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GLVR1, a Receptor for Gibbon Ape Leukemia Virus, Is Homologous to a Phosphate Permease of *Neurospora crassa* and Is Expressed at High Levels in the Brain and Thymus

STEPHEN V. JOHANN,[1] JAMES J. GIBBONS,[2] AND BRYAN O'HARA[1]*

Molecular Biology Research Section[1] and Department of Immunology,[2] Medical Research Division, American Cyanamid Company, Pearl River, New York 10965

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The human gene GLVR1 has been shown to render mouse cells sensitive to infection by gibbon ape leukemia virus. This indication that the GLVR1 protein acts as a virus receptor does not reveal the protein's normal physiological role. We now report that GLVR1 is homologous to *pho-4*$^+$, a phosphate permease of *Neurospora crassa*, at a level sufficiently high to predict that GLVR1 is also a transport protein, although the substrate transported remains unknown. To characterize the gene further, we have cloned cDNA for the mouse homolog of the gene, *Glvr-1*. The sequence of the murine protein differs from that of the human protein in 10% of residues, and it may be presumed that some of these differences are responsible for the inability of gibbon ape leukemia virus to infect mouse fibroblasts. *Glvr-1* RNA is most abundant in mouse brain and thymus, although it is present in all tissues examined. The pattern of RNA expression found in mouse tissues was also found in rat tissues, in which the RNA was expressed at high levels in all compartments of the brain except the caudate nucleus and was expressed most abundantly early in embryogenesis. Thus, high-level expression of *Glvr-1* appears to be restricted to specific tissues and may have developmental consequences.

Retroviruses enter cells following specific interaction with receptors at the cell surface. This specificity allows division of retroviruses into interference groups, the members of any one group utilizing the same receptor (23, 28). Eight interference groups have been defined for retroviruses which can infect human cells (29), presumably identifying the existence of eight distinct integral-membrane receptor proteins. Other extensive experiments have defined a number of receptors in cells of murine and avian origin (1, 11, 28). The identities and normal physiological functions of most of these receptors remain unknown. Three retrovirus receptors have been cloned. CD4, the major receptor for human immunodeficiency virus (16, 19), is involved in the recognition of antigen presented in the context of class II major histocompatibility antigen. *Rec-1*, the receptor for the main group of murine ecotropic viruses, is responsible for the transport of cationic amino acids (10, 27). Finally, we have recently cloned a human cDNA, GLVR1, which confers sensitivity to infection by gibbon ape leukemia virus (GALV) on mouse cells, although direct binding of viral envelope glycoprotein to GLVR1 has not been demonstrated (20). The presumptive protein encoded in the cDNA consists of 679 residues with multiple potential transmembrane domains. The presence of these domains and the fact that the protein confers sensitivity to viral infection suggest that GLVR1 is an integral-membrane protein. As indicated by Southern analysis of DNAs from several animal species (20), the gene is highly conserved across species lines. This conservation is also evident in the ability of the virus to infect cell lines of almost all mammalian and avian species tested (28), in which process it is likely that the various homologs of the protein perform a similar function.

Because earlier searches of GenBank and NBRF data bases failed to reveal homology to known sequences, we were unable to propose a normal physiological role for GLVR1. We now report that the protein is homologous to *pho-4*$^+$, a recent deposit in the NBRF data base which is presumed to be a phosphate permease of *Neurospora crassa* (18). In order to achieve a general characterization of GLVR1 and to assess its tissue-specific expression, we have cloned cDNA for the murine homolog of the gene. We present here a comparison of the human and mouse sequences and an analysis of RNA expression patterns in mouse and rat tissues. The level of RNA was found to be highest in brain and second highest in thymus. In addition, the spleens of mice undergoing an acute graft-versus-host (GVH) reaction showed significantly more RNA than normal spleens, suggesting a possible role for the gene in inflammatory processes. In a small developmental survey, the level of RNA was found to be highest at day 10 of embryogenesis.

MATERIALS AND METHODS

Cloning and sequencing of the cDNA for mouse *Glvr-1*. A mouse thymus library (Stratagene 935303) in λ ZAP was screened with two *Eco*RI fragments containing bases 1 to 2659 of the human GLVR1 cDNA-containing clone pHGR6-1 (20). Hybridizing phage were plaque purified and their inserts were excised in pBluescript SK$^-$ (Stratagene) by coinfection with helper phage, as described by the manufacturers. Sequencing was performed by using single-stranded DNA templates and synthetic oligonucleotide primers (24, 26). Amino acid sequence comparisons were carried out as described previously (15) by using a deletion penalty of 12 or 8 and a gap penalty of 4 or 3 (for Fig. 1 and 2, respectively). Percent homology was the number of identical residues divided by the length of the longer of the two sequences under comparison, with unshared gaps counted as residues.

Construction and use of pOJ9. In order to identify the relevant open reading frame within human GLVR1 cDNA

* Corresponding author.

conferring sensitivity to infection by GALV, a construct (pOJ9) was made in which the ATG codons normally present in the cDNA upstream of the ATG initiating the long open reading frame were removed. The sequence CATCTT (bases 318 to 323 in the human cDNA [20]) was changed to the *Hin*dIII recognition sequence, AAGCTT, by in vitro mutagenesis. The *Hpa*I site at position 2490 was changed to a *Bgl*II site by linker addition. The *Hin*dIII-*Bgl*II fragment was cloned into the eukaryotic expression vector pcDNA1 (Invitrogen) between the *Hin*dIII and *Bam*HI sites. This vector therefore carries the long open reading frame of GLVR1 cDNA under control of a cytomegalovirus promoter.

To test pOJ9, NIH 3T3 cells, plated 1 day previously at 3×10^5 cells per 60-mm dish, were transfected with 1 μg of pSV2neo or with pSV2neo and 3 μg of pOJ9 or pSV2GR6 and carrier DNA to 20 μg of total DNA per dish, by $CaPO_4$ precipitation. pSV2neo confers resistance to the antibiotic G418 (25). pSV2GR6 contains the human GLVR1 cDNA, including the region with the three ATG codons upstream of the ATG initiating the long open frame, under control of the simian virus 40 early promoter (20). Three dishes were transfected with each precipitate. After 2 days, the cells were replated in medium containing G418 and colonies were allowed to form. Each of the nine dishes gave rise to 80 to 200 colonies. Colonies derived from each dish were pooled and replated in each of two 60-mm dishes at 10^5 cells per dish with 4 μg of Polybrene (Sigma) per ml. After 1 day, one dish from each pool was exposed to 1 ml of GALV (10^6 infectious particles per ml). After an additional 2 days, 10^4 cells from each pool were replated with 3×10^5 PG4 S^+L^- indicator cells (7). The small proportion of viruses used for the infection surviving to this stage were destroyed by trypsinization prior to replating with the indicator cells. Foci initiated by productively infected NIH 3T3 cells were counted after 5 days of cocultivation with PG4 cells.

RNA preparation and analysis. Total RNA was prepared from quick-frozen and disrupted tissues as described elsewhere (6) and subjected to northern (RNA) analysis. Hybridization was carried out at 42°C in 50% formamide, 5× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), 5× Denhardt's solution, 0.1% sodium dodecyl sulfate (SDS), and 200 μg of sonicated, denatured salmon sperm DNA per ml. The probe used was the entire pMGR1 labeled by nick translation with [^{32}P]dCTP. Washing was done at a final stringency of 0.1× SSC–0.1% SDS, at 65°C. For most mouse tissues, (C57BL/6 × DBA/2)F_1 hybrids were used. For spleen undergoing a GVH reaction, an F_1 hybrid was injected with 5×10^6 C57BL/6 spleen cells and the host spleen was removed for RNA preparation 10 days after injection. Rat tissues were from Sprague-Dawley rats and were prepared at the developmental stages indicated in the figures.

Nucleotide sequence accession number. A composite of the sequences from pMGR1 and pMGR2 (see below) is available from GenBank under accession no. M73696.

RESULTS

Homology of GLVR1 and *pho-4*$^+$. Figure 1 shows the homology between the amino acid sequences for GLVR1 and *pho-4*$^+$. In this figure, only identical residues are shaded, but a visual inspection reveals further numerous conservative differences. The homology between the two proteins can be divided into three areas. Residues 1 to 261 of GLVR1 and 1 to 250 of *pho-4*$^+$ are 31% identical and are highly hydrophobic. This region is followed in both proteins by long hydrophilic regions which show a much lower level

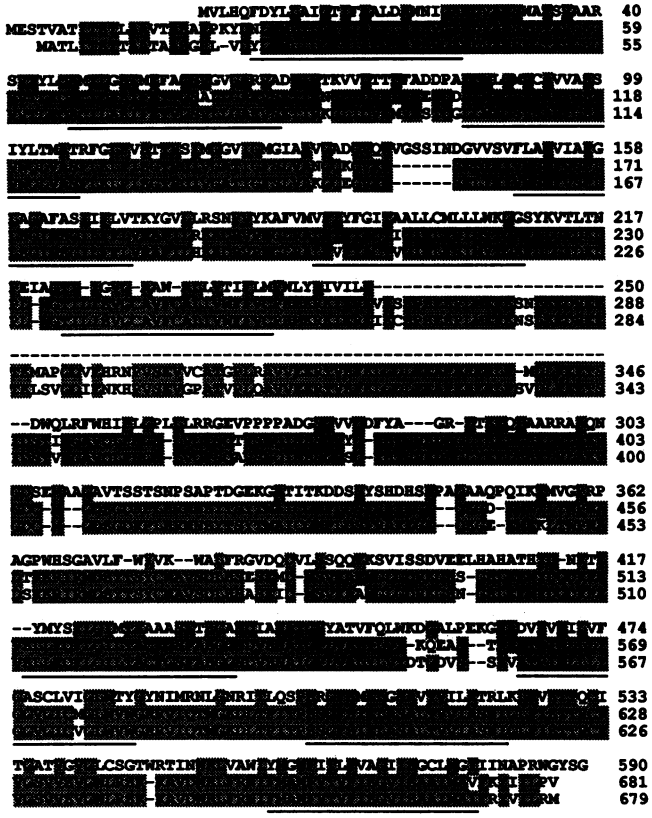


FIG. 1. Comparison of the protein sequences of *pho-4*$^+$ from *N. crassa* (top sequence), mouse *Glvr-1* (middle sequence), and human GLVR1 (bottom sequence). Residues that are identical between any two sequences are shaded. Gaps introduced for alignment are indicated by dashes. Potential transmembrane domains in the human sequence are underlined.

of homology (residues 262 to 511 of GLVR1 and 251 to 417 of *pho-4*$^+$). Finally, the carboxy-terminal regions again show notable homology, since they are 38% identical and highly hydrophobic (residues 512 to 679 of GLVR1 and 418 to 590 of *pho-4*$^+$).

The two proteins show a further structural relationship in that they each contain an internal repeat. Figure 2 compares the four sequences: GLVR1, residues 16 to 187; GLVR1, residues 508 to 679; *pho-4*$^+$, residues 1 to 177; and *pho-4*$^+$, residues 413 to 590. Pairwise comparisons of these four sequences reveal levels of homology spanning 16 to 34%. This internal repeat may have resulted from an ancient gene duplication.

Hydropathy plots for GLVR1 and *pho-4*$^+$ (Fig. 3A) are strikingly similar in the amino and carboxy termini. This similarity reflects not only the amino acid residues shared between the proteins but also the many differences which are conservative. Figure 3A also indicates regions which are likely to span the membrane. These regions were chosen with two points in mind. Firstly, it was presumed that a region which spans the membrane in one protein will also span the membrane in the other. Secondly, it was presumed that a region which spans the membrane in the first of the internal repeats will also do so in the second. These points allow certain domains which show only a small potential to span the membrane to be excluded or retained as transmembrane domains. This allows construction of a speculative

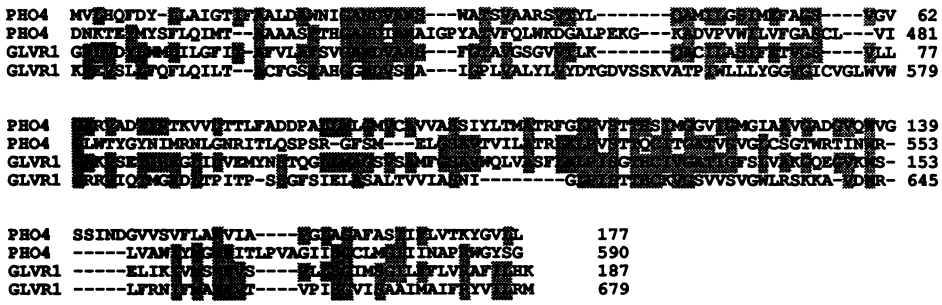


FIG. 2. A comparison of four sequences: *pho-4*$^+$, residues 1 to 177; *pho-4*$^+$, residues 413 to 590; GLVR1, residues 16 to 187; and GLVR1, residues 508 to 679. Residues identical to GLVR1 residues 16 to 187 are shaded. Gaps introduced for alignment are indicated by dashes.

topological map (Fig. 3B), giving a protein with 10 transmembrane domains. Hydrophobic regions not considered to cross the membrane for the reasons mentioned above are indicated in the figure as entering and leaving the membrane on the same side. Because GLVR1 residues 21 to 41 constitute a very good candidate for a transmembrane domain and because the region which they correspond to in *pho-4*$^+$ lacks a signal sequence upstream, residues that are amino terminal to GLVR1 residue 21 are expected to be orientated to the inner side of the membrane. As described below, human GLVR1 and mouse *Glvr-1* differ at 10% of their residues. On the assumption that some of these differences were responsible for the inability of mouse *Glvr-1* to allow infection by GALV, the coding region for mouse *Glvr-1* amino acid residues 553 to 560 was altered to match the corresponding human GLVR1 residues 550 to 558. This construct was found to render mouse cells sensitive to infection (data not shown). This suggests that GLVR1 residues 550 to 558 are critical for interaction with virus and are therefore likely to be situated outside the cell. If this model is correct, potential N-linked glycosylation sites in GLVR1 (residues 96, 371, 415, and 497) are situated on the inner side and therefore are not glycosylated. This arrangement of transmembrane regions and hydrophilic domains is not obviously similar to models for other proteins with multiple transmembrane domains. It is perhaps most similar to models for proteins such as that for the multidrug resistance gene (3) or that for the cystic fibrosis gene (22), which have six transmembrane domains, a large internal hydrophilic region, and a repeat of six transmembrane domains and the internal hydrophilic region. GLVR1 is similar to these proteins in having protein kinase C phosphorylation sites (30) at residues postulated to be internal (residues 84, 277, 318, 432, and 483; two further sites are present at proposed external residues 58 and 555). Protein kinase A phosphorylation sites (5) are present at proposed internal GLVR1 residues 277, 385, and 496. However, in contrast to the multidrug resistance and cystic fibrosis genes, no nucleotide binding domains are evident in GLVR1, and no sequence homology between these proteins and GLVR1 is detectable.

Cloning of murine *Glvr-1* and comparison with human GLVR1. Seven cDNA clones were obtained after screening of 200,000 plaques from a mouse thymus library with a GLVR1-specific probe, and their inserts were rescued as plasmids by coinfection with helper phage. One of these plasmids (pMGR1) contained an entire open reading frame similar to the open reading frame in the human cDNA and a substantial portion of upstream untranslated sequence. pMGR2 contained most of the open reading frame and, apparently, all of the 3′ untranslated sequence, as it has a short poly(A) stretch 15 bases after a polyadenylation signal. Mouse *Glvr-1* cDNA has the potential to encode a protein of 681 amino acid residues in its longest open reading frame (which is very similar in length to the presumed 679-residue human protein). An ATG codon with surrounding bases closely resembling the consensus sequence for translation start (12) initiates the open reading frame. Upstream of this codon, four other ATG codons are found (three upstream ATG codons are found in the human cDNA [20]). These have the potential to initiate translation of short peptides (up to 31 residues) which may have some as-yet-unknown function. Sequences conforming to the consensus sequence for polyadenylation are found in the 3′ end of the cDNAs of both species [that in the murine cDNA is followed by a short

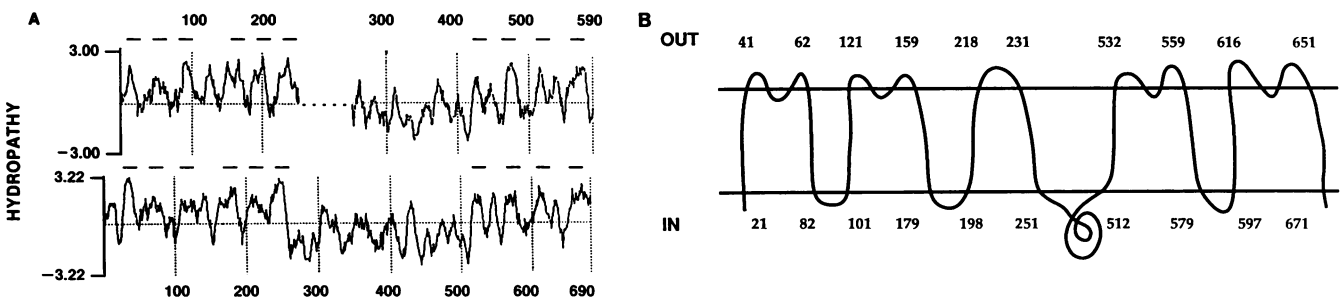
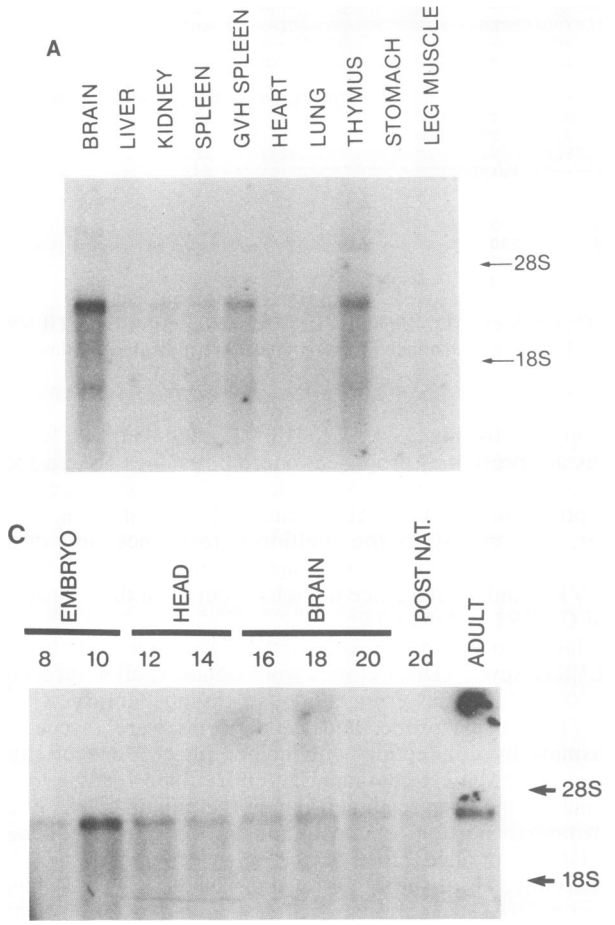


FIG. 3. (A) Hydropathy plots for *pho-4*$^+$ (top) and GLVR1 (bottom) generated by the program of Kyte and Doolittle (14). A gap was introduced in *pho-4*$^+$ to demonstrate the similarity between the amino and carboxy termini. Potential transmembrane domains are indicated by bars above each plot. (B) Speculative topological map of GLVR1 (not to scale). Residues at the ends of potential transmembrane domains are indicated by residue numbers. The long hydrophilic domain is shown between residues 251 and 512.



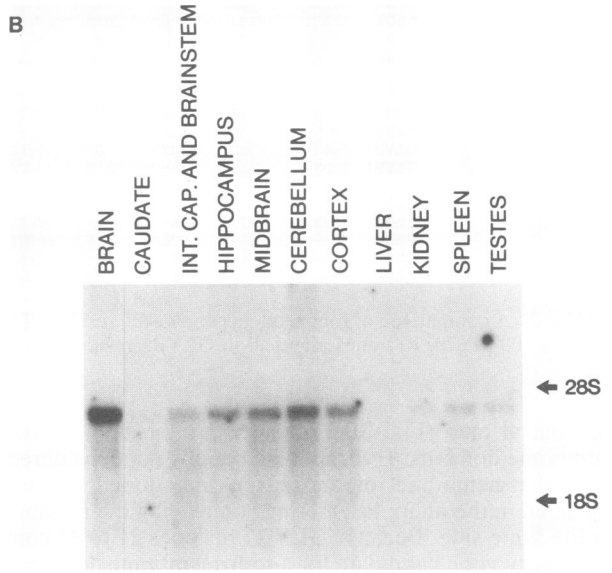


FIG. 4. (A) Northern blot of mouse tissue RNAs hybridized with a *Glvr-1*-specific probe (pMGR1). Each lane contains 15 μg of total RNA, except for the lane with RNA from stomach tissue, which contains 5 μg. (B) Northern blot of rat tissue RNAs hybridized with a *Glvr-1*-specific probe. Each lane contains 15 μg of total RNA. Total brain was derived from a single rat. Separate compartments of brain were pooled tissues from several rats. Int. Cap., internal capsule. (C) Northern blot of RNAs prepared from rat tissues taken at various stages of development and hybridized with a *Glvr-1*-specific probe. Each lane contains 15 μg of total RNA. Whole embryos, heads, and brains were taken at the indicated prenatal days. POSTNAT. 2d, 2 days after birth. Adult brain was from a 2-month-old rat.

poly(A) stretch, as already mentioned], identifying the signal which is likely to be used in both species. Overall, the DNA sequence homology between the human and mouse cDNAs is approximately 90% (data not shown).

Figure 1 shows the presumed protein sequence of *Glvr-1* and a comparison of the murine, human, and *pho-4*$^+$ protein sequences. The human and murine proteins differ at 10% of residues. The residues which differ are distributed throughout the protein but show a tendency to cluster in four areas. There is a region of considerable variation between the two proteins at the amino terminus. Residues 291 to 313 in the mouse differ considerably from those in the human protein. In the carboxy-terminal third, two areas are substantially different (residues 553 to 560 and 673 to 681 in the murine cDNA, compared with 550 to 558 and 671 to 679 in the human cDNA). The level of homology between *Glvr-1* and *pho-4*$^+$ is the same as that between GLVR1 and *pho-4*$^+$.

TABLE 1. Effect of pOJ9 on GALV infection of NIH 3T3 cells[a]

Plasmid combined with pSV2neo	No. of foci/3 × 10^4 cells with:	
	No GALV	GALV
None	ND[b]	0
pSV2GR6	0	382
pOJ9	0	1,000

[a] NIH 3T3 cells were transfected with the indicated plasmids and selected in G418. Pooled colonies were exposed to virus and tested for infection by using indicator cells as described in Materials and Methods.
[b] ND, not done.

Definition of the minimal open reading frame conferring sensitivity to viral infection. As mentioned above, both human and mouse cDNAs contained ATG codons upstream of the codon initiating the long open reading frame. In order to assess their significance, it was necessary to test the effect on function of removing them. The only demonstrated function associated with the locus is the ability to confer sensitivity to infection by GALV, and only the human cDNA will achieve this. Therefore, pOJ9, encoding the human cDNA but lacking ATG codons upstream of that initiating the long open reading frame, was constructed and tested for the ability to confer sensitivity to infection on mouse cells. Table 1 shows that NIH 3T3 cells, which are not normally susceptible to infection by GALV, become susceptible after transfection with pOJ9. The efficiency with which this was achieved was no less and in fact slightly better than the efficiency obtained with pSV2GR6. The latter plasmid contains most of the GLVR1 cDNA, including the long open reading frame and the three upstream ATG codons, and it has been previously shown to confer sensitivity to infection by GALV (20). This result establishes that expression of the protein from the first ATG in pOJ9, without the potential for coexpression of the small upstream open reading frames, confers the phenotype of sensitivity to infection.

***Glvr-1* RNA levels in mouse tissues.** The RNA levels for *Glvr-1* in mouse tissues were analyzed by northern analysis. Figure 4A shows that, with pMGR1 as probe, a single RNA species was readily detected in most tissues. Longer expo-

sures allowed detection of this RNA in all tissues examined, except perhaps stomach tissue. Despite the widespread presence of the RNA, there was considerable variation in level between tissues. The brain contained by far the highest level; this was severalfold higher than the next highest level, which was found in thymus tissue. Spleen undergoing a GVH reaction had a level of RNA approaching that found in the thymus and considerably higher than that found in normal spleen.

To determine which portion of the brain expressed high levels of RNA, all compartments of the brain were analyzed individually. Figure 4B shows that the RNA was found at a high level in rat brain in comparison with other tissues, which is in agreement with the results found with mouse tissues. Within the brain, RNA levels in most compartments were found to be high, notably so in the hippocampus, midbrain, cerebellum, and cortex. The caudate nucleus, in contrast, expressed low levels of *Glvr-1* RNA.

In order to examine *Glvr-1* expression during development, we analyzed RNA levels at several stages of rat embryogenesis. As can be seen in Fig. 4C, the RNA is expressed at day 10 much more abundantly than at day 8 of development of whole rat embryos. No fluctuation was found when whole heads were analyzed at days 12 and 14, nor (except for a gradual decline) when whole brains were taken at days 16, 18, and 20 and 2 days after birth. A higher level of RNA was found in 2-month-old adult brain than in brain from the later stages of embryogenesis.

DISCUSSION

Retrovirus receptors constitute a group of genes which have been studied extensively for the ability to allow infection by specific retroviruses. However, except for CD4 and *Rec-1*, their normal physiological roles remain obscure. This report, in detailing the homology between GLVR1 and *pho-4*$^+$, suggests a normal function for GLVR1. The function of *pho-4*$^+$ has been extensively investigated in *N. crassa*, in which it was found that the gene is required for high-affinity phosphate transport, renders cells sensitive to vanadate (a toxic analog of phosphate), and allows cells to grow under restrictive conditions requiring high-affinity uptake of phosphate (2, 17, 18). Thus, there is good evidence indicating that the gene encodes an integral-membrane protein which is responsible for high-affinity phosphate transport. GLVR1 and *pho-4*$^+$ display similar hydropathy plots, contain similar internal repeats, and are homologous in their primary structures. This homology includes not only the potential transmembrane domains but also several of the regions separating these domains. These similarities suggest that the two proteins have similar physiological functions. It is thus likely that GLVR1 is also a transport protein, although the substrate transported remains unknown. If the substrate is phosphate, then GLVR1 is likely to play a critical role in phosphate metabolism in man. It is interesting to note that GLVR1 has a similarity with *Rec-1* in that it appears to have a transport function.

Mouse fibroblasts in culture are resistant to infection by GALV, presumably because the virus cannot productively interact with *Glvr-1*. To investigate this and to assist in expression studies with mice, the murine *Glvr-1* cDNA was cloned and sequenced. The murine and human homologs of the gene were found to be 90% homologous, the differences between the two proteins being clustered, for the most part, in four areas. As alluded to in Results, some of these differences are the basis for the differential sensitivities of human and mouse cells to infection by GALV.

Glvr-1 RNA was detected at some level in almost all tissues. On the assumption that protein levels will generally reflect RNA levels, this suggests that the function of the gene is not peculiar to a specific cell type. This finding is in agreement with the sensitivity to infection in vitro of cells derived from a wide range of tissues (28). Despite the widespread distribution of the RNA, the levels varied widely among different tissues. The level was by far highest in most compartments of the brain. Because the internal capsule and brain stem are largely white matter and contain a level of RNA similar to levels found in several other tissues (e.g., cortex) which are rich in grey matter, it appears that *Glvr-1* expression is not favored in either white or grey matter. RNA levels for *Glvr-1* were also found to be high in the thymus and in the spleen of an animal undergoing a GVH reaction. GVH spleens display a distribution of cellular phenotypes different from that of normal spleens, containing two- to threefold more (donor) CD8$^+$ T cells and four- to sixfold more host macrophages than normal (4, 21; also unpublished observations). Furthermore, by day 8, the GVH spleen is a site of extramedullary hematopoiesis. It may therefore be that T cells in vivo or one of the other cell populations involved in the GVH response expresses high levels of *Glvr-1*. Given the high expression of *Glvr-1* in the GVH spleen and in the lymphopoietic thymus, it will be of interest to determine the level of expression of this gene in another hematopoietic organ, the bone marrow. These findings of high levels of RNA in brain, thymus, and GVH spleen demonstrate a substantial degree of tissue specificity in the expression of *Glvr-1* and suggest that the protein may be particularly important in neurophysiology and in T-cell function.

The locus was expressed at each stage of rat development in the tissues examined. A variation was found in whole embryos in early stages of embryogenesis, there being a notable increase in RNA levels at the 10-day stage compared with the 8-day stage. Thereafter, the RNA levels in heads and brains declined slightly until after birth, when it was found that adult brain expressed more RNA than was present in fetal or neonatal brain. These results suggest that the level of *Glvr-1* expression is developmentally regulated and may have developmental consequences.

We have recently shown that *Glvr-1* is tightly linked to the genes for interleukin-1 (*Il-1*) and the prion protein (*Prn-P*) on mouse chromosome 2 and is likely to be proximal to *Prn-P* (9). The data described here allow a comparison of *Glvr-1* with other loci mapped to this area which have not been cloned but for which expression characteristics are known. It is possible that *Glvr-1* is related or even identical to one of these other loci. In particular, the minor histocompatibility antigens H-3 and H-42 (8, 13) appear to be similar to *Glvr-1* in that these antigens are likely to be cell surface markers and are apparently widely expressed, which is also the case for *Glvr-1*, as we have shown here. The relationships between *Glvr-1* and markers in its immediate vicinity, including the minor histocompatibility antigens and several genes involved in development, remain to be determined.

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REFERENCES

1. **Bova, C. A., J. C. Olsen, and R. Swanstrom.** 1988. The avian retrovirus *env* gene family: molecular analysis of host range and antigenic variants. J. Virol. **62**:75–83.
2. **Bowman, B. J., K. E. Allen, and C. W. Slayman.** 1983. Vanadate-resistant mutants of *Neurospora crassa* are deficient in a high-affinity phosphate transport system. J. Bacteriol. **153**:292–296.
3. **Chen, C., J. E. Chin, K. Ueda, D. P. Clark, I. Pastan, M. M. Gottesman, and I. B. Roninson.** 1986. Internal duplication and homology with bacterial transport proteins in the mdr1 (P-glycoprotein) gene from multidrug-resistant human cells. Cell **47**:381–389.
4. **Gibbons, J. J., and J. Lucas.** 1989. Immunomodulation by low-dose methotrexate. I. Methotrexate selectively inhibits Lyt-2^+ cells in murine acute graft-versus-host reactions. J. Immunol. **142**:1867–1873.
5. **Glass, D. B., M. R. El-Maghrabi, and S. J. Pilkis.** 1986. Synthetic peptides corresponding to the site phosphorylated in 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase as substrates of cyclic nucleotide-dependent protein kinases. J. Biol. Chem. **261**:2987–2993.
6. **Glisin, V., R. Crkvenjov, and C. Byus.** 1974. Ribonucleic acid isolated by cesium chloride centrifugation. Biochemistry **13**: 2633–2639.
7. **Haapala, D. K., W. G. Robey, S. D. Oroszlan, and W. P. Tsai.** 1985. Isolation from cats of an endogenous type C virus with a novel envelope glycoprotein. J. Virol. **53**:827–833.
8. **Ishikawa, H., T. Hino, H. Kato, H. Suzuki, and K. Saito.** 1986. Cytotoxic T lymphocyte response to minor alloantigen in H-42b mice; clonal inactivation of the precursor cytotoxic T lymphocytes by veto-like spleen cells that express the H-42a antigen. J. Immunol. **137**:2080–2088.
9. **Kaelbling, M., R. Eddy, T. B. Shows, N. G. Copeland, D. J. Gilbert, N. A. Jenkins, H. P. Klinger, and B. O'Hara.** 1991. Localization of the human gene allowing infection by gibbon ape leukemia virus to human chromosome region 2q11-14 and to the homologous region on mouse chromosome 2. J. Virol. **65**:1743–1747.
10. **Kim, J. W., E. I. Closs, L. M. Albritton, and J. M. Cunningham.** 1991. Transport of cationic amino acids by the mouse ecotropic retrovirus receptor. Nature (London) **352**:725–728.
11. **Kozak, C. A., L. M. Albritton, and J. Cunningham.** 1990. Genetic mapping of a cloned sequence responsible for susceptibility to ecotropic murine leukemia viruses. J. Virol. **64**:3119–3121.
12. **Kozak, M.** 1986. Point mutations define a sequence flanking the AUG initiator codon that modulates translation by eukaryotic ribosomes. Cell **44**:283–292.
13. **Kurtz, M. E., R. J. Graff, A. Adelman, D. Martin-Morgan, and R. E. Click.** 1985. CTL and serologically defined antigens of the B2m, H-3 region. J. Immunol. **135**:2847–2852.
14. **Kyte, J., and R. F. Doolittle.** 1982. A simple method for displaying the hydropathic character of a protein. J. Mol. Biol. **157**:105–132.
15. **Lipmann, D. J., and W. R. Pearson.** 1985. Rapid and sensitive protein similarity searches. Science **227**:1435–1441.
16. **Maddon, P. J., A. G. Dalgleish, J. S. McDougal, P. R. Clapham, R. A. Weiss, and R. Axel.** 1986. The T4 gene encodes the AIDS virus receptor and is expressed in the immune system and the brain. Cell **47**:333–348.
17. **Mann, B. J., R. A. Akins, A. M. Lambowitz, and R. L. Metzenberg.** 1988. The structural gene for a phosphate-repressible phosphate permease in *Neurospora crassa* can complement a mutation in positive regulatory gene *nuc-1*. Mol. Cell. Biol. **8**:1376–1379.
18. **Mann, B. J., B. J. Bowman, J. Grotelueschen, and R. L. Metzenberg.** 1989. Nucleotide sequence of *pho-4*$^+$, encoding a phosphate-repressible phosphate permease of Neurospora crassa. Gene **83**:281–289.
19. **McDougal, J. S., M. S. Kennedy, J. M. Sligh, S. P. Cort, A. Mawle, and J. D. A. Nicholson.** 1986. Binding of HTLV-III/LAV to T4+ cells by a complex of the 110K viral protein and the T4 molecule. Science **231**:382–385.
20. **O'Hara, B., S. V. Johann, H. P. Klinger, D. G. Blair, H. Rubinson, K. J. Dunn, P. Sass, S. M. Vitek, and T. Robins.** 1990. Characterization of a human gene conferring sensitivity to infection by gibbon ape leukemia virus. Cell Growth Differ. **1**:119–127.
21. **Praloran, V., C. Raventos-Suarez, A. Bartocci, J. Lucas, E. R. Stanley, and J. J. Gibbons.** 1990. Alterations in the expression of colony-stimulating factor-1 and its receptor during an acute graft-versus-host reaction in mice. J. Immunol. **145**:3256–3261.
22. **Riordan, J. R., J. M. Rommens, B. Kerem, N. Alon, R. Rozmahel, Z. Grzelczak, J. Zielenski, S. Lok, N. Plavsic, J.-L. Chou, M. L. Drumm, M. C. Iannuzzi, F. S. Collins, and L.-C. Tsui.** 1989. Identification of the cystic fibrosis gene; cloning and characterization of complementary DNA. Science **245**:1066–1073.
23. **Rubin, H.** 1961. The nature of a virus-induced cellular resistance to Rous sarcoma virus. Virology **13**:200–206.
24. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74**:5463–5467.
25. **Southern, P. J., and P. Berg.** 1982. Transformation of mammalian cells to antibiotic resistance with a bacterial gene under control of the SV40 early region promoter. J. Mol. Appl. Genet. **1**:327–341.
26. **Vieira, J., and J. Messing.** 1987. Production of single-stranded plasmid DNA. Methods Enzymol. **153**:3–11.
27. **Wang, H., M. P. Kavanaugh, R. A. North, and D. Kabat.** 1991. Cell-surface receptor for ecotropic murine retroviruses is a basic amino-acid transporter. Nature (London) **352**:729–731.
28. **Weiss, R., N. Teich, H. Varmus, and J. Coffin (ed.).** 1984. RNA tumor viruses, vol. 1. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
29. **Weiss, R. A., M. A. Sommerfelt, M. O. McClure, P. R. Clapham, and J. N. Weber.** 1989. Retrovirus receptors on human cells. UCLA Symp. Mol. Cell. Biol. **90**:67–74.
30. **Woodget, J. R., K. L. Gould, and T. Hunter.** 1986. Substrate specificity of protein kinase C. Eur. J. Biochem. **161**:177–184.