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The Dual-Function Hamster Receptor for Amphotropic Murine Leukemia Virus (MuLV), 10A1 MuLV, and Gibbon Ape Leukemia Virus Is a Phosphate Symporter

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Previously, we showed that the amphotropic receptor homolog in hamster cells functions as a receptor not only for amphotropic murine leukemia viruses and 10A1 murine leukemia virus but also for gibbon ape leukemia virus (C. A. Wilson, K. B. Farrell, and M. V. Eiden, J. Virol. 68:7697–7703, 1994). Here, we demonstrate that this receptor functions as a sodium-dependent P_i transporter and that Na-P_i uptake can be specifically blocked following infection with either amphotropic murine leukemia virus, 10A1 murine leukemia virus, or gibbon ape leukemia virus.

Most hamster cells are refractory to infection by oncoretroviruses, notably murine leukemia viruses (MuLVs) and gibbon ape leukemia virus (GaLV) (16). The Chinese hamster lung cell line E36 is unique among hamster cells in its susceptibility to infection by several different MuLVs (xenotropic, amphotropic [A-MuLV], and 10A1) as well as GaLV (3). Most mammalian cells appear to have distinct receptors for A-MuLV and GaLV, as shown by receptor interference assays (13, 18). In contrast, E36 cells exhibit an overlap in GaLV and A-MuLV receptor utilization. Cells infected by GaLV are resistant to superinfection by not only GaLV but also A-MuLV (3). This finding led to the determination that E36 cells express a form of the A-MuLV receptor (termed EAR) which can facilitate infection by not only A-MuLV but 10A1 MuLV and GaLV as well (19). This receptor is similar to the A-MuLV receptors recently isolated from human and rat cells (approximately 90% amino acid identity) (7, 14) and also shares homology with the human GaLV receptor, GLVR1 (58% amino acid identity) (9).

It has recently been demonstrated that GLVR1 and Ram-1, the rat receptor for A-MuLV, function as cell surface sodium-dependent transporters of P_i (5, 11). The normal function of EAR protein has not been determined. Given the unique ability of EAR protein to function as a receptor for GaLV, 10A1 MuLV, and A-MuLV, studies were carried out to determine whether EAR also functions as a cell surface transporter of P_i.

Functional characterization of EAR protein. To determine if EAR functions as a phosphate transporter, a retroviral vector was used to introduce and express EAR cDNA in CHO-K1 hamster cells and murine NIH 3T3 cells (19). CHO-K1 cells lack functional A-MuLV receptors, and NIH 3T3 cells lack functional GaLV receptors. Therefore, cell surface expression of EAR in CHO-K1 and NIH 3T3 cells was confirmed by acquired susceptibility to A-MuLV- and GaLV-enveloped vectors, respectively (reference 19 and data not shown). Control and EAR-expressing cells were then assessed for phosphate uptake as previously described (10). The expression of EAR cDNA in CHO-K1 cells resulted in a significant augmentation of basic P_i uptake compared with that of control CHO-K1 cells. For example, phosphate uptake of 2,563 ± 63 pmol of P_i per min per mg of protein was observed in control cells compared with 5,503 ± 40 pmol of P_i per min per mg of protein in transfected cells (Fig. 1). An even more striking increase was observed in EAR-expressing NIH 3T3 fibroblasts, which displayed 10-fold augmentation of Na-P_i transport compared with that of control NIH 3T3 cells (780 ± 216 versus 7,126 ± 449 pmol of P_i per min per mg of protein [Fig. 1]).

Viral infection blocks EAR-specific Na-P_i transport. We previously showed that cells infected with GaLV exhibit a complete blockade of GLVR1-mediated P_i transport (11). Since EAR can function as a receptor for A-MuLV, 10A1 MuLV, and GaLV, we sought to determine what effect infection by each of these viruses had on EAR-specific Na-P_i transport. CHO-K1/EAR cells were infected with either A-MuLV (subtype 4070A, obtained from Janet Hartley, National Institute of Allergy and Infectious Diseases), 10A1 MuLV (obtained from Alan Rein, National Cancer Institute, Frederick Cancer Research and Development Center), or GaLV (subtype SEATO). Productive infection was determined by measuring levels of reverse transcriptase activity in cell media, as previously described (17). As shown in Fig. 2, uninfected CHO-K1/EAR cells exhibited an approximately twofold increase in P_i uptake over control CHO-K1 cells (5,442 versus 2,485 pmol of P_i per min per mg of protein). Infection with GaLV resulted in a return to the level of P_i transport observed in control CHO-K1 cells (2,778 pmol of P_i per min per mg of protein), as did infection with either A-MuLV or 10A1 MuLV (2,587 or 2,383 pmol of P_i per min per mg of protein, respectively). Thus, infection by any of these viruses results in the loss of EAR-specific Na-P_i transport in CHO-K1.

As a control for nonspecific effects on Na-P_i transport associated with the expression of a viral receptor or with retroviral infection of CHO-K1 cells, we measured changes in

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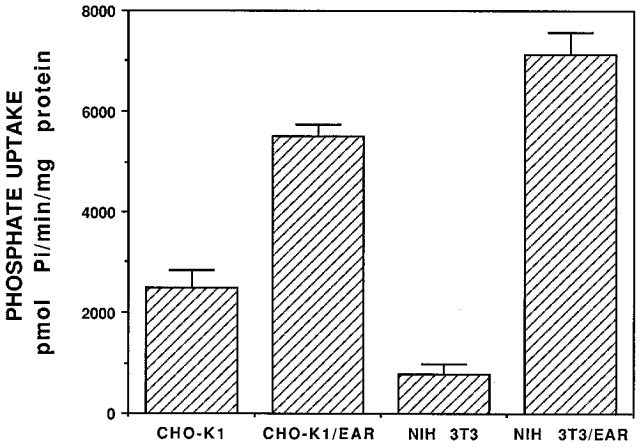

FIG. 1. Phosphate uptake in CHO-K1 and NIH 3T3 cells expressing EAR cDNA. Phosphate uptake was measured during a 2-min uptake incubation at 37°C in CHO-K1 and NIH 3T3 cells and EAR-expressing CHO-K1 (CHO-K1/EAR) and NIH 3T3 (NIH 3T3/EAR) cells. Data are means ± standard errors of triplicate determinations.

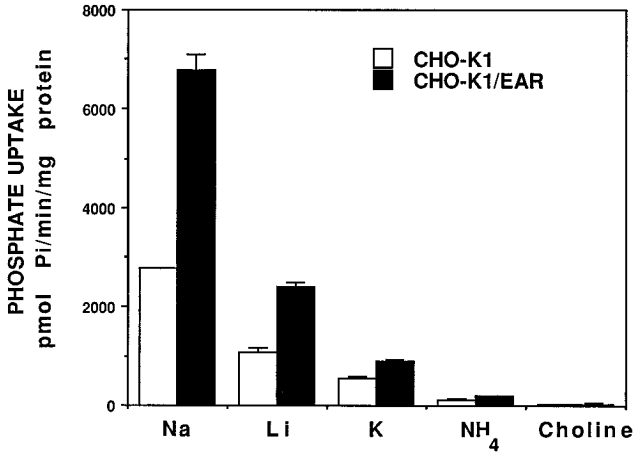

FIG. 3. Effect of monovalent cations on short-term phosphate uptake in CHO-K1 and CHO-K1/EAR cells. Each cation (at a concentration of 137 mM) was used separately as a chloride salt in uptake medium (pH 7.0) containing 0.1 mM $KH_2{}^{32}PO_4$ to determine phosphate transport during a 2-min incubation at 37°C. Data are means ± standard errors of triplicate determinations.

P_i uptake in CHO-K1 cells that express cDNA encoding the receptor for ecotropic murine leukemia virus (E-MuLV) (CHO-K1/ecoR) (16) and infected them with the Moloney strain of E-MuLV. As shown in Fig. 2, no significant increase in the level of phosphate uptake was observed in either CHO-K1/ecoR cells or CHO-K1/ecoR cells infected with E-MuLV compared with that of control CHO-K1 cells. These results demonstrate that the increase in P_i uptake observed in CHO-K1 cells is a specific event related to the expression of EAR cDNA and is not observed in CHO-K1 cells which express unrelated viral receptors and/or transporters.

Biochemical characterization of the EAR Na-P_i transporter. EAR protein is similar in overall topology to the human and rat A-MuLV receptors, GLVR2 and Ram-1, respectively, as well as the human GaLV receptor, GLVR1, but shares the highest degree of homology in terms of amino acid conservation to A-MuLV receptors (7, 9, 14, 19). Since EAR can function as a viral receptor for GaLV, we were interested in determining whether the functional parameters of EAR-specific phosphate uptake are similar to those for the GLVR1 phosphate symporter. We found several functional differences in EAR- and GLVR1-specific phosphate uptake. The results in Fig. 3 show that endogenous P_i transport in CHO-K1 cells and EAR-specific P_i transport strongly depend on the presence of Na^+ in the uptake medium. However, unlike the strict requirement of sodium ions for GLVR1-specific P_i transport (11), other monovalent cations support to a lesser degree both endogenous CHO-K1 P_i transport and EAR-mediated P_i transport (Na > Li > K > NH_4 > choline).

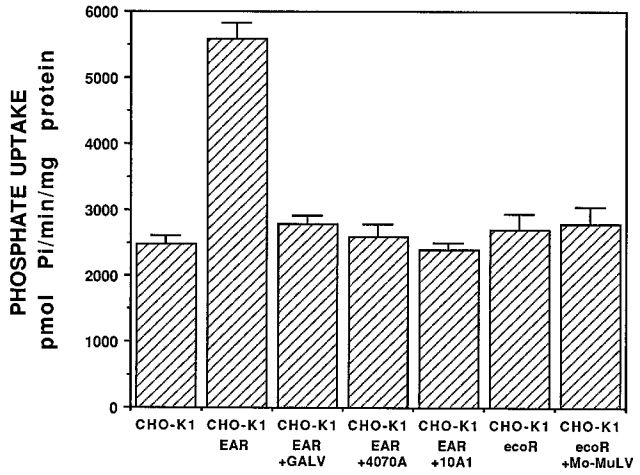

FIG. 2. Effect of virus infection on EAR-specific phosphate uptake. Phosphate uptake was measured during a 2-min uptake incubation at 37°C in CHO-K1 cells, EAR-expressing CHO-K1 cells (CHO-K1/EAR), and E-MuLV receptor-expressing CHO-K1 cells (CHO-K1/ecoR). CHO-K1/EAR cells infected with the indicated viruses and CHO-K1/ecoR cells infected with the Moloney strain of E-MuLV that were positive for the production of virus-related active reverse transcriptase enzyme were also included in the phosphate uptake assay. Data are means ± standard errors of triplicate determinations.

The phosphate transporter identified in human kidney cortex has a pH optimum of 8.0 for phosphate uptake (6), while mouse fibroblasts and GLVR1 Na-P_i transporters exhibit dual optima, with peaks at pH 6.5 and 7.5 (11). To determine the optimal pH for Na-P_i uptake in CHO-K1 and CHO-K1/EAR cells, experiments were carried out in uptake media with pH values ranging from 6.0 to 8.5. Both CHO-K1 and CHO-K1/EAR cells have their maximal uptake of phosphate at pH 7.0, with dramatic decreases in P_i uptake at pH 8.0 and 8.5 (Fig. 4).

The kinetic parameters (V_{max} and K_m) of phosphate uptake were determined for the EAR Na-P_i transporter as well as for the endogenous transporter(s) expressed in CHO-K1 cells within the concentration range of 0.06 to 2.0 mM P_i (Fig. 5). The time course determined for Na-P_i uptake was linear for up to 4 min at each phosphate concentration (data not shown). The V_{max} for the CHO-K1 endogenous transporter and the expressed EAR transporter (3.1 and 2.5 nmol of P_i per min per mg of protein, respectively) was somewhat higher than that reported for the human GaLV receptor, GLVR1 (1.9 nmol of P_i per min per mg of protein [11]), and in the same range as that reported for Ram-1 (2.67 nmol of P_i per min per mg of protein [5]). However, the capacity for phosphate by the hamster transporter is approximately 10 times higher than that previously reported for phosphate uptake in NIH 3T3 fibroblasts (V_{max} = 0.25 nmol of P_i per min per mg of protein [10]). The affinities for P_i determined for the endogenous CHO-K1 sodium-dependent transporter (K_m = 80 μM) and for EAR-specific phosphate transport (K_m = 90 μM) are higher than

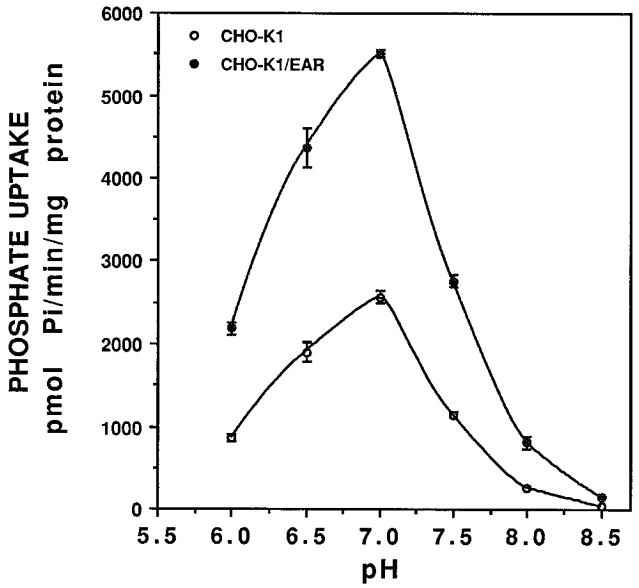

FIG. 4. Effect of pH on short-term (2-min) phosphate uptake in CHO-K1 and CHO-K1/EAR cells. The pH of the uptake medium was adjusted to indicated values, and short-term (2-min) sodium-dependent phosphate uptake in CHO-K1 and CHO-K1/EAR cells was determined as described in Materials and Methods. Data are means ± standard errors of triplicate determinations.

those reported for the human kidney cortex Na-P_i transporter (K_m = 170 μM) (6) and the endogenous NIH 3T3 Na-P_i transporter (K_m = 220 μM) (10). However, the affinity for phosphate determined for hamster transporters (the endogenous CHO-K1 homolog and EAR) is slightly lower than that previously determined for the human GLVR1 Na-P_i transporter (K_m = 53 μM) (11).

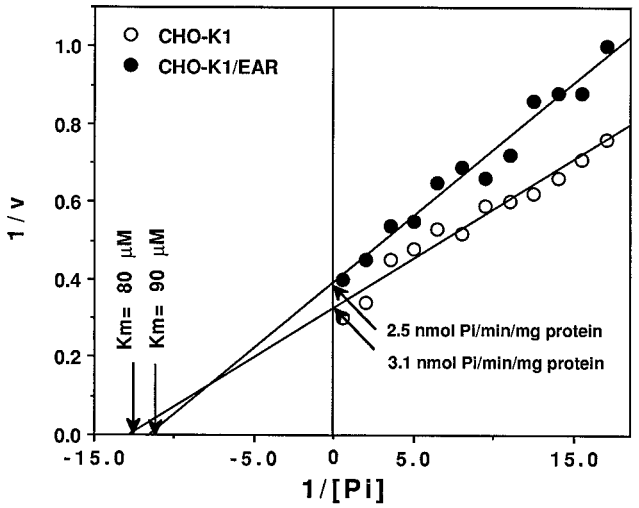

FIG. 5. Determinations of kinetic parameters (K_m and V_{max}) of sodium-dependent phosphate transport in control CHO-K1 and CHO-K1/EAR cells. Short-term sodium-dependent [^{32}P]phosphate transport experiments were carried out for CHO-K1 and EAR-expressing CHO-K1 cells with phosphate concentrations between 0.06 and 2.0 mM. Sodium-dependent phosphate uptake in the presence of the EAR transporter was determined by subtracting the amount of P_i uptake in CHO-K1 cells from the amount of P_i uptake in CHO-K1/EAR cells. Lineweaver-Burk linearization of experimental data represents the apparent kinetic parameters of the endogenous transporter in CHO-K1 cells and the transport parameters of introduced EAR. Lines were fit to datum points by least-squares analysis with the Cricket Graph program. Similar results were obtained in two independent experiments, each performed in duplicate. Data are means of four determinations.

Comparison of EAR- and GLVR1-specific P_i uptake reveals that EAR has a somewhat lower affinity for P_i but a higher V_{max} than GLVR1 (2.9 and 1.9 nmol of P_i per min per mg of protein, respectively). Ram-1 protein was shown to have a lower affinity but a higher capacity for P_i than GLVR1 protein (5), suggesting that EAR protein may be more similar to Ram-1 than to GLVR1 in terms of its functional parameters for phosphate uptake. In general, the kinetic parameters measured for the endogenous CHO-K1 transporter and the EAR transporter are quite similar, consistent with their common derivation from Chinese hamster cells.

We demonstrate in this report that EAR protein functions as a sodium-dependent phosphate transporter. Furthermore, productive infection by A-MuLV, 10A1, or GaLV results in a complete blockade of EAR-specific P_i transport. It is not known how viral infection blocks receptor-specific P_i transport. A comparison of the envelope glycoproteins of A-MuLV and 10A1 MuLV with those of GaLVs (approximately 35% amino acid identity) (1, 12) provides no basis for defining a region common to these viruses which is not also shared by other members of the Mn^{2+}-preferring family of oncoretroviruses (1). Therefore, it remains possible and even likely that the A-MuLVs and GaLVs interact with different regions of the EAR receptor. Of the 10 transmembrane domains present in both GLVR1 and EAR, 6 allow the formation of amphypathic alpha-helices (reference 11 and data not shown). Further organization of these alpha-helices may result in a barrel-like structure which contains an aqueous pore in the center similar to that hypothesized for the glucose transporter family (8). According to the general kinetic properties of membrane transporters, the pore opens alternately to either the extracellular surface or intracellular surface. Viral infection may block P_i transport by blocking the ability of phosphate and/or sodium to interact with the pore (either directly or by steric hindrance); however, the blockade of EAR-specific P_i uptake by viruses as divergent as GaLV and A-MuLV suggests that transport may be inhibited by a nonspecific mechanism. The trapping of transporter protein by viral envelope glycoproteins in either the endoplasmic reticulum or Golgi apparatus has been shown to result in interference to viral infection by some retroviruses (2, 15). Perhaps a similar mechanism plays a role in the virally induced block to P_i uptake shown here.

Phosphate is critical to a cell, and its presence regulates many cell functions (such as enzyme regulation, deoxynucleoside triphosphate levels, energy-requiring reactions, and signal transduction). Most mammalian cells and tissues appear to express proteins homologous to both of the GaLV and A-MuLV receptors (4, 5, 7). The observed differences between GLVR1 and EAR in terms of their kinetics and presumably their regulation of P_i uptake may provide an explanation as to why cells contain multiple cell surface P_i transporters. Viral infection of cells expressing GLVR1 or EAR results in a complete blockade of viral receptor-mediated phosphate transport, yet there is no apparent cytopathology. Thus, the presence of multiple Na-P_i transporters on the surfaces of mammalian cells provides an auxiliary means for P_i uptake to occur in the presence of viral infection and the concomitant loss of specific viral receptor and/or transporter function.

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