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A Herpesvirus Saimiri Membrane Protein Required for Interleukin-2 Independence Forms a Stable Complex with p56^{lck}

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ORF-2, a 32-kDa viral protein expressed by herpesvirus saimiri-transformed lymphocytes, is essential for transformation and is expressed on the plasma membrane of transformed cells. The current work now shows that most (approximately 80%) of ORF-2 resides in the cytoplasm, while only a small portion protrudes from the cell surface. Expressed as a glutathione *S*-transferase fusion protein, ORF-2 was found to interact with a 56-kDa cellular protein in untransformed, herpesvirus saimiri-transformed, and Jurkat lymphocytes. Microsequencing proved that this protein is the lymphocyte-specific tyrosine protein kinase p56^{lck}. Two regions of ORF-2 were found to be required for p56^{lck} interaction. Current evidence suggests that the interaction of ORF-2 with p56^{lck} plays a key role in the specific transformation of T lymphocytes to an interleukin-2-independent phenotype.

Herpesvirus saimiri (HVS) is a member of the gammaherpesvirus group and is commonly found in South and Central American squirrel monkeys. HVS has been divided into three subgroups based on transforming ability: A, B, and C (31). HVS subgroup C is nonpathogenic in its natural host but is highly oncogenic when injected into New Zealand White rabbits. Animals succumb to polyclonal leukemias and lymphomas within 30 days. HVS group C can also transform human peripheral blood lymphocytes (PBLs) in vitro to an interleukin-2 (IL-2)-independent phenotype (4, 27). These cells can be maintained in tissue culture for as long as 1 year without the addition of IL-2 and without periodic restimulation as required by normal human PBLs, which die in about 2 months (5, 27).

The genome of HVS is structurally similar to that of other herpesviruses, i.e., Epstein-Barr virus and cytomegalovirus. It contains a central 113-kb region of unique DNA and a variable number of 1.4-kb terminal repeat segments at each end (7). A conserved region of unique DNA found at the right-hand junction of the HVS genome has been shown to be required for transformation (16, 30). This region of strain 484-77 (group C) has been shown to code for several small nuclear RNAs (15), a viral dihydrofolate reductase (41), and two unique proteins: ORF-1 and ORF-2 (16, 25). Previous studies have shown that the deletion of either ORF-1 or ORF-2 abolishes the expression of an IL-2-independent phenotype of infected T cells, oncogenicity, and IL-2 production (10, 27, 29). ORF-1 has been shown to be a collagen-like protein with an apparent molecular mass of 19 to 22 kDa (28).

We have recently shown that ORF-2 of strain 484-77 (32 kDa) is expressed on the cell surface of HVS-transformed T cells (25). We now demonstrate that the orientation of ORF-2 in the membrane is such that the majority of the protein is intracellular and also show that ORF-2 binds to several proteins between 45 and 66 kDa in cell extracts from transformed and untransformed human PBLs as well as Jurkat cells. One of these proteins has been confirmed to be p56^{lck}. We have identified a region of ORF-2 important for binding p56^{lck} by deletion analysis.

Our first goal in further characterizing the ORF-2 protein was to determine its orientation in the plasma membrane. Computer-analyzed hydropathy showed ORF-2 to be mostly hydrophilic (amino acids [aa] 1 to 184), except for the last 30 residues, which are mostly hydrophobic (aa 185 to 214). Most likely, it is this hydrophobic tail that inserts into the outer plasma membrane in transformed T lymphocytes. Therefore, the bulk of the protein (the N terminus) either is facing the outer surface or is located on the cytoplasmic side of the cell membrane. Protease cleavage analysis showed that V8 protease cleavage sites existed only in the N-terminal region of ORF-2. This asymmetry allowed the orientation of ORF-2 in the membrane to be determined. Either HVS 484-transformed rabbit lymphocytes were cell surface labeled, or plasma membranes were prepared, labeled, and subsequently digested with V8 protease.

Cells (5×10^7) were harvested, washed twice with phosphate-buffered saline (PBS), and then resuspended at 10^8 cells per ml in 1 mM sulfo-LC-NHS-biotin [sulfosuccinimidyl-6-(biotinamido) hexanoate; Immunopure NHS-LC-Biotin; Pierce Chemical, Rockford, Ill.] for 35 min at room temperature to biotin label cell surface proteins. This compound is impermeable to the plasma membrane, reacted with only primary amine groups, and was therefore covalently linked to lysine and arginine residues on the cell surface (12). Plasma membranes were prepared as follows. Cells (5×10^7) were washed once with PBS and lysed with hypotonic lysis buffer (10 mM Tris [pH 7.5], 1 mM EDTA plus protease inhibitors: 2 μg of leupeptin per ml, 2 μg of aprotinin per ml, 1 mM phenylmethylsulfonyl fluoride). The lysate was spun at 1,000 × *g* for 10 min to remove unbroken cells and nuclei. The pellet was then extracted again. Supernatants were combined and spun at 20,000 × *g* for 25 min to collect plasma membranes. The plasma membrane proteins were biotinylated as described above. After labeling cells or membranes, 10^7 cells or cell membranes were digested with V8 protease in 250 μl of PBS with the appropriate amount of V8 (Calbiochem, San Diego, Calif.) added at 37°C for 1 h. Preparations were then washed twice with PBS, and proteins were extracted in extraction buffer (hypotonic buffer plus 150 mM NaCl, 1% Nonidet P-40 [NP-

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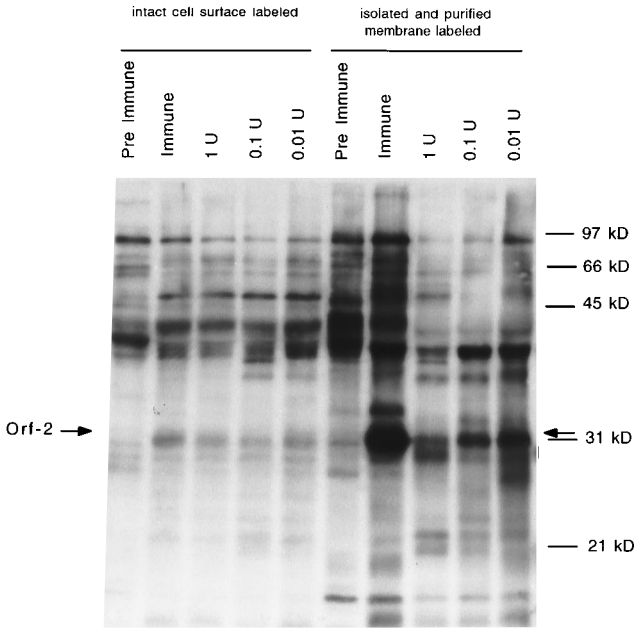

FIG. 1. Determination of orientation of ORF-2 by digest with V8 protease. Intact HVS-484-transformed rabbit cells (4×10^7) and purified membranes from 4×10^7 HVS 484-transformed rabbit cells were biotinylated with Immunopure NHS-LC-Biotin (Pierce Chemical) for 35 min at room temperature. Cells and membranes were washed once with PBS and resuspended in PBS with 10 U of V8 protease (Calbiochem) at 37°C for 30 min. Proteins were extracted with extraction buffer for 30 min at 4°C. Immunoprecipitation was performed with 5 μl of rabbit polyclonal ORF-2 antibody (or with preimmune serum) per 10^7 cell membranes; immune complexes were precipitated with 50 μl of 1:1 protein A-Sepharose. Immune complexes were run on an SDS–12% PAGE gel and electroblotted, and biotinylated proteins were visualized (Western-Light chemiluminescence system; Tropix). Each lane represents cell membranes from 10^7 cells.

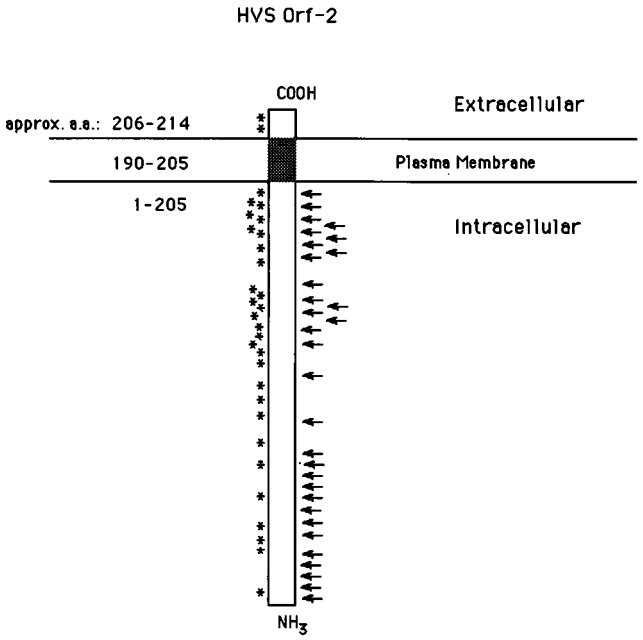

FIG. 2. Proposed orientation of HVS ORF-2 in the plasma membrane of HVS-transformed cells. The shaded portion represents the most hydrophobic region of ORF-2 as determined by computer analysis (DNASTAR, Madison, Wis.). A potential biotinylation site, either a lysine or an arginine residue, is denoted by an asterisk. The arrows represent potential V8 protease cleavage sites.

40]) for 30 min at 4°C. Extracts were then subjected to immunoprecipitation as follows. All manipulations were done at 4°C. Extract from 10^7 cells was precleared with 10% (vol/vol) Pansorbin cells (Calbiochem) for 1 h, and then 5 μl of rabbit anti-ORF-2 polyclonal antiserum or preimmune serum was added for 1 h. Immune complexes were precipitated with 50 μl of protein A-Sepharose (Sigma) beads (1:1 slurry in PBS) for 1 h. Beads were washed twice with RIPA buffer (50 mM Tris [pH 7.5], 150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate [SDS]) and once with 10 mM Tris–0.1% NP-40. Beads were then boiled for 5 min in SDS loading buffer and loaded onto an SDS–12% polyacrylamide gel electrophoresis (PAGE) gel. After electrophoresis, the gel was electroblotted onto Immunobilon-P^{SQ} polyvinylidene difluoride (PVDF) membrane (Millipore, Bedford, Mass.). Immunoprecipitated proteins were visualized by a chemiluminescent detection system (Western-Light; Tropix, Bedford, Mass.) (2).

Figure 1 shows immunoprecipitation of ORF-2 before and after V8 treatment from labeled plasma membranes (Pre Immune and Immune lanes were not treated with V8 protease). The majority of ORF-2 protein was digested by the V8 protease treatment as shown by the lack of the 32-kDa ORF-2 band after treatment. Figure 1 also shows the effect of V8 on cell surface-labeled ORF-2 versus that of labeled ORF-2 obtained from membrane preparations. V8 treatment of cell surface-labeled ORF-2 had no effect. In contrast, ORF-2 from membrane preparations was digested, and this digestion correlated with the amount of V8 used. These results provide good evidence that ORF-2 is localized in the membrane as shown in Fig. 2. Evidence for this proposed orientation is also given by the amount of labeling of cell surface-labeled ORF-2 compared with the amount of labeling of ORF-2 in membrane preparations. There are approximately 15 times more potential biotinylation sites on the N-terminal side of the membrane spanning-region than on the C-terminal end (29 versus 2; determined by counting the number of lysine or arginine residues). Therefore, labeling membrane preparations should produce a much stronger signal than cell surface labeling. The results shown in Fig. 1 are consistent with this prediction. Scanning with a densitometer showed the labeling of the membrane-prepared ORF-2 to be 12 to 15 times more intense than the cell surface-labeled ORF-2 (data not shown). This is what is expected because of the increase in availability of potential biotinylation sites with the prepared membranes. Additionally, the cell surface could not be labeled with ^{125}I (data not shown), which is consistent with the observation that there are no tyrosine residues in the carboxyl portion of ORF-2.

Since HVS 484 can transform human PBLs in vitro (4, 27) and ORF-2 is indispensable for this transformation (27), we sought to find what factors in human PBLs might interact with ORF-2. Because we found that the majority of the ORF-2 protein resides within the cytoplasm of HVS-transformed lymphocytes, experiments were next undertaken to identify cytoplasmic proteins interacting with ORF-2. A fusion protein of glutathione *S*-transferase (GST) and the antigenic region (determined by computer analysis to be aa 31 to 150) of ORF-2 was constructed as previously described (25) and was used in in vitro binding assays. A second fusion protein containing GST fused to a full-length ORF-2 (aa 1 to 214) was also constructed. These fusion proteins were purified as described previously (25, 37) and used in in vitro binding assays as follows. Whole blood was obtained from healthy donors, and PBLs were sep-

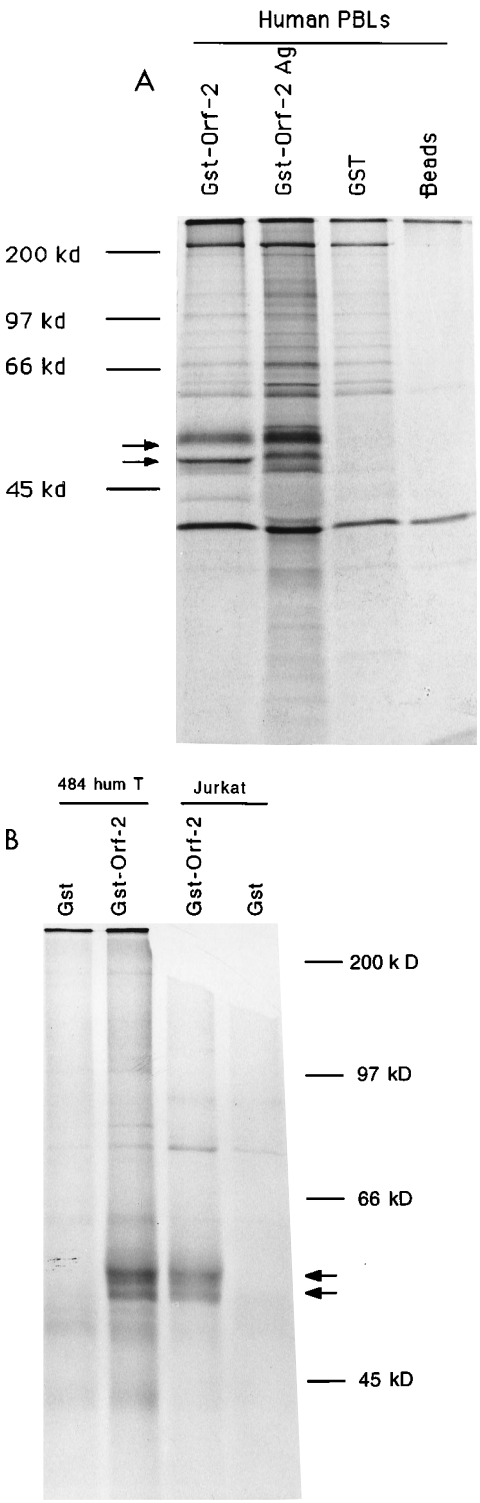

FIG. 3. In vitro binding assay with GST–ORF-2 fusion proteins identifies at least two proteins in Jurkat, HVS-infected, and uninfected lymphocytes. (A) Human phytohemagglutinin-stimulated PBLs (10^7) maintained in 50 U of IL-2 per ml (10 days after isolation) were [^{35}S]methionine labeled in AIM V medium (Gibco) for 2 h. Cells were washed and lysed with extraction buffer. Lysates were precleared with 10 μg of GST bound to glutathione agarose beads for 1 h at 4°C and then 2 μg of fusion protein purified on glutathione agarose beads (37) for 1 h at 4°C. Beads were then washed twice with RIPA buffer and once with 0.1% NP-40–10 mM Tris (pH 7.5), boiled in 50 μl of loading buffer, and loaded onto an SDS–12% PAGE gel. Interacting proteins were visualized by autoradiography. Arrows indicate the major interacting proteins. (B) The interaction of GST–ORF-2 with cellular lysates from HVS 484-transformed human T cells and Jurkat cells. Cells were labeled, and an in vitro binding assay was performed as described for panel A. Arrows indicate two major interacting proteins. The 484 hum T is a CD8 T-cell line immortalized in vitro with strain 484-77 (27).

TABLE 1. Results of internal sequencing of an ORF-2-binding protein[a]

Tryptic sequence	Initial yield (pmol)	Lck sequence[b]	Residues
xxSLE	2	ANSLEPEPWFFK	119–130
xFxQNxxxVVx	5	DFDQNQGEVVK	169–182
NLDNGGFYISP	10	NLDNGGFYISPR	185–196
IxxPxL	2	ITFPGLHELVR	197–207
ExPRE	3	EVPRETLK	239–246
LxxGxFGxV	8	LGAGQFGEVWM	251–269
IADFGLA	4	IADFGLAR	380–387
xxExN	3	LIEDNEYTAR	388–397

[a] Shown are the HPLC-separated microsequenced fragments of a tryptic digest of an ORF-2-binding protein approximately 55 to 56 kDa in molecular mass. Identified residues are given as capital letters, while undetermined residues are shown as x. Column 2 shows the estimated initial yield of each fragment from HPLC. Columns 3 and 4 show the tryptic fragments from p56^{lck} with which the sequenced peptide aligns. The tryptic fragments from p56^{lck} were identified with a computer-simulated digest (DNASTAR).

[b] Accession number (GenBank) X13529.

arated on Histopaque-1066 (Sigma). PBLs were stimulated for 2 days with 5 μg of phytohemagglutinin per ml (Sigma) and were grown in RPMI 1640 (Gibco)–10% fetal bovine serum, supplemented with 50 U of recombinant human IL-2 per ml to grow out T lymphocytes. PBLs (10^7) at least 10 days in culture were labeled with 200 μCi of [^{35}S]methionine (NEN) for 2 h in methionine-free AIM V medium (Gibco BRL, Grand Island, N.Y.). Cells were washed once with cold PBS and lysed at 4°C for 30 min in extraction buffer. Lysate (10^7 cells) was precleared with approximately 10 μg of control GST bound to 50 μl of glutathione-linked agarose beads for 1 h at 4°C. Lysate was then incubated with purified fusion protein (approximately 2 μg) on glutathione-agarose beads, control GST protein (approximately 2 μg) on beads, or with beads alone. Bead volume was 50 μl, and all incubations were done at 4°C for 1 h. Beads were then washed twice with 1 ml of RIPA buffer for 10 min and once with 0.1% NP-40–10 mM Tris buffer and then boiled in SDS-PAGE loading buffer for 5 min. Released fusion proteins and any interacting proteins were run on an SDS–12% PAGE and autoradiographed.

Figure 3A shows results of the in vitro binding assay with human PBLs. Several cellular proteins between 45 and 66 kDa appear to specifically interact with both the full-length ORF-2 and ORF-2 antigen fusion proteins. To see whether similar interacting proteins existed in other cells, HVS 484-transformed human PBLs (27) and Jurkat cells were next used in an in vitro binding assay. Again, two major proteins between 45 and 66 kDa bound specifically to the full-length ORF-2 fusion protein as shown in Fig. 3B. Since the cells used in these experiments were T cells (HVS-transformed PBLs and Jurkat cells) or predominately T cells (human PBLs grown in IL-2), it is likely that the same proteins between 45 and 66 kDa are being bound in each experiment.

To microsequence the interacting protein, the binding assay was performed on a large scale with 1 to 2 μg of ligand and cell lysate from 4 to 5 liters of Jurkat cells. GST–ORF-2 interacting proteins were blotted on PVDF membrane (Millipore), and the protein bands were excised. Microsequencing of the most

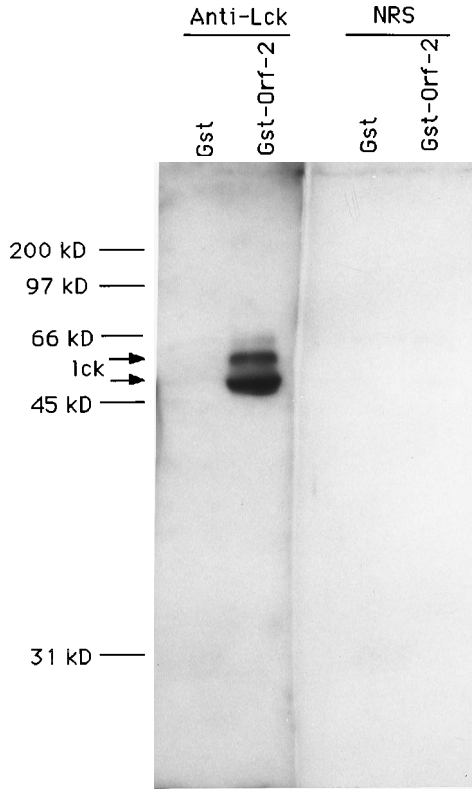

FIG. 4. GST–ORF-2 interacts with both forms of Lck (p56 and p60) from Jurkat cells. An in vitro binding assay with GST–ORF-2 fusion protein was performed as described earlier. The interacting proteins were separated on SDS-PAGE and electroblotted to PVDF membrane (Millipore). An anti-p56^{lck} rabbit polyclonal antiserum was used in a Western blot of the interacting proteins. The Western-Light (Tropix) chemiluminescence system was used to visualize protein-antibody complexes. A normal rabbit serum (NRS) was used in a control blot.

abundant band indicated that the approximately 55- to 56-kDa protein was N terminally blocked. The protein was therefore digested with trypsin (14), and fragments were separated by high-pressure liquid chromatography (HPLC). Sequence analysis was performed on several peaks which appeared likely to be pure fragments of the highest yield (shown in Table 1) on an Applied Biosystems 477A protein sequencer. The fragment with the clearest sequence (NLDN in Table 1) was used in a search of GenBank with the BLAST server (3). All of the fragments that were sequenced were found to align with the lymphocyte-specific tyrosine kinase p56^{lck} (Table 1).

To confirm that one of the interacting proteins was indeed p56^{lck}, Western blots (immunoblots) were performed on blotted proteins found to interact with the ORF-2 fusion proteins. In vitro binding assays were performed as before (but without metabolic labeling) with Jurkat cells. The interacting proteins were run on SDS-PAGE and electroblotted onto PVDF membrane, and a Western blot with an anti-p56^{lck} rabbit polyclonal antibody (UBI, Lake Placid, N.Y.) was done. Antibody-protein complexes were visualized with the Western-Light chemiluminescence system from Tropix. Figure 4 shows that the GST–ORF-2 fusion protein does interact with p56^{lck} and with a 60-kDa protein. The two bands seen represent the two known reported isoforms of Lck: a 56-kDa form and a hyperphosphorylated form which migrates at 60 kDa (11). While most Western blots showed both forms (p56 and p60) of Lck, some blots (e.g., Fig. 5) showed only the p56 form of Lck, although the p60 form of Lck was detected only after a long-term exposure. Because Lck has been shown to be regulated during the cell cycle (11), we attribute the absence of the p60^{lck} in some experiments to a variable in the growth conditions of the cells. The p56 form of Lck is present in the G_1 stage of lymphocyte cell growth. When the cells are induced to the S stage, the p60 form appears (11). This p60 form is due to phosphorylation at serines 42 and 59 of Lck (11).

To confirm that at least one of the [^{35}S]methionine-labeled proteins observed to interact with the GST–ORF-2 fusion protein was indeed p56^{lck} an SDS-PAGE gel was run with ^{35}S-labeled and unlabeled ORF-2 interacting proteins. The unlabeled portion of the gel was Western blotted with the anti-p56^{lck} antibody (Fig. 5). As shown in lanes 2 and 3, a major protein of approximately 56 kDa interacts with the ORF-2 fusion proteins. This migrates exactly with the p56^{lck} protein which interacts with the same ORF-2 fusion proteins identified by Western blot (lanes 6 and 7). This gives substantial support to the idea that at least one of the cellular proteins which interacts with ORF-2 is p56^{lck}.

To identify the region of ORF-2 responsible for binding to p56^{lck}, GST–ORF-2 fusion proteins containing deletions in the ORF-2 open reading frame were made (Fig. 6). Fusion proteins were purified on glutathione agarose beads as previously described (25, 37). A total of 1 to 2 μg was used in in vitro binding assays of Jurkat cell lysate as described earlier. As before, interacting proteins were also subjected to Western blot for p56^{lck}. Results shown in Fig. 5A (lanes 4, 5, 9, and 10) indicate that ORF-2 deletions 1 and 2 abolish the interaction with p56^{lck} found with the full-length or antigenic portion of ORF-2 (compare lanes 7 and 8 with 9 and 10). This is corroborated by the loss of the major 56-kDa band of ORF-2 metabolically labeled interacting proteins (lanes 4 and 5).

ORF-2 contains two domains which may play a role in Lck binding. One is an 8-residue sequence with 75% similarity to the regulatory domain of Src family kinases (ORF-2, aa 106 to 113, LKSFLEKY), and the other is a potential SH3 binding motif (ORF-2, aa 132 to 141, MPKPTLPPRP) (6). The results from deletions 1 and 2 indicated that the Src kinase homology domain and the potential SH3 binding site might be required for binding to Lck. To test this hypothesis, deletions 3 and 4 were constructed as GST fusion proteins (Fig. 6) and used in in vitro binding assays. The results of these experiments (Fig. 5B) support the hypothesis that both regions are required for Lck binding as shown by the interaction of Lck with deletion 3 but not deletion 2 or 4.

As ORF-2 is an essential element for HVS to be able to transform T lymphocytes (27), it is of interest to know its position in the plasma membrane. The V8 protease experiments provided substantial evidence that the bulk of the ORF-2 protein is retained in the cytoplasm as depicted in Fig. 2. This particular orientation of ORF-2 may have functional consequences regarding cellular factors that might interact with this protein and possibly aid in the transformation of T cells.

In vitro binding assays showed that two major proteins (p56 and p60) interact with ORF-2 fusion proteins. The major proteins observed in the binding assay are specific and bind with high affinity because of the fact that they remain bound after a stringent wash with RIPA buffer. Upon longer exposure of the binding assay autoradiograph, several minor proteins between 45 and 66 kDa also appeared (data not shown). The 56-kDa band was found to be p56^{lck} by microsequencing. This finding was confirmed with an anti-p56^{lck} antibody in Western blotting experiments. The two major ORF-2 interacting proteins present in human PBLs, HVS-transformed human PBLs, and

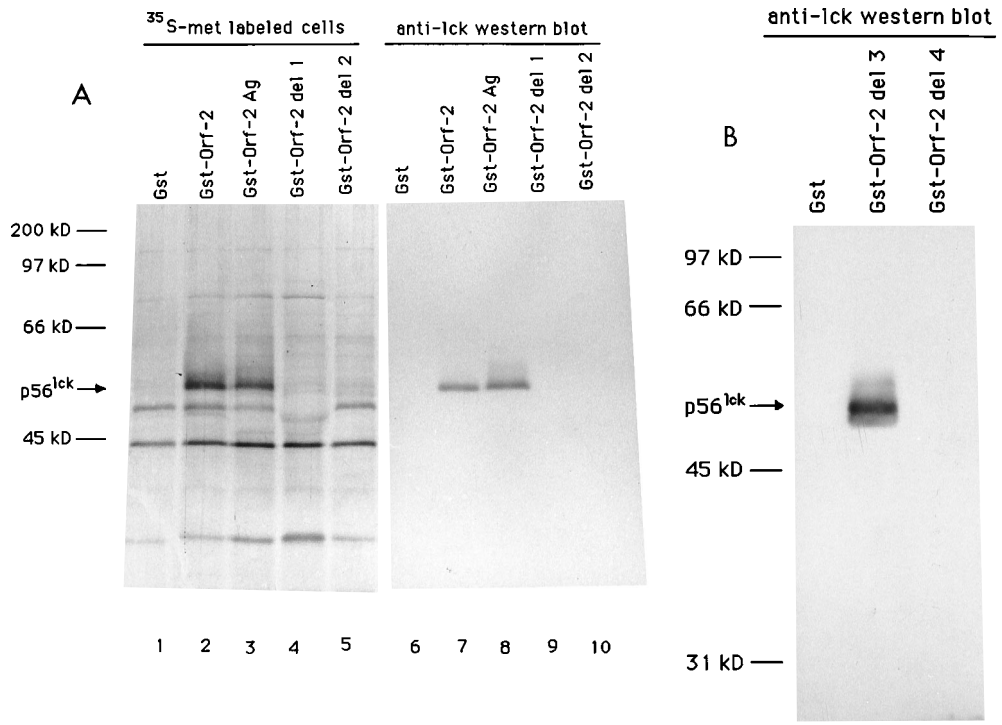

FIG. 5. Identification of a region of ORF-2 responsible for binding p56^{lck}. Several GST fusion proteins containing various regions of HVS 484 ORF-2 were constructed and used in in vitro binding assays as described earlier. Either binding assays were done with metabolically labeled Jurkat cells and the interacting proteins were electrophoresed, transferred to PVDF, and autoradiographed (panel A, lanes 1 to 5), or the assays were done with cold Jurkat cells and the interacting proteins were electrophoresed, transferred to PVDF, and Western blotted with an anti-Lck antibody (UBI) (panel A, lanes 6 to 10, and panel B).

Jurkat cells (Fig. 3) represent the two forms of Lck, p56 and p60, as evidenced by Western blot (Fig. 4).

Our finding that HVS strain 484 ORF-2 interacts with p56^{lck} is in good agreement with similar data recently published by Biesinger et al. (6), who have shown that the kinase p56^{lck} interacts with the ORF-1 protein from another HVS strain (strain 488) by different approaches than ours. ORF-1 (renamed Tip for tyrosine kinase interacting protein) from HVS 488 is a homolog of the ORF-2 protein found in HVS strain 484 (approximately 72% similarity). Biesinger et al. hypothesized that two regions of Tip may be important in its interaction with Lck. One is a region homologous to the regulatory domain of Src family kinases, and the other is an SH3 binding consensus motif (XPXXPPΨXP). Both of these homologous regions also exist in HVS 484 ORF-2 (aa 106 to 113 and aa 132 to 141, respectively) (Fig. 6).

The first kinase homology domain shares some 60% similarity with Src family kinases (i.e., Src, Fyn, Yes, etc.). The region of the *src* family kinases where this homology exists is located at the C-terminal portion of the SH1, or kinase, domain and just in front of the regulating tyrosine residue (33). We have shown that this domain is required for the interaction with Lck. This domain is thought to be responsible for the phosphorylation of Src family kinase substrates (33).

The other domain required for ORF-2 to bind Lck is a proline-rich region (MPKPTLPPRP). This region is homologous to the SH3 binding region identified by Ren et al. (34). Lck has an SH3 domain (39), and it is most likely through this

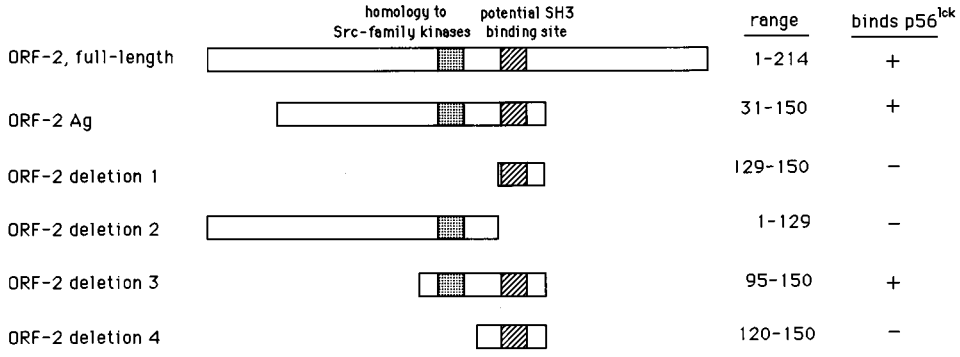

FIG. 6. Summary of deletions constructed from HVS 484 ORF-2 and interaction with Lck. Deletions 1 and 2 were constructed by deleting a restriction fragment from a GST–ORF-2 construct and religating the DNA. Deletions 3 and 4 were constructed by using PCR-amplified fragments from ORF-2 and cloning them downstream of GST as fusion proteins. All constructs were verified by restriction analysis, PCR, and fusion protein production.

domain that Lck binds ORF-2. Little is known about SH3 binding and its consequences on cellular function, although SH3 has been shown to be involved in binding various other Src family kinases and is probably involved in the regulation of their activity (33).

Biesinger et al. have shown that the ORF-2 homolog in HVS strain 488 (Tip) can be a substrate for Lck kinase activity in vitro (6). While we have not yet shown ORF-2 to be phosphorylated, a similar situation probably exists. This phosphorylation is then probably mediated through the two domains shown here to be the minimal requirement for Lck interaction.

Lck is a lymphocyte-specific protein tyrosine kinase and has been found in stable complex with CD4 (36) and CD8 (40). Lck has been shown to be essential for T-cell receptor (TCR) signal transduction (23, 38). It is believed that CD4 or CD8 delivers Lck into the proximity of the TCR-CD3 complex to allow it to phosphorylate the zeta chain of CD3, which then allows for the recruitment and phosphorylation of ZAP 70 to continue the downstream signal transduction (22). Lck also is important in the signaling pathway of IL-2. It has been shown to bind to the β chain of the IL-2 receptor (IL-2R) and can phosphorylate it in vitro (17). IL-2 has been shown to transiently stimulate the kinase activity of Lck in human T cells (18). A constitutively activated Lck can upregulate antigen-independent IL-2 production in T cells (26). This dual role of Lck, its involvement in TCR-CD3 as well as IL-2R signal transduction, makes Lck an important regulator in the growth maintenance of T cells.

Other viruses do express viral regulatory proteins which interact with a cellular protein tyrosine kinase such as Lck. The Epstein-Barr virus LMP2 interacts with and is a substrate for Src family protein tyrosine kinases (8). It has also been shown that the induction of $p56^{lck}$ may be a prerequisite for transformation by Epstein-Barr virus (9). Other tumor viruses also have been shown to interact with Src family kinases; the middle T antigen of the polyomavirus is an example of one of these (13, 19, 24). Even such divergent viruses as human immunodeficiency virus have evolved interactions with cell signaling proteins. It has been recently discovered that the gp120 of human immunodeficiency virus disrupts the CD4-$p56^{lck}$ complex. This disruption is hypothesized to be involved in inhibition of TCR-CD3 signaling (20).

While the downstream targets of $p56^{lck}$ have not been fully elucidated, it is known that $p56^{lck}$ must be myristylated at the N terminus to achieve its highest activity to allow for association with the inner leaflet of the plasma membrane (35). Lck activity is also greatly enhanced by dephosphorylation at tyrosine 505, probably by CD45 (21). We speculate that ORF-2 may be responsible for the recruitment of Lck to the plasma membrane (as CD4, CD8, or IL-2R), where it becomes myristylated and activated by CD45. Upon activation, $p56^{lck}$ can either transduce a TCR-CD3 signal via phosphorylation of CD3 zeta chain or transduce a growth signal utilized by the IL-2R probably through activation of the Shc-Grb2-Sos pathway (32). It is also possible that both could occur simultaneously. We hypothesize that ORF-2 causes Lck to be constitutively activated, which leads to IL-2-independent growth of HVS-transformed T cells. Importantly, deletions of ORF-2 resulted in loss of the IL-2-independent phenotype of infected cells (27). Note also that HVS-transformed T cells do not require periodic restimulation like normal PBLs, which gives a hint that ORF-2–Lck is involved in TCR signaling as well. It is also of interest that transgenic mice carrying a constitutively active form of Lck developed thymic tumors (1). This may well correspond with T-cell-specific lymphogenesis by HVS in which the activation of Lck may play a role. Experiments are under way to determine the effects of ORF-2 on the signaling pathways mediated by Lck. Such work may also bring about a better understanding of the regulation and function of $p56^{lck}$ as well.

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