acceptor 1 or donor 2-acceptor 2 pattern has been described previously (13). The "i" constructs with these two splicing patterns were made by substitution of a *Bgl*II fragment (nucleotides 3450 to 4212) from an MVMi genomic clone. The bottom row shows the expression construct for authentic VP2-i, which was made by introducing a frameshift in the VP1 unique coding region of pMVMi-6 (7) by filling in at the *Hin*dIII site (at nucleotide 2650).

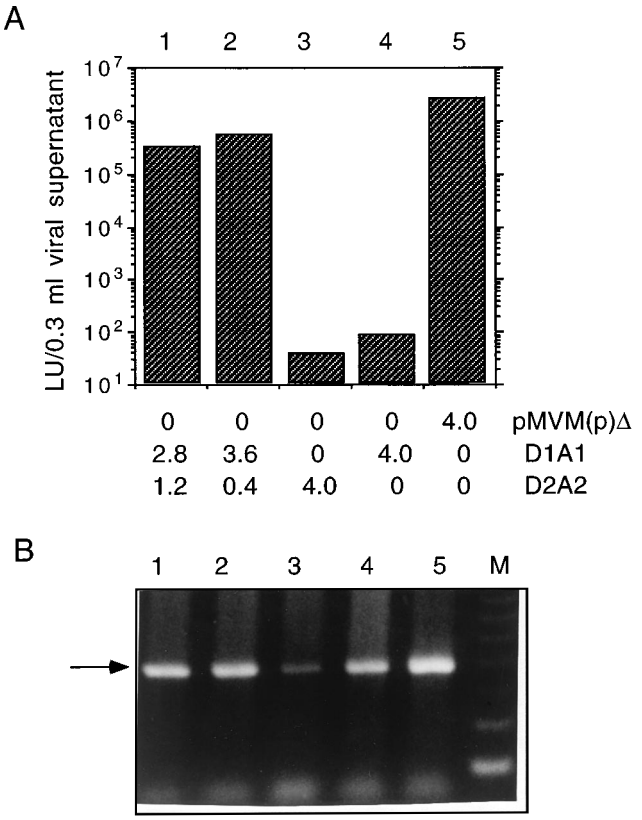

FIG. 2. (A) Transduction of NB324K cells with viral supernatants from producer cells that had been transiently transfected with the indicated helpers together with pGLuLUCΔSV (2.5 μg). Numbers at the bottom are micrograms of helper plasmid used in each transfection. The recipient cells were assayed for transduced luciferase activity at 2 days postinfection. The ordinate shows this activity in light units (LU), assayed with a commercial kit (Promega). Values of $<10^2$ LU do not differ significantly from background values. (B) PCR to detect packaging of the transducing genome using primers for the luciferase gene (primer sequences available on request). Samples of viral supernatants were incubated with DNase (100 μg/ml; 3 h at 37°C) which was then inactivated at 64°C for 15 min. The samples were then subjected to 30 cycles of PCR and analyzed by electrophoresis in a 1.5% agarose gel with ethidium staining. The arrow indicates the amplified luciferase fragment (453 bp); the marker (lane M) was a 123-bp ladder (Life Technologies). We assume that the packaged DNA became available, as a PCR template, by virion disruption in the 2-min, 94°C preincubation step. Controls (not shown) established complete removal of any contaminating plasmid by the DNase. Lane numbers match bar numbers in panel A.

expression plasmid, and 7 μg of VP2 expression plasmid. Transducing virus was harvested at 3 days following electroporation by combining the culture medium with an extract of the cells made by freezing and thawing three times. After low-speed centrifugation, the viral supernatants were stored at −20°C. Transducing activity was determined (7, 8) by infection of NB324K or A9 cells, grown to ≈30% confluence in 6-cm-diameter dishes, and assay of luciferase activity in extracts of the recipient cells at the times indicated.

Genomic clones of MVMp capable of expressing only one of the capsid proteins (i.e., either VP1 or VP2) have been described previously (13). The helper plasmids used here (Fig. 1) were replication-defective derivatives of these clones. The original clones were made by substituting, into an MVMp infectious clone, cDNA sequences derived from the mRNAs encoding VP1 and VP2 containing, respectively the D2A2 and D1A1 splices (13). Recombinant viruses were generated by cotransfection of pGLuLUCΔSV (Fig. 1) with the helper plasmids singly or in pairs. Transducing activity was then assayed by exposure of fresh, recipient NB324K cells to the viral supernatants from the transfected producer cells. As shown in Fig. 2A, use of the VP1 and VP2 expression plasmids in combination generated substantial activity, amounting to 20 to 50% of that observed with the single helper, pMVM(p)Δ (7). The latter expresses both VP1 and VP2 using the normal splicing patterns. Neither of the prespliced helpers transfected singly with pGLuLUCΔSV gave rise to significant transducing activity (Fig. 2A). We also examined the viral supernatants for the presence of packaged (DNase-resistant) GLuLUCΔSV

TABLE 1. Luciferase transducing activity, in NB324K cells (nonrestrictive) or murine A9 fibroblasts, of GLuLUCΔSV virus preparations packaged by various combinations of MVMp- or MVMi-derived capsid proteins VP1 and VP2

Expt	VP1 source	Time (h) post-infection	Transducing activity[a] (light units/0.3 ml of viral supernatant) in:			
			NB324K cells		A9 cells	
			VP2-p	VP2-"i"[b]	VP2-p	VP2-"i"[b]
1	VP1-p	30	227,000	10,200	33,800	160
		54	216,000	36,500	30,700	150
	VP1-"i"	30	311,000	6,490	43,600	(65)
		54	345,000	20,000	33,800	(28)
2	VP1-p	50	441,000	35,800	22,100	180
	VP1-"i"	50	442,000	97,800	23,100	370

[a] Luciferase activity was determined in extracts of recipient cells at the indicated times after infection with viral supernatants (0.3 ml/6-cm-diameter dish) from producer cells expressing capsid proteins in the combinations indicated. Proteins were expressed by transfection with the helper plasmids shown in Fig. 1, as described in the text. Standard and Promega luciferase assays were used, respectively, for NB324K and A9 extracts. (The Promega assay is ≈5.5-fold more sensitive.) Values in parentheses are not significantly above background levels. The values shown are single determinations for experiment 1 and the means of two to four determinations for experiment 2.

[b] VP2-i was used in experiment 2.

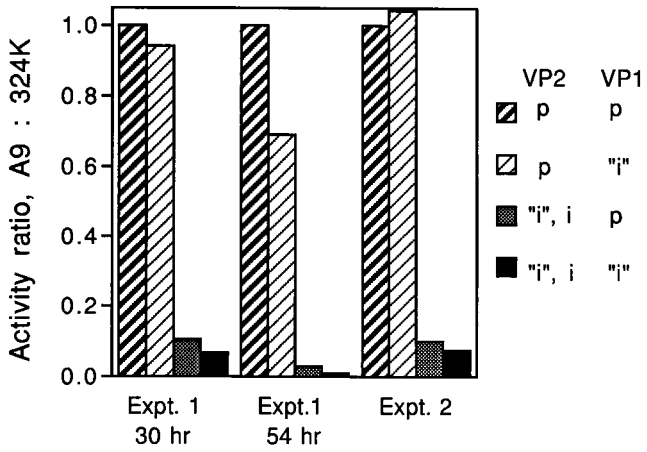

FIG. 3. Graphic presentation of results from Table 1. The ratio of transducing activity in A9 to that in NB324K cells is plotted for each combination of helpers, after the VP2-p–VP1-p combination is normalized to 1.0 for each experiment.

DNA, detected by PCR with luciferase-specific primers. As shown in Fig. 2B, this DNA was detected in all cases, although only weakly when the VP1 expression plasmid (D2A2) was used alone. These results are consistent with previous findings that VP2, but not VP1, is capable of efficient capsid formation and packaging of DNA into noninfectious virions.

The above observation that transducing virions could be generated efficiently by triple cotransfection of the recombinant genome with separate helpers expressing VP1 or VP2 provided the basis for the following experiments in which virions containing different combinations of MVMp- or MVMi-derived capsids were tested for transducing activity. Expression plasmids for MVMi-related VP1 or VP2 were constructed (see legend to Fig. 1) by substituting DNA encoding the lymphotropic determinant of the MVMi capsid into the pMVMp D2A2 or D1A1 helper constructs. The expression products of the resulting constructs are referred to as VP1-"i" and VP2-"i". Additionally, a helper plasmid expressing authentic VP2 of MVMi (VP2-i) was made by introducing a frameshift mutation in the region encoding the VP1 unique sequence (Fig. 1).

Viral supernatants from NB324K cells that had been cotransfected with pGLuLUCΔSV and various combinations of the helper constructs were tested for transducing activity in both NB324K cells and murine A9 fibroblasts as recipient cells. The results are shown in Table 1 and summarized in Fig. 3. Experiments 1 and 2 differ in the use of VP2-"i" and VP2-i, respectively. The first point to note is that reasonably efficient transduction of the nonrestrictive NB324K cells was obtained with all helper combinations, whether homologous or heterologous (Table 1). In these recipient cells, the source of VP1 made little difference, although there was some effect of the source of VP2. In particular, VP2-"i" generated less transducing activity for NB324K cells than did VP2-p. Less decrease was observed with the authentic VP2-i in combination with VP1-"i" (Table 1). The decrease in luciferase activity in NB324K cells with VP2-"i" versus VP2-p was usually <20-fold, and we have not investigated this further.

Much more dramatic effects of the source of VP2 were observed in transduction of A9 fibroblasts. In these cells, virions generated with MVMi-related VP2 showed greatly reduced activity (100- to 500-fold less than those with VP2-p [Table 1]). This is more clearly illustrated in Fig. 3, in which the transducing activity in A9 cells is plotted as a fraction of the activity seen in NB324K cells for the corresponding combinations of helper constructs. It can be seen that the relative A9 transducing activity was always decreased by at least 10-fold for recombinants containing VP2-i or VP2-"i." In contrast, this activity was not significantly affected by the source of VP1 (whether VP1-p or VP1-"i").

It is also apparent from Table 1 that recombinant virions containing VP2-p gave substantially lower transducing activity in A9 cells than in NB324K cells, although the difference was much less than with MVMi-related VP2. The results of plasmid transfection experiments (not shown) suggest that this stems from more efficient expression of the reporter construct in NB324K cells, rather than a higher efficiency of infection. Thus, after electroporation with plasmids containing the luciferase reporter driven by either the LuIII P4 promoter or the simian virus 40 early promoter, the level of expression by NB324K cells was >100-fold higher than that by A9 cells. (Parallel transfections with a β-galactosidase expression plasmid [pCMVβ] indicated a similar electroporation efficiency for both cell types.)

VP1 and VP2 of minute virus of mice differ in primary sequence only by the unique N-terminal region of VP1 (142 amino acids). As discussed previously (13), the latter contains several possible nuclear localization signals. Since VP1 is required for virion infectivity, it is plausible that this region is involved in nuclear delivery of an infecting genome. Although it is attractive to hypothesize that the success of this process might require interaction of cellular factors with the viral allotropic determinant carried by VP1, VP2 perhaps being dispensable after penetration of the cell, our results appear to exclude this hypothesis, at least for the fibrotropic determinant in transduction of A9 cells, since they implicate VP2-p as the functional carrier of this determinant.

We thank Jon Carlson, Joe Corsini, and Solon Rhode for valuable discussions and Lisa Burger for technical assistance.

This work was supported by NIH grants CA52085 (to I.H.M.) and AI21302 (to D.J.P.) and by a generous donation from the Monfort

Foundation. A.L.S. received support from a Cancer Student Fellowship, University of Colorado Cancer Center.

REFERENCES

1. **Antonietti, J. P., R. Sahli, P. Beard, and B. Hirt.** 1988. Characterization of the cell type-specific determinant in the genome of minute virus of mice. J. Virol. **62:**552–557.
2. **Ball-Goodrich, L. J., and P. Tattersall.** 1992. Two amino acid substitutions within the capsid are coordinately required for acquisition of fibrotropism by the lymphotropic strain of minute virus of mice. J. Virol. **66:**3415–3423.
3. **Cotmore, S. F., and P. Tattersall.** 1987. The autonomously replicating parvoviruses of vertebrates. Adv. Virus Res. **33:**91–174.
4. **De Wet, J. R., K. Y. Wood, M. DeLuca, D. R. Helinski, and S. Subramani.** 1987. Firefly luciferase gene: structure and expression in mammalian cells. Mol. Cell. Biol. **7:**725–737.
5. **Gardiner, E. M., and P. Tattersall.** 1988. Evidence that developmentally regulated control of gene expression by a parvoviral allotropic determinant is particle mediated. J. Virol. **62:**1713–1722.
6. **Gardiner, E. M., and P. Tattersall.** 1988. Mapping of the fibrotropic and lymphotropic host range determinants of the parvovirus minute virus of mice. J. Virol. **62:**2605–2613.
7. **Maxwell, I. H., C. J. Long, J. O. Carlson, S. L. Rhode III, and F. Maxwell.** 1993. Encapsidation of a recombinant LuIII parvovirus genome by H1 virus and the fibrotropic or lymphotropic strains of minute virus of mice. J. Gen. Virol. **74:**1175–1179.
8. **Maxwell, I. H., F. Maxwell, S. L. Rhode III, J. Corsini, and J. O. Carlson.** 1993. Recombinant LuIII autonomous parvovirus as a transient transducing vector for human cells. Hum. Gene Ther. **4:**441–450.
9. **McMaster, G. K., P. Beard, H. D. Engers, and B. Hirt.** 1981. Characterization of an immunosuppressive parvovirus related to the minute virus of mice. J. Virol. **38:**317–326.
10. **Spalholz, B. A., and P. Tattersall.** 1983. Interaction of minute virus of mice with differentiated cells: strain-dependent target cell specificity is mediated by intracellular factors. J. Virol. **46:**937–943.
11. **Tattersall, P., and J. Bratton.** 1983. Reciprocal productive and restrictive virus-cell interactions of immunosuppressive and prototype strains of minute virus of mice. J. Virol. **46:**944–955.
12. **Tullis, G. E., L. R. Burger, and D. J. Pintel.** 1992. The trypsin-sensitive RVER domain in the capsid proteins of minute virus of mice is required for efficient cell binding and viral infection but not for proteolytic processing in vivo. Virology **191:**846–857.
13. **Tullis, G. E., L. R. Burger, and D. J. Pintel.** 1993. The minor capsid protein VP1 of the autonomous parvovirus minute virus of mice is dispensable for encapsidation of progeny single-stranded DNA but is required for infectivity. J. Virol. **67:**131–141.
14. **Willwand, K., and B. Hirt.** 1993. The major capsid protein VP2 of minute virus of mice (MVM) can form particles which bind to the 3′-terminal hairpin of MVM replicative-form DNA and package single-stranded viral progeny DNA. J. Virol. **67:**5660–5663.