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The Envelope Glycoprotein of an Amphotropic Murine Retrovirus Binds Specifically to the Cellular Receptor/Phosphate Transporter of Susceptible Species

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A rat cDNA (rRam-1), which was cloned on the basis that it enables Chinese hamster ovary (CHO) cells to be infected by amphotropic host range murine retroviruses, was recently found to encode a widely expressed Na\(^{+}\)-phosphate symporter (M. P. Kavanaugh, D. G. Miller, W. Zhang, W. Law, S. L. Kozak, D. Kabat, and A. D. Miller, Proc. Natl. Acad. Sci. USA 91:7071–7075, 1994). CHO cells express the hamster homolog of Ram-1 but are resistant to amphotropic retroviruses. Although the amphotropic envelope glycoprotein gp70 bound weakly onto control CHO cells, CHO/rRam-1 cells had novel high-affinity binding sites, and the resulting strongly adsorbed gp70 was only slowly removed from cell surfaces, with a half-life of greater than 6 h. CHO/rRam-1 cells were also specifically and efficiently killed by exposure to amphotropic gp70 followed by antiserum to gp70 in the presence of complement. Infection with an appropriately pseudotyped form of amphotropic retrovirus 4070A did not perturb control CHO cells or inhibit their phosphate transport. In contrast, 4070A infection of CHO/rRam-1 cells caused major alterations including cell-cell fusions, a specific 40% down-modulation of the rRam-1 component of phosphate transport, and complete interference to superinfection by amphotropic viruses. The 4070A virus-infected CHO/rRam-1 cells retained a substantial cell surface pool of rRam-1 that functioned as a viral receptor but not as a viral receptor. We conclude that amphotropic gp70 binds more strongly to rRam-1 than to the homologous hamster protein and that this stable attachment is necessary for infection, interference, membrane fusion, and pathogenesis.

A cDNA that encodes the putative cell surface receptor for amphotropic host range murine leukemia viruses (MuLVs) was independently cloned from rats on the basis that it conferred susceptibility to infection on Chinese hamster ovary (CHO) cells (24) and from humans on the basis of partial homology to the cell surface receptor (Glvr-1) for Gibbon ape leukemia viruses (37) and for feline leukemia viruses of subgroup B (36). The recombinant rat amphotropic receptor will be abbreviated rRam-1, in distinction to homologous Ram-1 proteins of other species. Weak homology of Glvr-1 to a fungal phosphate transporter (15) had suggested that Glvr-1 might be a member of a possibly diverse family of transporters, and this hypothesis was recently confirmed by direct evidence that both Ram-1 and Glvr-1 are major Na\(^{+}\)-phosphate symporters in many cells and tissues (17, 28).

Because infections by some viruses, including retroviruses, may require species- or cell-specific membrane-associated accessory factors or coreceptors (7, 12, 13, 20, 38, 41), it cannot be inferred that Ram-1 or Glvr-1 directly mediates virus adsorption onto cells. Amphotropic MuLVs infect almost all mammalian species, and the mechanism for CHO cell resistance is unclear. Previous evidence suggested that amphotropic MuLVs bind relatively poorly onto CHO cells (11, 17), but this could be due to low cell surface expression of the hamster homolog of Ram-1, due to mutations in the hamster protein that reduce its affinity for virus, or due to a coreceptor deficiency. Treatment of CHO cells with inhibitors of asparagine-linked glycosylation cause the cells to become susceptible to amphotropic MuLV infections (25, 26, 43), and lectin-resistant CHO cell mutants that synthesize glycoproteins with truncated oligosaccharides are also susceptible (26). Additionally, hamster cells secrete a factor that inhibits their infection by amphotropic MuLVs (25, 26). This evidence has implied that hamster cells are not absolutely resistant to amphotropic MuLVs and that cell surface molecules needed for their infection may be masked by glycosylation or by a secreted factor. RNA blot analysis has shown that CHO cells and susceptible NIH 3T3 mouse fibroblasts contain similar amounts of Ram-1-related mRNAs (24).

Interactions of retroviruses with cell surface receptors have been implicated not only in virus entry into cells but also in interference to superinfection, immunosuppression, mitogenesis, and cytopathology (9, 14, 27, 29, 30, 32–34, 40). Amphotropic MuLV infection of some cells causes substantial cell killing and cell-cell fusion (e.g., see below). Recently, we found that expression of an amphotropic env gene but not an ecotropic env gene caused an approximately 40 to 50% reduction in phosphate transport by mouse NIH 3T3 fibroblasts (17), suggesting that the mouse homolog of Ram-1 might be a major phosphate transporter in these cells and that its activity or expression might be partially or fully blocked by amphotropic env glycoproteins. However, that study was ambiguous because NIH 3T3 fibroblasts express multiple phosphate transporters and because there are no negative control mouse fibroblasts that lack amphotropic receptors. The previous results would be compatible with a nonspecific effect of amphotropic env glycoproteins on membrane potential or on intracellular Na\(^{+}\) or K\(^{+}\) concentrations. For all of these reasons, we have used CHO cells to more thoroughly analyze interactions of rRam-1 with amphotropic env glycoproteins. Our results suggest that the viral glycoprotein interacts more strongly with rRam-1 than with Glvr-1.
with the homologous hamster protein. Interference to super-
infection is achieved without eliminating rRam-1 from cell
surfaces.

MATERIALS AND METHODS

Cell clones. Normal CHO fibroblasts are resistant to infection by both am-
photropic and ecotropic murine retroviruses (24, 37). However, for the studies
reported here we used a clone of CHO cells (CERD-C9 cells) that expresses
recombinant ecotropic receptors (39). rRam-1 DNA was subcloned into the ex-
session site of the retroviral vector pSSF (1). The pSSF-Ram-1 plasmid was
transfected into a coculture of 6/2 ecotropic packaging cells (21) and PA12
amphotropic packaging cells (21) for helper-free amplification of helper-free
SFF-Ram-1 virions (19, 1). These virions were used to infect the CERD-C9 line
of CHO cells. Cell clones from the latter culture were isolated by limiting
dilution, and clones were screened for rRam-1 expression by testing for suscep-
tibility to amphotropic virus infection. The selected clones, called CHO/rRam-1,
which had been previously described for other cellsurface antigens (11). In this case, the
samples were maintained in Dulbecco’s modified Eagle medium with 10% fetal bovine
serum.
Binding assays. Amphotropic gp70 virus was obtained by centrifugation of
1.5 liters of medium from a culture of infected mouse BALB/c 3T3 fibroblasts
(11). The gp70 was then released from the virions by freeze-thawing and was
recovered in the supernatants after sedimentation of the residual viral particles
as described elsewhere (11, 35). The amphotropic gp70 virus preparations (aliquots
were adjusted to 4 mg of total protein per ml and were frozen at −80°C) main-
tained their receptor-binding activities through repetitive freeze-thaw cy-
cles. For analyses, gp70 preparations were incubated with cell cultures for 2 h
at 37°C in amounts specified for each experiment. After removal of unbound gp70,
the cells were rinsed with fresh medium and were then incubated for 1 h at 37°C
with a 1:200 dilution of a goat antimouse serum to Friend ecotropic viral gp70
cross-reacts strongly with amphotropic gp70 (10, 19). After the cells were rinsed
with fresh medium, the adsorbed gp70-antibody complexes were detected by
ten methods. In one method, cells were incubated with fluorescein-labeled
rabbit anti-goat immunoglobulin G (GIBCO BRL, Grand Island, NY) and
maintained in Dulbecco’s modified Eagle medium with 10% fetal bovine
serum.

Additional evidence for gp70 binding was obtained by using
[125I]protein A instead of fluorescein-labeled secondary antibody
and by titrating this radioactivity assay with different concen-
trations of the gp70 preparation (Fig. 2). The antisera to
 gp70 and [125I]protein A were present in large excesses. Al-
though control CHO cells bound gp70 with relatively low af-
finity, CHO/rRam-1 cells bound substantially larger amounts
in a manner that saturated as the gp70 concentration in-
creased. Thus, CHO/rRam-1 cells have a class of relatively
high affinity sites for gp70 that are absent from control CHO
cells.

When incubated with gp70 and then with antibody to gp70
and with complement, CHO/rRam-1 cells were rapidly
and specifically killed (Fig. 3). The cells, which had been labeled
by incorporation of L-[35S]methionine (Amersham, Arlington
Heights, Ill.) at 50 μCi/ml (1,000 Ci/mmol) for 2 h followed by a 2-h chase
in fresh complete medium to incubation with the other reagents.

Other methods. All other methods have been previously described. These
include assays for cellular uptake of different concentrations of [35S]methionine (18), least
squares fitting of the resulting curves to a Michaelis-Menten equation by a
computer program (Kaleidograph) (38), immunoprecipitation, and Western blot
methods for distinguishing amphotropic and ecotropic gp70 glyco-
proteins (10, 19). Quantitative virus infection assays used helper-free retroviruses
with amphotropic envelope glycoproteins that encode human growth hormone
(19, 39). In this assay, the infected cells with growth hormone are detected
by immunofluorescence, and the multiplicity of infection is then calculated from the
fraction of infected cells. Protein concentrations were measured by the Coom-
assie blue dye method (Bio-Rad, Richmond, Calif.).

RESULTS

CHO/rRam-1 cells have novel high-affinity binding sites for
amphotropic gp70 that are absent from control CHO cells. To
study the role of rRam-1 in virus binding, we incubated CHO
cells (clone CERD-C9) and its CHO/rRam-1 derivative for 2
h with amphotropic gp70 and subsequently for 1 h with a
specific antisera to gp70 and then for 1 h with a fluorescein-
conjugated secondary antibody. CHO/rRam-1 cells became highly
fluorescent in this gp70-dependent reaction, whereas control
CHO cells bound relatively little gp70 (Fig. 1).

In contrast, infection of CHO/rRam-1 cells with the
4070A virus reproducibly caused a substantial and highly signi-

if (n = 6) down-modulation in phosphate transport. Thus,
down-modulation caused by 4070A virus infection is
specific for the rat protein rRam-1. Similarly, the weaker inhi-
bition caused by extracellular gp70 also appears to be specific to rRam-1.

**Interference to amphotropic MuLV superinfection.** Consistent with previous studies using other cells and retroviruses (3, 6, 9, 36, 40), infection of CHO/rRam-1 or mouse BALB/c 3T3 fibroblasts with amphotropic MuLV 4070A caused complete (greater than 400-fold) resistance to superinfection with a helper-free amphotropic host range virus (SFF-hGH) (19) that encodes human growth hormone (Table 1). This efficient interference to superinfection could not be explained simply by the partial down-modulation of cell surface rRam-1 (e.g., as in Fig. 6B), because CHO/rRam-1 cell clones with relatively little rRam-1 are efficiently infected in the conditions of our studies (Table 2). For example, CHO/rRam-1 clone 40 cells express

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**FIG. 1.** Immunofluorescence assay for binding of amphotropic envelope glycoprotein gp70 onto cells. Cells were seeded in an eight-well chamber slide (5 × 10⁴ cells per well) the day before. Cells were incubated for 2 h at 37°C with or without of the 4070A gp70 preparation (0.4 mg of protein per ml) followed by sequential 1-h incubations with goat antiserum to gp70 (1:200) and then with fluorescein-conjugated rabbit anti-goat immunoglobulin G (1:200). The cultures were fixed and mounted for fluorescence microscopy. The photographs are gp70-specific immunofluorescence (left) and the corresponding cells as seen by phase-contrast microscopy (right). The panels show CHO/rRam-1 clone 13 incubated with (A and a) or without (B and b) 4070A gp70 and control CHO cells incubated with (C and c) or without (D and d) 4070A gp70.
only about 18% as much rRam-1 as clone 13 or clone 54 cells but are infected with approximately the same efficiency.

**Cytopathic effects of amphotropic MuLV 4070A.** The CHO cell clone (clone CERD-C19) was ideal for this project because it contains receptors for ecotropic mouse retroviruses and could consequently be easily infected with helper-free virions that encode rRam-1 (see Materials and Methods). Similarly, by passing amphotropic MuLV 4070A through φ2 ecotropic retroviral packaging cells, we obtained a pseudotyped 4070A virus that was highly infectious for these CHO and CHO/rRam-1 cells, thereby enabling controlled comparisons.

An unexpected result of amphotropic 4070A virus infections was the initial extensive cytopathology and cell-cell fusion that resulted in the CHO/rRam-1 cultures (e.g., compare the infected and uninfected cultures in Fig. 7A and B, respectively). Approximately 40% of the nuclei in the infected CHO/rRam-1 culture occurred in syncytia. In contrast, as substantiated below, the infected CHO cells were resistant to this syncytium formation (see below). Moreover, infection with the 4070A virus caused a substantial selective reduction in growth rate of CHO/rRam-1 cells. 4070A gp70 was adsorbed onto CHO/rRam-1 cells for 2 h at 37°C at a 1:10 dilution. The cells were then rinsed thoroughly and incubated in fresh complete culture medium for various times prior to incubation for 1 h with antisera to gp70 and then for 1 h with [125I]protein A as described in the legend to Fig. 2. Each datum point is the mean of replicate assays (n = 2).

**FIG. 2.** Radioactive assay for binding of amphotropic gp70 onto CHO cells that contain or lack rRam-1. Cells expressing or lacking rRam-1 were seeded (2 × 10⁵ cells per 2 cm²) in wells of a 24-well culture plate the day before the assay. Wells were incubated with 0.25 ml of culture medium that contained various dilutions of the stock 4070A gp70 preparation for 2 h at 37°C. After being rinsed with complete medium, the cultures were incubated with goat antiserum to gp70 (1:200) for 1 h and then in medium with [125I]protein A (0.4 μCi/ml) in a volume of 0.25 ml per well for 1 h at 37°C. After being washed, the cells were solubilized in 0.5 ml of 0.1 N NaOH. A small aliquot was used to determine protein concentration, and the samples were then counted in a gamma counter. Each datum point is the mean of replicate assays (n = 2). The data are unsuitable for estimation of binding constants because the gp70 preparations were impure and contained an unknown proportion of active molecules.

**FIG. 3.** Cytotoxic assay for amphotropic gp70 binding to CHO cells that lack or contain rRam-1. Cells, which had been seeded (2 × 10⁵ cells per 25 cm²) in tissue culture flasks 2 days before the assay, were labeled for 2 h with [35S]methionine. The cultures were washed, incubated in complete medium for 2 h at 37°C, and then incubated for another 2 h in 2 ml of complete medium with or without a 0.2 ml supplement of the 4070A gp70 preparation. All cultures were washed and incubated with goat antiserum to gp70 (1:200) for 30 min prior to supplementation with a 1:12 dilution of rabbit complement assays at the indicated. At the end of the time course, the cultures were solubilized in 0.2% sodium dodecyl sulfate, and aliquots were assayed for radioactivity. The cells were CH/ram-1 incubated with gp70 (solid circles), CHO/rRam-1 (open circles), CHO incubated with gp70 (open squares), and CHO (open triangles). Each datum point is the mean of replicate assays (n = 2).

**FIG. 4.** Slow rate of removal of adsorbed gp70 from surfaces of CHO/rRam-1 cells. 4070A gp70 was adsorbed onto CHO/rRam-1 cells for 2 h at 37°C at a 1:10 dilution. The cells were then rinsed thoroughly and incubated in fresh complete culture medium for various times prior to incubation for 1 h with antisera to gp70 and then for 1 h with [125I]protein A as described in the legend to Fig. 2. Each datum point is the mean of replicate assays (n = 2).

**FIG. 5.** Phosphate transport activities of control CHO cells and of CHO cells that express rRam-1. The initial rates of ⁴⁰P uptake are plotted as a function of total P concentration. The lines were drawn by least-squares computer fit to the Michaelis-Menten equation (Kaleidograph program) as previously described (38). The Km and Vmax values attributable to rRam-1 were 270 ± 70 μM and 130 ± 10 nmol/mg of protein per h, respectively. These values were determined from a plot in which the control CHO values were subtracted from the data for the CHO/rRam-1 cells. Each datum point is the mean of replicate assays (n = 2).
the CHO/rRam-1 cells. This difference in effect of 4070A virus on CHO and CHO/rRam-1 cells was not due to a difference in efficiency of infecting these cultures. As seen by immunofluorescence microscopy or Western blotting for viral antigens, the CHO and CHO/rRam-1 cells were equally infected by the pseudotyped 4070A virus.

We observed that the cell-cell fusions and retarded growth rate described above gradually diminished after several weeks in the 4070A virus-infected CHO/rRam-1 cultures. Our interpretation is that the cell-cell fusion resulted from interaction of virus-infected cells with uninfected CHO/rRam-1 cells and that the fusion ceased after the virus had spread into all cells in the culture. Recovery then ensued when the polykaryons and polyploid cells were overgrown. The virus-infected cultures used for Fig. 6B and in Table 1 had all recovered from the initial cytopathic phase of the infection process as seen by microscopic inspection.

We verified several predictions of the cell-cell fusion model proposed above. Specifically, the 4070A virus-infected CHO cells and the infected CHO/rRam-1 cells that had undergone cytopathic recovery fused extensively when they were mixed 1:1 with uninfected CHO/rRam-1 cells but not when they were mixed with uninfected CHO cells. For example, Fig. 7 shows the consequences of mixing uninfected CHO/rRam-1 (Fig. 7C) or CHO (Fig. 7D) with the 4070A-infected CHO cells. In addition, we labeled the cellular DNAs by incorporation of bromodeoxyuridine and then used a fluorescent antibody to detect the labeled nuclei by microscopy (3). This analysis clearly showed that the polykaryons were synctia that formed by fusion of bromodeoxyuridine-labeled 4070A virus-infected cells with unlabeled uninfected CHO/rRam-1 cells.

We also attempted to determine whether the presence of

### Table 1. Interference to amphotropic retroviral superinfections

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Infection efficiency&lt;sup&gt;a&lt;/sup&gt; (MOI)</th>
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</thead>
<tbody>
<tr>
<td>CHO/rRam-1</td>
<td>0.80 ± 0.009</td>
</tr>
<tr>
<td>CHO/rRam-1/MuLV 4070A</td>
<td>&lt;0.0002</td>
</tr>
<tr>
<td>Mouse BALB</td>
<td>0.30 ± 0.033</td>
</tr>
<tr>
<td>Mouse BALB/MuLV 4070A</td>
<td>&lt;0.0002</td>
</tr>
<tr>
<td>CHO</td>
<td>&lt;0.0002</td>
</tr>
<tr>
<td>CHO/MuLV 4070A</td>
<td>&lt;0.0002</td>
</tr>
</tbody>
</table>

<sup>a</sup> The cell lines, which were uninfected or infected with amphotropic MuLV 4070A prior to analysis, included CHO/Ram-1 (clone 13), mouse BALB/c 3T3 fibroblasts, and control CHO cells.

<sup>b</sup> Measured as described previously (39), using an amphotropic helper-free pseudotype of SFF-hGH, a virus that encodes human growth hormone. Infected cells were detected 48 h after infection by growth hormone-specific immunofluorescence (19). Medium containing virus (0.5 ml/2 cm<sup>2</sup>) was incubated with cells in a 24-well culture plate. Because virus was in excess and was only partially adsorbed during incubation with the cell cultures, infection efficiency is a function of virus concentration and of cell susceptibility (16, 39). Multiplicity of infection (MOI) was calculated from the fraction (P<sub>n</sub>) of cells that lacked growth hormone, using the equation MOI = − log P<sub>n</sub>0.44 (19, 39). MOI differs from the fraction of growth hormone-positive cells by correcting for the fact that cells in highly infected cultures are often multiply infected. Errors are standard deviations based on counting statistics. For CHO/rRam-1 (clone 13) cells, 78 cells were positive among 995 counted; for BALB/c 3T3 fibroblasts, 75 were positive among 290 counted. For each of the other cultures, approximately 5,000 cells were scanned without any positives being detected.

### Table 2. CHO/rRam-1 clones with different quantities of rRam-1 are equally susceptible to amphotropic virus infection

<table>
<thead>
<tr>
<th>Cell clone</th>
<th>rRam-1 level (cpm/mg of protein)</th>
<th>Infection efficiency (MOI)&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHO</td>
<td>0 ± 240</td>
<td>0.004 ± 0.002</td>
</tr>
<tr>
<td>CHO/rRam-1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clone 13</td>
<td>7,100 ± 1,600</td>
<td>0.79 ± 0.055</td>
</tr>
<tr>
<td>Clone 54</td>
<td>6,900 ± 420</td>
<td>0.49 ± 0.034</td>
</tr>
<tr>
<td>Clone 38</td>
<td>3,050 ± 640</td>
<td>0.52 ± 0.044</td>
</tr>
<tr>
<td>Clone 19</td>
<td>2,850 ± 490</td>
<td>0.52 ± 0.038</td>
</tr>
<tr>
<td>Clone 40</td>
<td>1,450 ± 350</td>
<td>0.50 ± 0.036</td>
</tr>
</tbody>
</table>

<sup>a</sup> The CHO clone CERD-C9 was infected with helper-free ectopic pseudotype virus SFF-rRam-1 to obtain a population of CHO/rRam-1 cells (see Materials and Methods). The CHO/rRam-1 clones were then isolated by limiting dilution.

<sup>b</sup> Binding of amphotropic gp70 was measured in conditions of gp70 excess, using the antibody and [<sup>125</sup>I]protein A method (see Fig. 2). The background of control CHO cells (1,300 cpm) was subtracted from the data shown. Errors are the range for duplicate assays.

<sup>c</sup> Measured by using the amphotropic virus that encodes human growth hormone and expressed as multiplicity of infection (MOI) as described in Table 1, footnote b. The differences in rRam-1 expression do not significantly correlate with infection efficiencies over this range of rRam-1 levels. Presumably, at lower rRam-1 levels the MOI would decline.
rRam-1 perturbed processing of amphotropic MuLV 4070A env glycoproteins. This analysis was complicated by the cytopathology and recovery that occur in the cultures following virus infection (see above), and clear effects of rRam-1 on processing were not observed.

**DISCUSSION**

**Role for rRam-1 in cell surface binding of amphotropic MuLV env glycoproteins.** Our evidence supports the hypotheses that the gp70 envelope glycoprotein of amphotropic host range MuLVs binds to a cell surface pool of rat rRam-1 Na⁺-phosphate symporter protein that is only slowly being endocytosed or degraded and that binding to the homologous hamster transporter is relatively weak. Adsorption of this gp70 onto CHO cells, as seen by three sensitive assays, is highly dependent on rRam-1 expression (Fig. 1 to 3). rRam-1 cDNA expression causes increased phosphate transport activity (Fig. 5) and adds novel relatively high-affinity gp70 binding sites to cell surfaces (Fig. 2). After attachment to these rRam-1-dependent sites, the gp70 remains accessible for many hours to antibodies or other reagents added to the culture media, and it appears to be lost from cell surfaces with a half-life of greater than 6 h (Fig. 4). Therefore, rRam-1 contributes to formation of a complex with amphotropic gp70 that has an extremely slow rate of dissociation. In agreement with these suggestions, expression of amphotropic env glycoprotein specifically down-modulates phosphate transport by rRam-1 but has no effect on the phosphate transporters of control CHO cells (Fig. 6). Extracellular amphotropic gp70 also seems to act as a weak specific inhibitor of the rRam-1 transporter (Fig. 6). Although these results are all consistent with direct binding of gp70 onto rRam-1, we emphasize that gp70-receptor complexes have not been identified or isolated and that rRam-1 might be merely a subunit or cofactor for the gp70 binding pathway. Moreover, the specific changes in the homologous hamster transporter that mask or otherwise prevent its strong interaction with amphotropic gp70 remain unknown. Nevertheless, our results establish that rRam-1 contributes to formation of relatively strong stable bonds with amphotropic MuLV env glycoproteins and not merely to postbinding steps of infection.

This investigation was initiated using a single clone of CHO cells (clone CERD-C19), thus reducing effects that might derive from clonal variation of these cells. As seen by immunofluorescence microscopy, none of these control CHO cells bound amphotropic gp70 to a detectable extent (e.g., Fig. 1), and similar results were obtained with other CHO cell lines that were analyzed (12). Moreover, all CHO/rRam-1 subclones that were derived from the CERD-C19 cells bound the gp70 strongly and were highly susceptible to infection and to cytopathic effects of amphotropic retroviruses.
Interference to superinfection. Amphotropic MuLV 4070A infection partially down-modulates the rRam-1 component of phosphatase transport in CHO/rRam-1 cells (Fig. 6). Initially, this infection causes substantial cytopathology and cell-cell fusion (Fig. 7) and the apparent down-modulation can approach approximately 80% of the rRam-1 component of transport (Fig. 6A), whereas after more complete cytopathic recovery, the extent of down-modulation is reduced to only approximately 30 to 50% (Fig. 6B). Because these recovered cultures may have a slight residual cytopathology and because amphotropic gp70 may directly inhibit rRam-1 transporter activity (Fig. 6A), it is likely that 4070A virus infection may cause somewhat less than 35 to 50% removal of cell surface rRam-1. Similarly, human immunodeficiency virus type 1 (HIV-1) env glycoproteins partially down-modulate CD4, and additional HIV-1 genes (yup and nef) are required for more complete CD4 removal (2, 8, 18, 42). Moreover, ecotropic MuLV infections partially down-modulate cell surface receptor expression (38). It has been suggested that gibbon ape leukemia virus infections strongly down-modulate Glv-1 receptor expression, but the data would not exclude a partial loss of receptor (28).

Partial down-modulation of rRam-1 caused by amphotropic MuLV infection could not per se account for the complete interference to superinfection that occurs in these cells (Table 1) because CHO/rRam-1 clones with relatively little rRam-1 expression are as efficient as clone 13 and clone 5A cells that have highest rRam-1 expression levels (Table 2). In agreement with previous evidence for other retroviruses (16, 39), these results suggest that almost complete removal of rRam-1 would be required to efficiently abrogate infection by a down-modulation mechanism. We infer that residual rRam-1 remaining on surfaces of 4070A virus-infected CHO/rRam-1 cells must be inactive as a viral receptor, probably because its virus binding site may be blocked by gp70. An important consequence of incomplete rRam-1 down-modulation would be the preservation of adequate phosphate uptake capability by infected cells that lack alternative phosphate transporter systems. Presumably, this would reduce cytopathology and benefit both virus and host.

Cytopathology by amphotropic MuLV 4070A. Infection of our CHO/rRam-1 cells with amphotropic MuLV 4070A initially causes a severe rRam-1-dependent cytopathology that involves a slowing of cell growth and extensive cell-cell fusions (Fig. 7). Our evidence strongly suggests that the fusion involves a coupling of infected cells that express amphotropic env glycoproteins with uninfected CHO/rRam-1 cells and that recovery occurs after all receptor-bearing cells become infected and the polyploid cells are substantially overgrown. Control CHO cells do not form syncytia in the same conditions (compare Fig. 7C and D). The reduced cytopathic effects that occur in control CHO cells infected with the same pseudotyped 4070A virus could involve weaker interactions of 4070A env glycoproteins with the homologous partially masked (25, 26, 43) hamster Ram-1 protein and/or changes that may not involve amphotropic env glycoproteins. Indeed, our preliminary studies suggest that the weak abnormalities that occur in our control CHO cell clone are caused by binding of the pseudotyped virus onto the cell surfaces and that they are independent of the 4070A viral genetic information (results not shown). The extensive cell-cell fusions caused by amphotropic virus 4070A infection of our CHO/rRam-1 cultures is compatible with other indications (22) that amphotropic MuLV membranes can fuse directly with surface membranes of susceptible cells. Our evidence that rRam-1 does not mediate rapid endocytosis of adsorbed gp70 (Fig. 4) also supports the idea that amphotropic virus may bind stably to rRam-1 and then infect by fusion of virion and cell surface membranes.


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