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Constitutive endocytosis of HLA class I antigens requires a specific portion of the intracytoplasmic tail that shares structural features with other endocytosed molecules

(internalization/coated pits/cell-surface molecules/cytoplasmic domain)

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ABSTRACT **HLA class I antigens present in the human leukemia T-cell line HPB-ALL are shown to be endocytosed in the absence of specific antibodies. In 1 hr, ≈10% of class I molecules initially present at the cell surface are found intracellularly. Genetically engineered mutants of the HLA-A2 gene lacking exon 6 or 7 or both were used to analyze whether the cytoplasmic region contributes to the internalization. The results indicate that amino acids encoded by exon 7 (spanning amino acid residues 323–340) are required for internalization, while deletion of exon 6 had no effect. In addition, a comparison of the cytoplasmic sequences of receptors that are known to be internalized via coated pits and the present data revealed that they share a structural feature that could constitute a specific signal required for endocytosis.**

A variety of molecules enter cells by a mechanism known as receptor-mediated endocytosis. This mechanism is used for a multiplicity of purposes, such as delivery of nutrients and growth factors [low density lipoprotein (LDL), transferrin, epidermal growth factor, and insulin], entry of some viruses and toxins, cellular uptake of plasma proteins (asialoglycoproteins, α_2-macroglobulin, immune complexes, and lysosomal enzymes), and transport of ligands across the cell (polymeric IgA and IgM) (1). For some molecules, the functional implications of their internalization are not known [e.g., T-cell receptors (2), CD4 (3)]. Molecular mechanisms involved in receptor-mediated endocytosis processes are not well understood. Several studies indicate that some structural features in the cytoplasmic region of receptors must be required for endocytosis (1). However, while there is solid evidence of the existence of specific structural signals in proteins that are targeted to some cellular compartments (nucleus, mitochondria, endoplasmic reticulum, and plasma membrane), no common structural feature has been described for those molecules internalized via coated pits (4).

Class I histocompatibility antigens are integral membrane glycoproteins present on all nucleated cells. They consist of a polymorphic major histocompatibility complex-encoded heavy chain of 45 kDa, noncovalently associated with β_2-microglobulin, an invariant 12-kDa polypeptide. Heavy chain anchors the molecule in the cell membrane and has a 34-amino acid intracytoplasmic region that is highly conserved among HLA and H-2 alleles (5). Class I antigens play a central role in the immune defense by binding antigenic peptides, which are subsequently presented to specific cytolytic T lymphocytes (6, 7). The location within the cell and the mechanism of binding of foreign peptides are unknown. In the presence of specific antibodies, class I antigens are internalized via coated pits. This process occurs in T lymphocytes and macrophage/monocyte type cells but not on fibroblasts or B lymphocytes. Its function is also unknown (8–10). In this paper, the role of the intracytoplasmic region of HLA-A2 in its internalization in the absence of antibodies (as is likely to occur *in vivo*) is examined.

MATERIALS AND METHODS

Internalization Assay. Cells ($3–4 \times 10^7$) were iodinated with $Na^{125}I$ on ice by the lactoperoxidase method (2). After washing, they were resuspended in culture medium at 2×10^6 cells per ml and divided into the appropriate number of aliquots (4×10^6 cells per tube). They were either maintained on ice or incubated at 37°C for various times and then placed on ice for all subsequent steps. Cells were pelleted and resuspended in 200 μl of culture medium. To each aliquot, 2 μl of ascites was added and the tubes were incubated for 1 hr with short occasional gentle shakings. Tubes were then centrifuged and cells were washed three times with culture medium to remove the excess antibody not bound. At the last wash, cell viability was checked and usually was >90%. Cells were lysed for 45 min by the addition of 0.5 ml of lysis buffer (10 mM Tris·HCl, pH 7.5/150 mM NaCl/1 mM EDTA/2% Nonidet P-40) containing the lysate derived from 2×10^7 nonlabeled cells. Insoluble cell debris was pelleted by centrifugation for 15 min at 13,000 × *g*. To the supernatant, 150 μl of a 10% suspension of fixed *Staphylococcus aureus* cells (Pansorbin, Calbiochem) was added and incubated for 30 min. After centrifugation, the pellet was kept on ice and 50 μl of Pansorbin was added to the supernatant. After 30 min, the tubes were centrifuged and the pellets were added to those previously obtained. These immunoprecipitates contain the labeled molecules present at the cell surface. To recover the labeled molecules that the antibodies did not bind (internalized molecules), 2 μl of ascites was added to the supernatants and after 1 hr of incubation on ice the immune complexes formed were immunoprecipitated by the addition of 200 μl of Pansorbin. All immunoprecipitates were washed as described (11).

HLA-A2 Mutants. The HLA-A2 gene used is contained in a 6.5-kilobase *Eco*RI fragment. A 4.5-kilobase *Xba* I subfragment containing all exons of the HLA-A2 gene was subcloned in pUC13 vector (p13A2X). This plasmid has unique restriction enzyme cutting sites at the introns located between exons 5 and 6 (*Afl* II site), 6 and 7 (*Nco* I site), and 7 and 8 (*Sph* I site) of the HLA-A2 gene. This feature was used to construct three mutants lacking exon 6, 7, or both, respectively. It was assumed that splicing signals are not altered after DNA manipulations. Thus, the mutant lacking exon 6 (designated HLA-A2Δ6) was made by cutting the plasmid p13A2X with the enzymes *Afl* II and *Nco* I and then filling in the protruding ends and religating the resulting plasmid with T4 ligase. The initial whole HLA-A2 gene was

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Abbreviation: LDL, low density lipoprotein.

reconstructed by joining the *Xba* I fragment to the rest of the gene subcloned in pUC8 vector. Mutants lacking exon 7 (HLA-A2Δ7) and exons 6 and 7 (HLA-A2Δ6,7) were similarly made. Wild-type and mutated HLA-A2 genes were subcloned into the *Eco*RI site of the vector pSV2neo. Each plasmid was linearized by treatment with *Bam*HI. Cells (4×10^6) were electroporated in the presence of 20 μg of linearized plasmids by using a BRL electroporator. On day 2 after transfection, the cells were placed in selective medium containing active G418 (600 μg/ml) (GIBCO). On day 20, cells expressing HLA-A2 antigens at the surface were stained with the HLA-A2 specific monoclonal antibody 4B3 and fluorescein isothiocyanate-conjugated goat $F(ab')_2$ anti-mouse IgG (Tago) and were analyzed and sorted by an EPICS V fluorescence-activated cell sorter. The size of the different HLA-A2 proteins was assayed by SDS/PAGE after labeling cells with $Na^{125}I$ and immunoprecipitating HLA-A2 antigens with 1 μl of ascites containing the antibody 4B3 and 150 μl of Pansorbin.

RESULTS AND DISCUSSION

Assay for Internalization of Class I Major Histocompatibility Complex Molecules. Because antibody-mediated endocytosis of class I molecules has been reported to occur in T cells (8), the T leukemia cell line HPB-ALL (12) was used. Since antibodies against class I antigens, used as a "ligand" in earlier studies, might affect or influence the endocytic process, an internalization assay has been developed that relies on distinguishing those molecules present at the cell surface from those present inside the cell. It is based on the method used previously to study biosynthesis of class I antigens (13).

The validity of the internalization assay was established on the basis of the following criteria: (*i*) It must distinguish between molecules present at the cell surface and those inside the cell. (*ii*) It should be useful to study internalization of other receptors, such as the transferrin receptor, whose internalization kinetics have been characterized in detail (2, 14). (*iii*) It should show a temperature dependency. The first assumption was addressed by applying the assay to cells bearing radiolabeled class I antigens either only at the surface or inside. HPB-ALL cells were labeled only at the surface by iodination as described and were kept at 0°C. After immunoprecipitation, all radioactivity was recovered in the fraction corresponding to external molecules, as was expected (Fig. 1*A*). Newly synthesized class I molecules take 30–60 min to reach the cell surface (13). Thus, HPB-ALL cells were pulsed with [^{35}S]methionine for 10 min and chased with an excess of nonlabeled methionine at 0, 30, and 60 min. At these times, cells were placed on ice and the external and internal molecules were precipitated for each aliquot. As was expected, for the aliquot chased at a time <30 min, all radiolabeled class I molecules were recovered in the immunoprecipitate assigned as containing the internalized molecules (Fig. 1*B*). Therefore, the assay discriminates molecules present at the cell surface from those present inside the cells. Fig. 1*C* shows two internalization experiments for the transferrin receptor carried out at 21°C or 37°C in the presence of transferrin contained in fetal calf serum. At 21°C, the fraction of internalized receptors was negligible, while at 37°C, at 60 min ≈50% of transferrin receptors were inside the cells. These data are in agreement with the kinetics described (2, 14) and fulfill the temperature-dependency requirement.

Constitutive Internalization of HLA Class I Antigens in HPB-ALL Cells. Internalization of HLA class I antigens was investigated in HPB-ALL cells and showed a clear temperature and time dependence (Fig. 2). At 37°C, the fraction internalized reached a plateau at 40–60 min. Densitometric analysis revealed that at equilibrium ≈10% of HLA molecules were found intracellularly. Isoelectrofocusing of surface and internalized fractions after 60 min showed that the various locus- and allele-specific class I heavy chains in HPB-ALL were internalized to the same extent (data not shown). The invariant distribution between extracellular and intracellular molecules after 40–60 min is consistent with several interpretations—e.g., (*i*) the existence of a dynamic steady state involving recycling of the internalized molecules

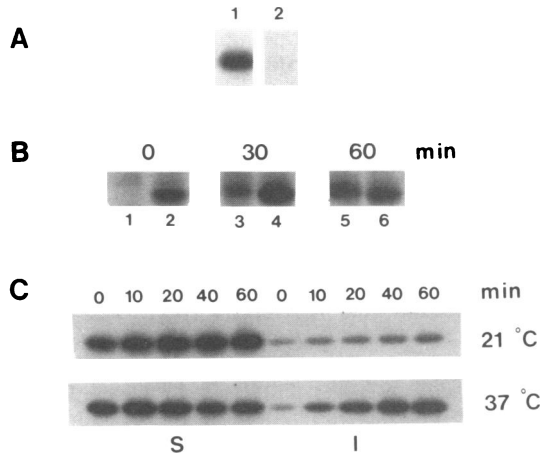

FIG. 1. Validation of the internalization assay. (*A*) Quantitative immunoprecipitation of HLA class I antigens present at the cell surface. HPB-ALL cells after ^{125}I labeling on ice were subjected to the internalization assay. The antibody W6/32 was used for the immunoprecipitations. Only the bands corresponding to the heavy chains of the fraction of HLA antigens present at the surface (lane 1) and intracellularly (lane 2) are shown. Bands for the β_2-microglobulin showed the same relative intensity as their corresponding heavy chains (not shown). (*B*) Quantitative immunoprecipitation of HLA class I antigens present inside the cells. HPB-ALL cells were pulsed with 0.5 mCi (1 Ci = 37 GBq) of [^{35}S]methionine for 10 min and then either kept on ice or warmed (chased) for 30 and 60 min. After those times, cells were placed on ice, and the internalization assays were carried out as described. The antibody W6/32 was used for the immunoprecipitations. Only bands corresponding to the heavy chains are shown. Lanes 1, 3, and 5 are derived from the first immunoprecipitation round. Lanes 2, 4, and 6 are from the second immunoprecipitation round. (*C*) Time and temperature dependence of the internalization of the transferrin receptor. Surface-iodinated HPB-ALL cells were either held on ice (time 0) or warmed at 21°C or 37°C for various times and then returned to ice. Internalization assays were performed as indicated using the transferrin receptor-specific antibody 5E9 for the immunoprecipitations. S and I, fractions derived from the cell surface or intracellularly, respectively.

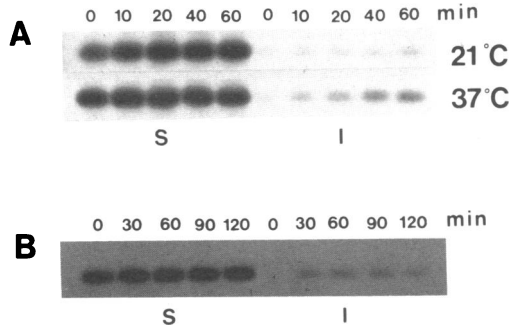

FIG. 2. HLA class I antigens are constitutively endocytosed in the T-cell line HPB-ALL. (*A*) Internalization of HLA class I antigens shows a time and temperature dependence. Surface-iodinated HPB-ALL cells were either held on ice (time 0) or warmed at 21°C or 37°C for various times and then returned to ice. Internalization assays were performed as indicated by using the antibody W6/32 for the immunoprecipitations. Only the bands corresponding to the heavy chains are shown. S and I, fractions derived from the cell surface or intracellularly, respectively. (*B*) Same as in *A*, except longer incubation times at 37°C were used.

back to the surface, as has been described for other endocytosed molecules (1); (*ii*) distinct physical locations for different fractions of the class I antigens, only one of which may be internalized; or (*iii*) a distinct chemical form for the fraction of molecules that is internalized [e.g., a phosphorylated form (5)]. The fraction of class I HLA antigens phosphorylated has been estimated as 0.1–0.6 in different cell lines (15, 16). The kinetics of internalization described for HLA class I antigens in the cell line HPB-ALL does not necessarily reflect what might occur on other cell types and on resting and activated peripheral blood T lymphocytes.

Amino Acids Encoded by Exon 7 of HLA-A2, but Not Those of Exon 6, Are Necessary for Endocytosis. Most amino acids of the cytoplasmic region of HLA antigens are encoded by exons 6 (11 amino acids) and 7 (16 amino acids). Exon 8 encodes only 1 amino acid, and the 3′ end of exon 5 encodes the 5 N-terminal amino acids of the cytoplasmic tail (17). To examine the contribution of the amino acids encoded by exons 6 and 7 to the internalization of the HLA-A2 molecule, three mutants lacking the amino acids encoded by exon 6 (designated HLA-A2Δ6), exon 7 (HLA-A2Δ7), or both (HLA-A2Δ6,7) were constructed. The predicted cytoplasmic amino acid sequences for these mutants are shown in Fig. 3*A*. HLA-A2 gene constructs were subcloned into the vector pSV2neo and transfected into HPB-ALL cells. Cells bearing comparable levels of surface HLA-A2 molecules, as determined by staining with antibody 4B3, were sorted for each of the transfectants and were used for subsequent experiments.

The structure of HLA-A2 molecules present at the surface of different transfectants was investigated by immunoprecipitation (Fig. 3*B*). HLA-A2Δ6 and HLA-A2Δ7 products each showed a smaller size than wild-type HLA-A2, consistent with their respective amino acid deletions. Unexpectedly, two bands were revealed for the mutant HLA-A2Δ6,7. The lower band fits the expectation for an HLA-A2 molecule lacking the amino acids encoded by exons 6 and 7. The upper band appears even larger in size than the wild-type HLA-A2 molecule. This product may be the result of a partially anomalous alternative splicing in the HLA-A2 gene. Although splicing signals at the exon–intron junctures were kept intact when mutants were made, lack of other DNA structural features present within depleted introns could affect the efficiency of the splicing process. Thus, this HLA-A2 product could contain a cytoplasmic domain of 30–50 amino acids, with an amino acid sequence unrelated to those shown by HLA antigens. This high molecular weight HLA-A2 antigen did not affect the internalization assay, because this material is clearly distinguishable from the true HLA-A2Δ6,7 mutant.

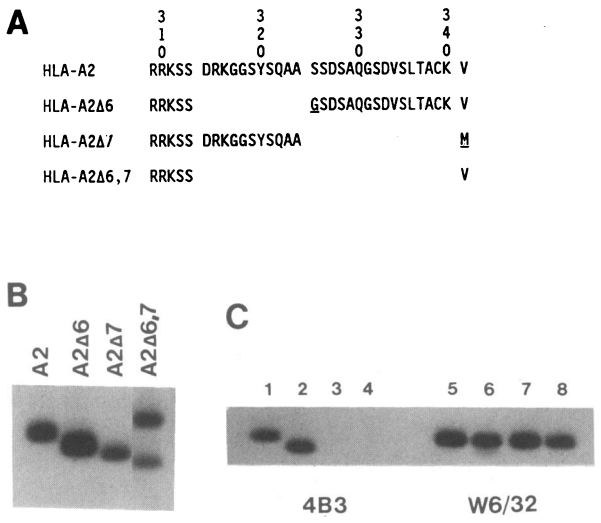

FIG. 3. HLA-A2 mutants. (*A*) Predicted cytoplasmic amino acid sequences. The amino acids (single-letter code) at position 325 in mutants HLA-A2Δ6 and HLA-A2Δ7 (underlined) are predicted to change as a result of the newly engineered junction between exons 5 and 7, and 6 and 8, respectively. Both amino acid changes are conservative and are not likely to affect the conclusions of this report. (*B*) Size analysis. ^{125}I-labeled HPB-ALL cells transfected with the HLA-A2 gene and the mutant HLA-A2Δ6, HLA-A2Δ7, and HLA-A2Δ6,7 genes were lysed, and the different HLA-A2 species were immunoprecipitated by using the antibody 4B3 and analyzed by SDS/PAGE. Only the bands corresponding to the heavy chains are shown. (*C*) Phosphorylation. HPB-ALL cells transfected with the HLA-A2 gene and the mutant HLA-A2Δ6, HLA-A2Δ7, and HLA-A2Δ6,7 genes were labeled with 1 mCi of carrier-free [^{32}P]orthophosphate as described (18), except treatment with phorbol 12-myristate 13-acetate was omitted. After lysing the cells, HLA-A2 antigens were immunoprecipitated by using the antibody 4B3. Lanes: 1, HLA-A2; 2, HLA-A2Δ6; 3, HLA-A2Δ7; 4, HLA-A2Δ6,7. Supernatants containing endogenous HLA antigens were then immunoprecipitated by using the antibody W6/32 (lanes 5–8).

Class I antigens are phosphorylated *in vivo* at serine residue 335 (encoded by exon 7) (5). Phosphorylation of the different HLA-A2 species was therefore examined to confirm the nature of the deletions (Fig. 3*C*). HLA-A2 and HLA-A2Δ6 molecules were phosphorylated to a similar extent, but neither HLA-A2Δ7 nor HLA-A2Δ6,7 was labeled. However, as expected, endogenous class I antigens were phosphorylated in all cell lines (Fig. 3*C*).

Internalization experiments (Fig. 4 *A–D*) showed that only HLA-A2 and HLA-A2Δ6 were internalized in a proportion comparable to that shown by endogenous HLA antigens present in HPB-ALL cells (Fig. 2*A*). For HLA-A2Δ7 and HLA-A2Δ6,7 the fraction of internalized molecules did not undergo any substantial increase when compared with the background levels obtained at 0 min. These observations were further confirmed by immunoprecipitating different aliquots with the antibody W6/32. This antibody recognizes the endogenous HLA antigens as well as the HLA-A2 molecules. HLA-A2Δ6, HLA-A2Δ7, and HLA-A2Δ6,7 can

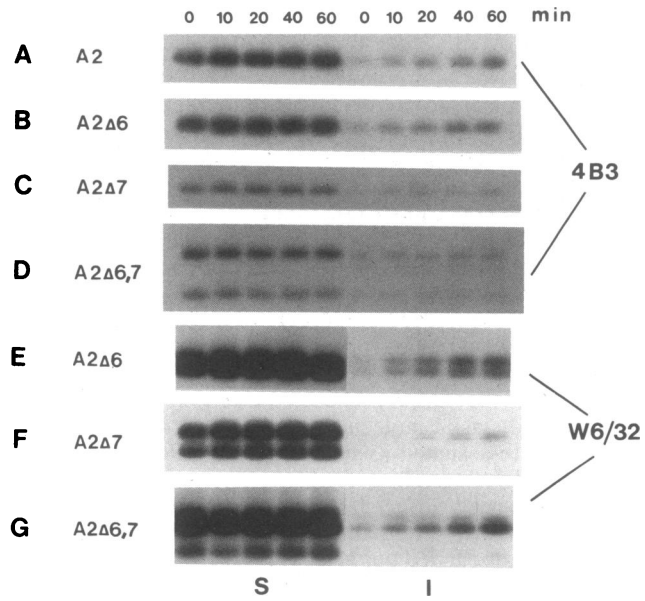

FIG. 4. Amino acids encoded by exon 7, but not exon 6, are necessary for internalization. Results of the internalization assays performed in HPB-ALL cells transfected with the HLA-A2 gene and the mutant HLA-A2Δ6, HLA-A2Δ7, and HLA-A2Δ6,7 genes. Immunoprecipitations were carried out by using the antibodies 4B3 (*A–D*) or W6/32 (*E–G*). HPB-ALL cells were transfected with the wild-type HLA-A2 gene (*A*), the HLA-A2Δ6 gene (*B* and *E*), the HLA-A2Δ7 gene (*C* and *F*), or the HLA-A2Δ6,7 gene (*D* and *G*). Only the bands corresponding to the heavy chains are shown. S and I, fractions derived from the cell surface or intracellularly, respectively.

be distinguished by size from the endogenous antigens. The endogenous HLA antigens thus provide an internal positive control for internalization. While in all cases endogenous HLA antigens were internalized, only the mutant HLA-A2Δ6 showed an internalization rate comparable to them (Fig. 4 *E–G*). It should be noted that the high molecular weight mutant of HLA-A2 was not internalized. A comparative densitometric analysis of the internalized fractions for the different HLA-A2 mutants and for the endogenous HLA antigens is shown in Fig. 5. The level of surface expression does not affect the internalization rate, since the noninternalized mutant HLA-A2Δ7 showed the same level of expression as the wild-type HLA-A2. Therefore, it can be concluded that amino acids encoded by exon 7 are necessary to provide the signals required for endocytosis of the HLA-A2 molecule, while those encoded by exon 6 are not required. As has been found for some cytoplasmic deletion mutants of LDL and transferrin receptors, the defect in endocytosis of the mutants HLA-A2Δ7 and HLA-A2Δ6,7 is probably due to their inability to concentrate in coated pits and to induce the formation of these structures (19, 20). Given the high degree of homology between different HLA antigens in their cytoplasmic regions (5), it is likely that this also applies to other class I antigens. Internalization-deficient HLA molecules, such as HLA-A2Δ7 and HLA-A2Δ6,7 will be valuable as negative controls for those experiments designed to shed light on the biological role of endocytosis of HLA antigens.

Cytoplasmic Domains of Molecules That Are Endocytosed via Coated Pits Share a Common Structural Feature. Proteins possess conserved signals that contribute to targeting them to specific cellular compartments, such as nucleus, mitochondria, and endoplasmic reticulum (4). Conserved structural features that mediate clustering of receptors in coated pits have not been discovered. However, for some molecules there are some data on the role of their cytoplasmic domains in endocytosis. Thus, for example, only cytoplasmic amino acids spanning the region 791–812 of the LDL receptor are required for endocytosis (21). Particularly interesting is tyrosine residue 807 that can only be substituted by an aromatic residue to keep internalization of the receptor (21). A deletion between cytoplasmic amino acids 6 and 41 impairs endocytosis of the transferrin receptor (22). The viral protein hemagglutinin is internalized via coated pits when cysteine residue 543 is substituted by tyrosine (23). It is noteworthy that the cytoplasmic portion of hemagglutinin is only 10 amino acids long. Change of tyrosine residue 501 to serine interferes with the internalization of the vesicular stomatitis virus G protein (22). Changing of a single tyrosine in the cytoplasmic region of the polyimmunoglobulin receptor impedes efficient endocytosis of this receptor (23).

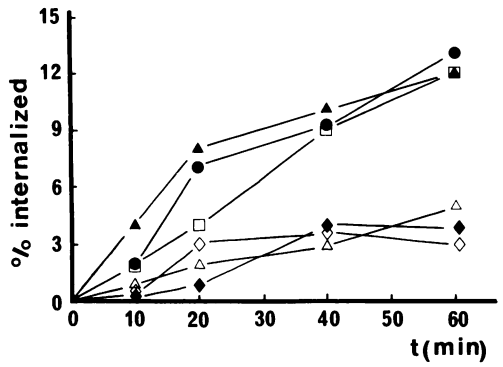

FIG. 5. Densitometric quantitation of internalization of endogenous HLA class I molecules in HPB-ALL cells (see Fig. 2*A*) and of transfected HLA-A2 and its mutants (Fig. 4 *A–D*). The percentage of internal molecules at time 0 (background level) was subtracted from the percentage of internal molecules at any given time. ●, HLA-A2; ▲, HLA-A2Δ6; △, HLA-A2Δ7; ◆, HLA-A2Δ6,7; ◇, HLA-A2 high molecular weight form obtained upon transfecting HPB-ALL cells with the HLA-A2Δ6,7 gene; □, endogenous HLA class I antigens.

In an attempt to find out whether molecules endocytosed via coated pits share any structural features, their cytoplasmic regions were compared. If such a conserved feature exists, cytoplasmic regions of endocytosed receptors should share some structural feature present in the 10-amino acid long cytoplasmic sequence of the hemagglutinin mutant that is endocytosed (23). Alignment of amino acid sequences was carried out with those available sequences and positions known to be involved in mediating endocytosis. Sequences of molecules that were compared included receptors for human LDL (24), human transferrin (25), bovine mannose 6-phosphate (26), rat asialoglycoprotein (27), and rabbit polyimmunoglobulin (28), as well as hemagglutinin mutant (23), vesicular stomatitis virus G protein (29), CD4 (30), and class I histocompatibility antigen HLA-A2 (Table 1). Amino acid residues numbered in this figure as +1 to +5 showed a striking homology, especially those between positions +1 and +4. Thus, residues designated at +1 are either an aromatic amino acid or glycine (mouse class I histocompatibility antigens have either threonine or serine at this position). Residues represented as +2 are partially polar and are strong hydrogen bond formers (glutamine, serine, and threonine). A one-amino acid gap was introduced between residues +1 (tyrosine) and +3 (arginine) in sequences of the hemagglutinin and polyimmunoglobulin receptor to maximize homology with the rest of the sequences. Residues numbered +3 are positively or negatively charged, and amino acids present at position +4 are either hydrophobic or threonine. Amino acids at position +5 are usually not hydrophobic. No other apparent sequence homology in the flanking regions could be seen. The presence of glycine instead of an aromatic residue at position +1 of HLA antigens could explain why internalization of these molecules is so low when compared with internalization of other molecules. Substitution of glycine for tyrosine at position +1 markedly reduces the rate of internalization of the LDL receptor (21).

Table 1. Cytoplasmic domains of molecules endocytosed via coated pits share a common structural feature

Molecule	Sequence		
	12345		
HA*	538 NGSLQY–RICL	547	(538–547)
LDLr (h)	802 FDNPVYQKTTEDEVHI	817	(790–839)
TRr (h, m)	15 GEPLSYTRFSLARQVD	30	(1–65)
VSV G	496 KKRQIYTDIEMNRLGK	511	(483–511)
HLA-A2 (h)	326 SDSAQGSDVSLTACKV	341	(309–341)
M6Pr (b)	204 PHLAFWQDLGNLVADG	219	(191–257)
AsGr (r)	1 TKDYQDFQHLDNEN	14	(1–38)
Pol Igr (rb)†	663 VSIGSY–RTDISMSDL	677	(653–755)
CD4 (h)	423 QCPHRFQKTCSPI	435	(396–435)

Sequences aligned are referenced in the text. Positions of amino acids (single-letter code) comprising their cytoplasmic regions are indicated, as well as those corresponding to the sequence segments displayed. Species from which the molecules listed were obtained are indicated as follows: h, human; m, mouse; r, rat; b, bovine; rb, rabbit. –, One-amino acid gaps introduced to maximize homology among the sequences compared. HA, hemagglutinin; LDLr, LDL receptor; TRr, transferrin receptor; VSV G, vesicular stomatitis virus G protein; MGPr, mannose 6-phosphate receptor; AsGr, asialoglycoprotein receptor; Pol Igr, polyimmunoglobulin receptor.

*Hemagglutinin sequence from a mutant that is endocytosed. It has tyrosine at position 543 instead of cysteine (23).

†The change of a single tyrosine (position in the sequence not indicated) within the cytoplasmic domain of the polyimmunoglobulin receptor impairs efficient endocytosis of this receptor (20). Two tyrosine residues occur within the cytoplasmic portion (28). Only the one here shows homology with the other receptors within the region numbered 1–5.

This five-amino acid long pattern was also found within the sequence of the IgE receptor beginning at amino acid 6 (residues Tyr-Ser-Glu-Ile-Glu) (31). T-cell antigens CD5 (32) and CD8 (33) do not have this pattern in their amino acid sequences, and neither is endocytosed (2, 34). The sequence is also absent in both the α and β chains of T-cell receptors (35, 36), which are known to be internalized (2). However, the associated polypeptides CD3 γ (37) and CD3 δ (38) clearly have this sequence at positions 149–153 (Tyr-Ser-His-Leu-Gly) and 139–143 (Tyr-Ser-His-Leu-Gln), respectively. Within the large cytoplasmic domains of the insulin and EGF receptors (39, 40), the pattern was located between positions 1322 and 1326 (Tyr-Thr-His-Met-Asn) and 944 and 948 (Phe-Ser-Lys-Met-Ala), respectively. Both of these segments are located outside their respective protein kinase domains. Class II histocompatibility antigens, which are internalized, at least in B lymphocytes, do not display the pattern within the sequences of their α and β chains (41). These molecules, however, are associated with a third polypeptide known as the invariant chain (42). This interaction could bypass the absence of the conserved segment present in the other endocytosed molecules. Alternatively, internalization of class II antigens might require another type of amino acid signal.

Some interesting characteristics of the conserved region are as follows: (*i*) It is relatively polymorphic; no two sequences display the same five amino acid residues. Nevertheless, hydrophobicity of their constituent amino acids is fairly conserved in all the sequences. This feature is analogous to what occurs in some sequence signals that mediate protein targeting to cellular compartments. (*ii*) It is not found at any common relative location within the cytoplasmic portion of the molecules compared. (*iii*) It seems to be very conserved in different mammalian species.

The 100/50-kDa accessory coat proteins (43, 44) have been proposed to promote coat assembly and coated pit formation by their interaction with receptor cytoplasmic domains (19, 45, 46). Our observation suggests that the conserved structural pattern found within the cytoplasmic domains of molecules endocytosed via coated pits could constitute a consensus structural signal that mediates internalization of receptors by interacting with the 100/50-kDa accessory coat proteins. This point is consistent with recent experiments that show that a peptide derived from the cytoplasmic domain of the endocytosed hemagglutinin mutant (23) competes with the cytoplasmic region of the LDL receptor in the interaction with a purified fraction of the 100/50-kDa accessory proteins (46). Apart from those residues comprising this putative consensus signal, other boundary sequences or amino acid residues could affect the avidity of those interactions. Polymorphisms for the 100/50-kDa coat proteins (44) and for the conserved receptor cytoplasmic sequences described would account for the distinct internalization rates displayed by the different molecular species endocytosed. If that were true, cell-specific expression and/or protein modification of accessory coat proteins could explain why internalization of class I histocompatibility antigens only takes place in certain cell types (e.g., T lymphocytes, macrophages) (8, 10). Site-directed mutagenesis of the cytoplasmic domains of different receptors, in combination with studies directed to examine the biochemical nature of the interaction between the endocytosed molecules and the 100/50-kDa coat proteins, would presumably allow testing the proposed hypothesis.

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