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Identification of the Myelin Protein Plasmolipin as the Cell Entry Receptor for *Mus caroli* Endogenous Retrovirus▽

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The Asian wild mouse species *Mus caroli* harbors an endogenous retrovirus (McERV) that is closely related to but distinct from the endogenous retrovirus family defined by the *Mus dunni* endogenous virus and the *Mus musculus* endogenous retrovirus. McERV could infect some cell types from humans, dogs, and rats, but not all, and did not infect any mouse cell line tested. Because of its interesting host range and proposed ancestral relationship to primate retroviruses and because none of the entry receptors for this family of retroviruses had been identified, we began a search for the McERV receptor. We determined the chromosomal location of the receptor gene in the human genome by phenotypic screening of the G3 human-hamster radiation hybrid cell line panel and confirmed the localization by assaying for receptor activity conferred by bacterial artificial chromosome (BAC) clones spanning the region. We next localized the gene more precisely in one positive BAC by assaying for receptor activity following BAC digestion with several restriction enzymes that cleaved different sets of genes, and we confirmed that the final candidate gene, plasmolipin (PLLP; TM4SF11), is the novel receptor by showing that the expression of the human PLLP cDNA renders hamster and mouse cells susceptible to McERV infection. PLLP functions as a voltage-dependent potassium ion channel and is expressed primarily in kidney and brain, helping to explain the limited range of cell types that McERV can infect. Interestingly, mouse PLLP also functioned well as a receptor for McERV but was simply not expressed in the mouse cell types that we originally tested.

An endogenous gammaretrovirus can be induced from cells of the Asian wild mouse *Mus caroli* by treatment of the cells with bromodeoxyuridine (22). This virus is distinct from the murine leukemia virus (MuLV) family of active retroviruses found in the laboratory mouse *Mus musculus* and appeared to be similar to exogenous retroviruses from gibbon apes (gibbon ape leukemia virus [GALV]) and woolly monkeys (simian sarcoma-associated virus [SSAV]). These similarities included a xenotropic host range (the ability to infect cells from species other than mice but not cells from mice) and reported cross interference between the viruses, indicative of a common cell entry receptor. It was therefore hypothesized that mice were the source of the exogenous simian viruses. Recently, a retrovirus induced by bromodeoxyuridine treatment of *Mus caroli* (*Mus caroli* endogenous retrovirus [McERV]) that has properties similar to those of the original virus studied over 30 years ago (22) was cloned and sequenced (C. Stocking, M. Ziegler, U. Bergholz, K. Weber, M. Eiden, and V. Prassolov, unpublished results). However, further analysis indicated that McERV does not use the cell entry receptor used by GALV and SSAV (Pit1; Slc20a1), weakening the argument that McERV is the progenitor of GALV and/or SSAV. McERV also did not use the receptors for xenotropic MuLV (Xpr1) (*Mus musculus*) or those for the endogenous feline leukemia virus RD114 (Slc1a4 and Slc1a5). While the McERV sequence showed relatively high similarity to those of GALV and SSAV, it was most closely related to *Mus dunni* endogenous virus (MDEV) (26) and the *Mus musculus* endogenous retrovirus MmERV (1), the cell entry receptors for which are unknown.

To further characterize the relationship between McERV and the simian retroviruses and to identify the potentially novel receptor used by McERV, we used human-hamster radiation hybrid (RH) cells and human genomic resources to identify the receptor for McERV on human cells. We and others have previously used human-hamster and mouse-hamster RH cell lines to identify human hyaluronidase 2 as a receptor for jaagsiekte sheep retrovirus (32), mouse transferrin receptor 1 as a receptor for mouse mammary tumor virus (36), and sodium-dependent *myo*-inositol transporter 1 as a receptor for the M813 MuLV from *Mus cervicolor* (17). However, one problem with the previously used approach is that phenotypic screening of RH cell lines typically localizes the receptor to a fairly large genomic region (~250 kb), and final identification of the receptor has involved substantial screening of candidate genes and some degree of luck. Indeed, for some retrovirus receptors, the RH approach accurately localized the receptor, but receptor identification relied on the screening of cDNA expression libraries (34). Here we have employed a deterministic approach involving the analysis of select human bacterial artificial chromosome (BAC) clones that are available for the entire human genome to make the final receptor identification. This analysis revealed that plasmolipin (PLLP), a major component of myelin that is also expressed on some epithelial cells, is the novel McERV receptor.

MATERIALS AND METHODS

Cell culture and virus production. Cells were grown in Dulbecco's modified Eagle medium with 7 to 10% fetal bovine serum except for the primary cultures

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of rabbit tracheal epithelial (RbTE) cells, which were grown in keratinocyte serum-free medium (Gibco) in fibronectin-coated cell culture dishes. The retroviral vector encoding green fluorescent protein (GFP) with an McERV pseudotype [GFP(McERV)] was harvested from 293 cells (39) infected with a biological clone of replication-competent McERV (C. Stocking, M. Ziegler, U. Bergholz, K. Weber, M. Eiden, and V. Prassolov, unpublished) and the retroviral vector SF91-ieGFP-PRE (R780) (38). Sequential medium harvests from these and other virus-producing cells were made every 12 to 24 h, starting the day after the cells became confluent, until the cells detached from the dishes. Virus-containing medium was filtered through 0.45-μm-pore-size filters to remove cells and debris and was used immediately or was frozen at −75°C until use.

BAC and plasmid clones. BAC clones were a gift from Barbara Trask (Fred Hutchinson Cancer Research Center) or were purchased from the BACPAC resources center (Children's Hospital and Research Center Oakland). Mouse and human PLLP (mPLLP and hPLLP, respectively) cDNA clones in the pCMV-Sport6 expression vector were purchased from Open Biosystems. The mouse and human cDNAs encode proteins that exactly match the GenBank C57BL/6 mPLLP reference sequence NP080661.1 and the hPLLP reference sequence NP057077.1, respectively. BAC and plasmid clone identity was confirmed by analysis of DNA fragment sizes after restriction enzyme digestion.

GFP(McERV) infection assays. Target cells were seeded at 5×10^4 cells per well (3.5-cm diameter) in six-well plates. The next day, the cells were exposed to 0.1 μl to 0.5 ml of the GFP(McERV) vector in a total of 2 ml medium containing 4 μg Polybrene per ml. The RH cells were fed with fresh medium the day after infection to minimize Polybrene toxicity. GFP-positive (GFP^+) foci were counted 2 to 3 days after infection. Prior to analysis, the culture medium was replaced with phosphate-buffered saline (containing calcium and magnesium) to eliminate background green fluorescence from the culture medium. RH cell lines judged susceptible to McERV infection showed from 10 to 10^5 GFP^+ foci per ml of virus added, and those judged negative for infection showed no GFP^+ foci (<2 per ml of virus).

Some cell lines were analyzed for GFP(McERV) infection by fluorescence-activated cell sorter (FACS) analysis 2 days after exposure to the virus in the presence of Polybrene. GFP(McERV) titers were similar when assayed by the GFP^+ focus assay or the FACS assay.

Transfection assay for receptor activity. Target cells were seeded at 5×10^4 cells per well (3.5-cm diameter) in six-well plates. The next day, the cells in each well were transfected with 2 μg test DNA and 0.5 μg of the plasmid pLAPSN (28) by using 7.5 μl of the TransIT-LT1 reagent (Mirus Bio, Madison, Wisconsin) according to the manufacturer's protocol. The pLAPSN plasmid expresses human placental alkaline phosphatase and was included to monitor the efficiency of transfection. The day after transfection, the cells in each well were trypsinized and divided at a ratio of 3:4 into a 10-cm-diameter dish and at a ratio of 1:4 into a well of a six-well plate. The next day, cells in the 10-cm dishes were infected by exposure to 2 ml GFP(McERV) virus mixed with 8 ml fresh medium and 4 μg Polybrene per ml. Two to three days after infection, GFP^+ foci were counted in the 10-cm dishes and the six-well plates were stained for heat-stable alkaline phosphatase activity as previously described (9).

For the transfection of restriction enzyme-digested BAC DNA, the DNA was digested for at least 4 h in a total volume of 10 to 20 μl and the enzymes were heat inactivated at 80°C for 20 min before transfection. A sample of each digested BAC DNA was analyzed by gel electrophoresis to check the extent of digestion and to ensure that DNA fragments of the expected sizes were generated.

To generate stable cell lines expressing hPLLP or mPLLP, the respective coding regions were cloned into the M5neo retroviral vector that also expresses neomycin phosphotransferase (20), and viruses produced from the vectors were used to infect A23 or NIH 3T3 cells, which were subsequently selected in G418 (400 μg/ml). GFP(McERV) infection frequencies on PLLP-expressing cells were analyzed by FACS analysis.

TABLE 1. Range of cells that are infectible by McERV[a]

Species	Target cells (reference or source)	Cell type	Titer (TU/ml) of GFP(McERV) vector
Human	293 (39)[b]	Neuronal	10^6
	HeLa (37)	Epithelial carcinoma	3×10^6
	RD (25)	Rhabdomyosarcoma	9×10^5
	HT-1080 (33)	Fibrosarcoma	<1
	Reh (13)	Acute lymphocytic leukemia	<100
	TF-1 (19)	Erythroleukemia	<100
African green monkey	COS (12)	Kidney	5×10^5
	Vero (46)	Kidney	3×10^4
Owl monkey	OMK (5)	Kidney	10^5
Dog	D-17 (35)	Osteosarcoma	10^5
	D-64	Marrow stromal fibroblast	<100
Cat	G355 (8)	Fetal glial	10^6
Cow	MDBK (24)	Kidney epithelial	10^6
Rabbit	RbTE (14)	Tracheal epithelial	10^6
Pig	PK-15 (ATCC)	Kidney epithelial	2×10^6
Mink	Mv1Lu (18)	Lung epithelial	10^6
Mouse	NIH 3T3 (ATCC)	Fibroblast	<1
	MDTF (21)	Fibroblast	<1
Rat	Rat1 (42)	Fibroblast	<100
	NRK (6)	Kidney	3×10^5
Chinese hamster	A23 (45)	Lung fibroblast	<1
	CHO-K1 (30)	Ovary	<1
	CHO-Lec8 (30)	Ovary	<1
	E-36 (30)	Lung	<1
Syrian hamster	BHK (23)	Kidney	<100

[a] The GFP(McERV) vector titer in numbers of GFP^+-focus transducing units (TU) per ml was measured as described in Materials and Methods. The sensitivity of the GFP^+-focus assay was 1 TU/ml, while that of the FACS assay was 100 TU/ml. Results are the means from at least two experiments. All cell lines that were not susceptible to McERV vector transduction were susceptible to either 10A1 MuLV pseudotype vector infection (human, dog, NIH 3T3, Rat1, and Syrian hamster cells; vector titers from 6×10^3 to 2×10^6) or MDEV pseudotype vector infection (MDTF and Chinese hamster cells; vector titers from 1×10^5 to 8×10^5), showing that the lack of McERV pseudotype vector transduction was not due to a general block of retroviral vector transduction unrelated to the Env protein.

[b] 293 cells were previously classified as human embryonic kidney epithelial cells (39).

RESULTS

Chromosomal localization of the McERV receptor. The ability of the GFP(McERV) vector to infect human cells but not hamster cells (Table 1) suggested that a screen for the putative McERV cell entry receptor could be carried out using the G3 panel of human-hamster RH cell lines (40). These hybrids were generated by fusing irradiated (10,000 rad) human lymphoblastoid cells with A3 (similar to A23 [45]) hamster lung fibroblasts. Irradiation randomly fragments the human DNA such that the resulting hybrids each carry about 18% of the human genome, in fragments with an average size of 4 megabases, in addition to a complete hamster genome. The initial assay of a few hybrid cell lines for susceptibility to infection by the GFP(McERV) vector showed that about one in five hybrids was infectible, indicating that the putative receptor was expressed at the expected frequency based on the amount of human DNA in each hybrid. The chromosomal location of the receptor was estimated by using software available on the Stanford Human Genome Center website (http://www-shgc.stanford.edu/seq/rhserverformnew.html), and after 40 of the 83 G3 panel hybrids were screened, the receptor localized to chromosome 16q13 closest to marker SHGC-34581, with a highly significant logarithm (base 10)-of-odds (LOD) score of 8.03 (Fig. 1). In addition, the

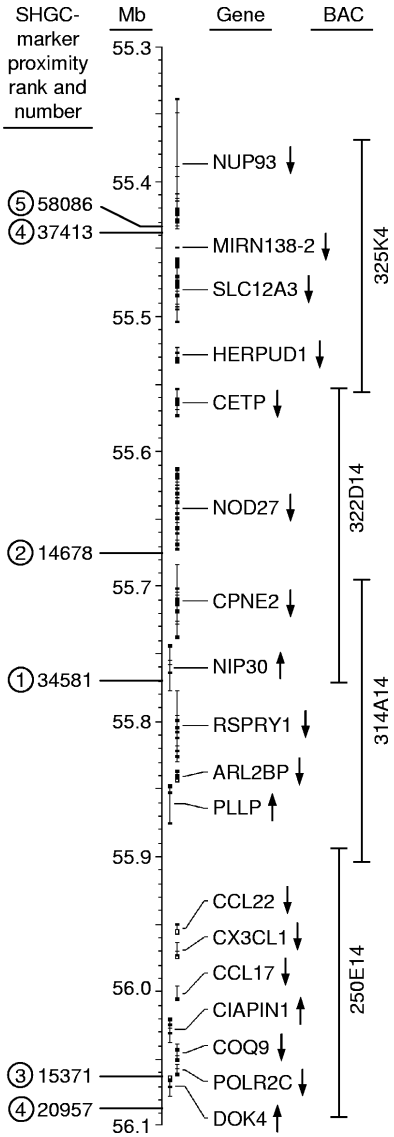

FIG. 1. McERV receptor localization to human chromosome 16q13. Analysis of 40 of the G3 RH cell lines linked the McERV receptor to the following unique SHGC markers, with the indicated LOD scores, ranked from highest to lowest: SHGC-34581, 8.03 (1); SHGC-14687, 7.46 (2); SHGC-15371, 6.35 (3); SHGC-20957 and SHGC-37413, 5.56 (4); SHGC-58086, 4.80 (5); SHGC-14315, 4.31 (6); SHGC-14216 and SHGC-247, 3.78 (7); and SHGC-20956, 3.69 (8). The positions of markers ranked 1 to 5 are shown on human chromosome 16. DNA sequence numbers are indicated in megabases starting from the tip of the p arm of chromosome 16 (human genome build 36.2). Known genes in this region are depicted with filled boxes indicating exons, open boxes indicating nontranslated regions in exons, lines indicating introns, and arrows indicating the direction of gene transcription. BAC clones spanning this region are indicated to the right. The original names of the clones begin with "RP11-," which has been deleted for simplicity. Data used to generate this figure are from the NCBI map viewer and the UCSC genome browser.

rest of the 10 top-scoring markers were all located close to SHGC-34581 on chromosome 16, with a decreasing LOD score correlating with an increasing distance from SHGC-34581 (Fig. 1). The final result was 101RRR000R 100R000000 0R0000RRR0 RR0R1RRRRR RR0RR11R01 100R010RRR RRRRR1RR0R RRRR1R0RR0 RR0, where 1 indicates an infectible hybrid, 0 indicates a resistant hybrid, and R indicates an untested hybrid.

Fine mapping of receptor localization by BAC assay. To more precisely map the McERV receptor, we assayed four BACs spanning a 700-kb interval surrounding marker SHGC-34581 (Fig. 1). BAC 314A14 showed high receptor activity after transfection into A23 hamster cells, while the other three showed no activity (Table 2). The positive BAC contained five annotated genes, one of which was a four-transmembrane protein (PLLP) that was the most likely receptor candidate based on predicted subcellular locations of the encoded proteins. To determine which gene actually had receptor activity, we assayed for receptor function after the digestion of BAC 314A14 with restriction enzymes that digested different sets of genes in the BAC (Table 2). Digestion with AatII, MluI, SacII, or SwaI did not reduce receptor activity in comparison to that of the undigested BAC DNA. Three of these enzymes cut the genes *CPNE2*, *RSPRY1*, and *ARL2BP*, and all four cut *NIP30*, while none cut the *PLLP* gene, indicating that *PLLP* encodes the receptor for McERV. In contrast, EcoRI cuts the *PLLP* gene in five places and HindIII cuts *PLLP* in two places, and both of these enzymes significantly reduced the receptor activity of BAC 314A14 DNA. Together, these data indicated that *PLLP*, or some unannotated gene in the same region, encoded the receptor for McERV.

Of interest, none of the BAC clones rendered NIH 3T3 cells susceptible to GFP(McERV) infection, even though a cotransfected plasmid (pLAPSN) was expressed as measured by histochemical staining for alkaline phosphatase. Transfection assays in which A23 and NIH 3T3 cells were exposed to aliquots of the same transfection mix containing BAC 314A14 and pLAPSN were repeated several times, and while exposure of the transfected A23 cells to GFP(McERV) induced hundreds of GFP^+ foci per 10-cm-diameter dish, no GFP^+ foci were detected in the transfected NIH 3T3 cells exposed to the GFP(McERV) vector.

The PLLP cDNA encodes the McERV receptor. To test whether the *PLLP* gene encodes the McERV receptor, we assayed whether the transfection of cells with plasmids that express the hPLLP or mPLLP cDNA from a human cytomegalovirus immediate early promoter could make resistant cells susceptible to GFP(McERV) vector infection. Indeed, A23

TABLE 2. McERV receptor activities of BAC clones[a]

BAC clone	Restriction enzyme digestion	No. of GFP^+ foci
325K4	None	5
322D14	None	5
314A14	None	520
250E14	None	10
314A14	AatII	670
	EcoRI	12
	HindIII	130
	MluI	1,070
	SacII	450
	SwaI	570

[a] A23 hamster cells were transfected with restriction enzyme-digested or undigested BAC clone DNA and were exposed to GFP(McERV), and GFP^+ foci were counted 3 days after virus exposure as described in Materials and Methods.

TABLE 3. hPLLP and mPLLP proteins function as receptors for McERV[a]

Target cells	Titer (TU/ml) of GFP vector of pseudotype:	
	McERV	10A1-MuLV
NIH 3T3	<100	1.1×10^6
NIH 3T3 + hPLLP	6.5×10^5	7.2×10^5
NIH 3T3 + mPLLP	1.4×10^6	1.7×10^5
NIH 3T3 + hSLC12A3	<100	9.7×10^5
A23	<100	9.2×10^5
A23 + hPLLP	4.2×10^6	9.6×10^5
A23 + mPLLP	4.1×10^6	6.8×10^5
A23 + hSLC12A3	<100	6.1×10^5

[a] NIH 3T3 mouse or A23 hamster cells stably transduced with retroviral vectors that express the indicated mouse or human proteins were exposed to a GFP vector with the indicated pseudotype, and transduction was measured by FACS analysis. Results are means from two experiments.

cells transfected with either expression vector became highly susceptible to GFP(McERV) vector infection, with ~80% of the cells expressing GFP after exposure of the transfected cells to the vector. Interestingly, NIH 3T3 mouse cells also became highly susceptible to GFP(McERV) vector infection following the transfection of the cells with either expression plasmid, with ~30% of the cells expressing GFP after exposure of the transfected cells to the vector. This result contrasts with the resistance of the NIH 3T3 cells to GFP(McERV) vector infection following the transfection of BAC 314A14 and indicates that the *PLLP* gene carried by the BAC is not properly expressed in NIH 3T3 cells.

We also generated stable A23 and NIH 3T3 cell lines expressing hPLLP and mPLLP, and all of these cell lines were highly susceptible to GFP(McERV) vector infection (Table 3). The McERV vector titers were at least as high as those of a GFP vector with a 10A1 MuLV pseudotype (Table 3). Stable cell lines expressing human SLC12A3, a multiple-membrane-spanning protein near PLLP (Fig. 1), remained uninfectable by the GFP(McERV) vector.

McERV can infect cells from many mammalian species but has a restricted tissue tropism. The results above show that mPLLP serves as a functional McERV receptor. This was surprising based on our initial infectivity results showing that rodent fibroblast cell lines were resistant to McERV infection (Table 1). The screening of additional cell lines showed that GFP(McERV) can infect cells from a variety of mammalian species but that not all cell types from a susceptible species are infectible (Table 1). For example, the normal rat kidney cell line NRK was infectible, but Rat1 fibroblasts were not. Several human cell lines were highly susceptible to GFP(McERV) infection, while others were completely resistant, with a difference in vector titers of up to $>10^6$. In general, neural, kidney, and most epithelial cells were infectible, while fibroblasts were not. Reverse transcription-PCR analysis confirmed that PLLP expression correlated with McERV infectibility (Fig. 2). PLLP is expressed primarily in brain and kidney epithelial cells (15); thus, the tropism observed in cultured cells roughly parallels the tissue distribution of PLLP expression in animals, consistent with the hypothesis that PLLP expression is the primary determinant of McERV entry into cells. PLLP orthologs (Fig. 3) are widely expressed in vertebrate species, but we have not tested whether these orthologs are functional as receptors for McERV in species other than mammals.

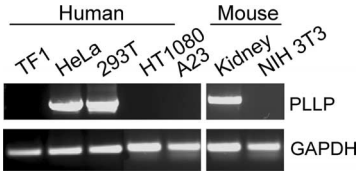

FIG. 2. Susceptibility of cells to McERV infection correlates with PLLP RNA expression. Reverse transcription-PCR detection of PLLP and GAPDH (glyceraldehyde-3-phosphate dehydrogenase) (positive control) in mRNA from cell lines or from mouse kidney was performed with primers designed for hPLLP, mPLLP, and GAPDH cDNAs. No PLLP-specific signal was detected for A23 cells using human (shown) or mouse (not shown) primers.

McERV and MDEV use different receptors for cell entry. Of the known functional Env proteins, the Env protein of MDEV shares the highest sequence identity (66%) with that of McERV. The cell entry receptor for MDEV is not known, so we sought to determine if MDEV utilizes the same receptor as McERV by using an interference assay (27). This assay is based on the principle that retrovirus infection typically results in a block of further infection by retroviruses that use the same entry receptor but does not interfere with infection by retroviruses that use other cell entry receptors. We found that McERV infection of 293 cells reduced the McERV vector infection rate by 200-fold but had no effect on MDEV vector infection (Table 4), indicating that MDEV does not require the McERV receptor for entry into 293 cells.

Some retroviruses, such as 10A1 MuLV, can use two receptors for cell entry (29), and similarly, it is possible that MDEV uses the McERV receptor and some other receptor for cell entry and thus is not blocked by prior infection of cells with McERV. To test this possibility, we measured McERV vector infection of cells previously infected with MDEV. We found that MDEV infection of 293 cells did not interfere with either MDEV or McERV vector infection (data not shown), an uninformative result likely due to poor MDEV replication or

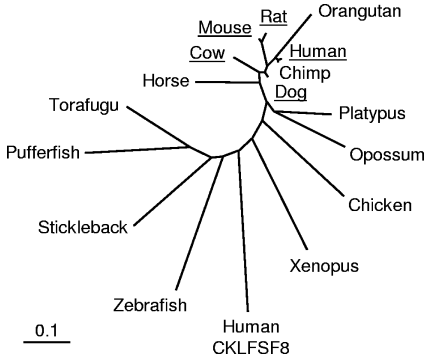

FIG. 3. Phylogeny of mammalian PLLPs and related proteins. Amino acid sequences of proteins closely related to mPLLP and hPLLP were obtained from the GenBank database using BLAST searches and were compared using ClustalW. Also shown is CKLFSF8, the most closely related non-PLLP protein. Species for which derived cell lines are susceptible to McERV infection are underlined. The rest of the species shown have not been tested for McERV susceptibility. The scale bar at the lower left indicates 10% amino acid divergence.

TABLE 4. Lack of interference between McERV and MDEV[a]

Target cells	Titer (TU/ml) of vector of pseudotype:	
	McERV	MDEV
293	8×10^5	2×10^5
293/GFP + McERV	4×10^3	3×10^5
D17	1.5×10^5	6×10^5
D17/LN + MDEV	1.7×10^5	6×10^3
PK-15	6×10^5	3×10^3
PK-15 + MDEV	6×10^5	<10

[a] MDEV infection of 293 and D17 cells was measured by using a vector encoding alkaline phosphatase (LAPSN) produced from G355 cat cells infected with MDEV (26). MDEV infection of PK-15 cells was measured using a vector encoding yellow fluorescent protein made from cells infected with MDEV. McERV infection of 293 cells was measured using the LAPSN vector produced from 293 cells infected with McERV. McERV infection of D17 and PK-15 cells was measured by using a GFP vector made from 293 cells infected with McERV. Results are the means from two experiments. TU, transducing units.

poor MDEV Env expression in the 293 cells. However, MDEV infection of D17 or PK-15 cells reduced the MDEV vector infection rate by 100- or >300-fold, respectively (Table 4), but had no effect on McERV vector infection, indicating that MDEV does not use the McERV receptor on D17 or PK-15 cells. Together, these results indicate that MDEV and McERV use distinct receptors on several cell lines, despite their high Env sequence similarity. This conclusion is supported by the different tissue tropisms of MDEV and McERV pseudotype vectors. In particular, MDEV vectors can efficiently transduce fibroblasts from many mammalian species (2, 26), while McERV vectors cannot (Table 1), a result consistent with the use of different cell entry receptors by these viruses.

DISCUSSION

Most receptors for simple retroviruses are expressed in a broad range of tissues. In contrast, PLLP shows a tissue-specific expression pattern that complicated the analysis of the host range of the virus. In particular, PLLP is not expressed in any of the fibroblast cell lines that we tested, including those of mice and other rodents, leading to the incorrect conclusion that McERV had a xenotropic host range. Analysis of cells from other tissues and cells expressing the mPLLP cDNA shows that McERV can actually infect a broad range of mammalian species, including mice and rats (Table 1). The broad host range of McERV in comparison to the xenotropic host range of GALV and SSAV, and the use of PLLP as a receptor by McERV in contrast to the use of Pit1 and/or Pit2 by GALV and SSAV, argue against the hypothesis that McERV is the ancestor of the simian retroviruses. Similarly, the recently identified koala retrovirus (16, 41), a close relative of GALV, can infect cells that are not susceptible to McERV infection (NIH 3T3, MDTF, BHK, and E-36 cells) and, like GALV, appears to use Pit1 as a receptor (31), arguing that McERV is not the ancestor of the koala retrovirus.

The phenotypic screen for the McERV receptor was remarkably successful and reached high statistical certainty after analysis of only 40 of the 83 available RH clones. Furthermore, the introduction of the single human BAC carrying the *PLLP* gene (BAC 314A14) into A23 cells rendered the cells highly susceptible to McERV infection. In contrast, NIH 3T3 mouse embryo fibroblasts remained completely resistant to McERV infection following introduction of the BAC carrying the *hPLLP* gene. Thus, while the *hPLLP* gene could be expressed in the A23 hamster cells, it was not expressed in the NIH 3T3 cells either because of a lack of transcriptional factors necessary for *PLLP* gene expression or the presence of specific repressors of *PLLP* gene expression. We do not know whether the resistance of unmodified A23 cells to McERV infection is due to a lack of hamster PLLP expression or to the expression of a PLLP that is not functional as a receptor. Our PCR data suggest that A23 cells do not express hamster PLLP (Fig. 2), but we have no positive control to prove that the PCR primers used can recognize the hamster PLLP cDNA sequence. We have screened a variety of hamster cell lines for susceptibility to McERV infection, but all were resistant (Table 1), a result that does not resolve the question of whether hamster PLLP is active as a receptor for McERV.

Hamacher et al. (15) previously localized the *hPLLP* gene to chromosome 16q13 by using *PLLP* gene-specific PCR and genomic DNA from the same G3 human-hamster RH cell lines that we have used here. Their analysis of DNA from all 83 RH cell lines localized the *PLLP* gene closest to the marker SHGC-34581, with an LOD score of 11.48. We localized the receptor for McERV closest to the same marker by using 40 RH cell lines with an LOD score of 8.03, consistent with the identification of PLLP as the McERV receptor. Of the 40 RH cell lines that we screened in our phenotypic assay, two gave results that were different from those of the PCR assay of Hamacher et al. In both cases, we detected a positive hybrid by phenotype, while the PCR-based approach did not score these hybrids as positive. Limiting the statistical analysis of the PCR results to the same 40 hybrids localized the *PLLP* gene closest to the SHGC-34581 marker but with an LOD score of 7.57, which is lower than our LOD score of 8.03. In addition, of the top 10 markers that linked most closely to the *PLLP* gene by PCR, only 7 were on chromosome 16, 2 were on chromosome 14, and 1 was on chromosome 1. In contrast, all of the markers that mapped closest to PLLP by our phenotypic analysis were on chromosome 16. Thus, the phenotypic localization of genes is at least as accurate as PCR-based analysis, a somewhat counterintuitive result because the presence or absence of the markers in the RH cell lines was originally established by PCR.

PLLP is an 18-kDa membrane-bound proteolipid that is listed as a member of the four-transmembrane superfamily (TM4SF11). This is the first TM4 protein to be identified as a retrovirus receptor, although other TM4 proteins have been found to serve as receptors for human hepatitis C virus, including the tetraspanin CD81 and claudins 1, 6, and 9 (7). However, PLLP is not related to these proteins and does not fit the consensus features of a tetraspanin (a small first and a large second extracellular loop with multiple cysteine residues and a glycine in the second loop). PLLP is instead closely related to the chemokinelike factor superfamily, especially member 8 (CKLFSF8) (Fig. 3). Furthermore, unlike the HCV TM4 receptors CD81 and claudin, plamolipin is predicted to have very short extracellular loops (8 and 14 to 18 amino acids) (11, 44), leaving little apparent space for virus binding. As is the case for hepatitis B virus, it is possible that McERV infection depends

on the presence of receptors in addition to the presence of PLLP, but as yet we have no evidence for this.

PLLP is a major component of myelin (4, 10), is present in the apical membranes of tubular epithelial cells, and has been detected in a variety of other tissues (3, 15). PLLP can be extracted from membranes using organic solvents and, upon its addition to synthetic lipid bilayers, can induce the formation of ion channels that are both voltage dependent and potassium specific (43). The disease, if any, induced by McERV infection in animals is not known, but McERV envelope protein interaction with PLLP in myelin might induce interesting neurological effects based on PLLP's role in ion transport and its presence in the central and peripheral nervous systems.

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