4F2 (CD98) Heavy Chain Is Associated Covalently with an Amino Acid Transporter and Controls Intracellular Trafficking and Membrane Topology of 4F2 Heterodimer

Title

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Citation
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Issue Date
1999-05-24

URL
https://doi.org/10.11501/3152544

Type
Thesis or Dissertation

Textversion
author

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(4F2抗原H鎖はアミノ酸トランスポートと会合し、細胞内輸送及び細胞膜における4F2抗原ヘテロダイマー形成を制御している。)
4F2 (CD98) Heavy Chain Is Associated Covalently with an Amino Acid Transporter and Controls Intracellular Trafficking and Membrane Topology of 4F2 Heterodimer


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Running title: Intracellular Trafficking and Membrane Topology of CD98

8 Figures and no Tables

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SUMMARY

4F2, also termed CD98, is an integral membrane protein consisting of a heavy chain linked to a light chain by disulfide-bond. We have generated a monoclonal antibody to the mouse 4F2 light chain and cloned the cDNA. It encodes a mouse counterpart of rat L-type amino acid transporter-1, and induces system L amino acid transport in Xenopus oocytes in the presence of 4F2 heavy chain. Transfection studies in mammalian cells have indicated that the 4F2 heavy chain is expressed on the plasma membrane on its own, whereas the 4F2 light chain can be transported to the surface only in the presence of 4F2 heavy chain. 4F2 heavy chain is expressed diffusely on the surface of fibroblastic L cells, while is localized selectively to the cell-cell adhesion sites in L cells expressing cadherins. These results indicate that the 4F2 heavy chain is associated covalently with an amino acid transporter and controls the cell surface expression as well as the membrane topology of the 4F2 heterodimer. Although 4F2 heavy and light chains are expressed coordinately in most tissues, the light chain is detected barely by the antibody in kidney and intestine, despite the presence of heavy chain in a complex form. The results predict the presence of multiple 4F2 light chains.
INTRODUCTION

4F2 antigen, also called CD98, has been originally identified as an activation antigen of lymphocytes (1). It is known to be rather ubiquitously expressed in many types of cells and notably in almost all tumor cell lines (2). Early biochemical studies have revealed that 4F2 antigen is a heterodimer consisting of a type 2 glycosylated integral membrane protein of around 80 kDa (heavy chain, hc) and a protein with apparent MW of 37 kDa (light chain, lc) linked by disulfide-bond (2, 3). Although 4F2 hc cDNA has been previously cloned (4, 5), 4F2 lc remained unidentified. 4F2 hc shares some 30% homology with a broad-specificity amino acid transporter (BAT), which activates system $\text{b}^{0,+}$-like amino acid transport (6, 7), and is shown to induce system $\text{y}^+\text{L}$ amino acid transport when expressed in Xenopus oocytes (8, 9). Since 4F2 hc has been indicated subsequently to induce multiple amino acid transport systems (10), there has been speculation that 4F2 hc is a specific activator of the transport systems rather than a carrier (11). Besides its relation to amino acid transport, a variety of functional implications have been made on 4F2. For instance, anti-4F2 hc antibody has been reported to inhibit growth of some tumor cells (12) and hematopoietic progenitor cells (13). Also it was indicated to be involved in the virus-induced syncytium formation as well as cell fusion of normal monocytes in the absence of virus infection (14, 15). More recently, 4F2 hc has been shown to reverse the dominant negative effect of overexpressed cytoplasmic domain of $\beta 1$ integrin on the ligand-affinity of integrin (16). Although these results imply the involvement of 4F2 antigen in diverse cellular activities, exact mechanisms underlying them remain unknown.

In the present study, we first have generated a monoclonal antibody to mouse 4F2 lc, and isolated the 4F2 lc cDNA by expression cloning using it. 4F2 lc consists of 512 residues, and is predicted to be a very hydrophobic protein with 11 or possibly 12 membrane-spanning regions. GenBank search has revealed that the cDNA is a mouse counterpart of the most recently reported rat gene termed L-type amino acid transporter-1, LAT1. LAT1 cRNA could induce system L amino acid transport in Xenopus oocytes in the presence of rat 4F2 hc, and has been suggested to be a 4F2 lc (17). We have confirmed that the mouse 4F2 lc induces high affinity amino acid transport with features of system L in the presence of mouse 4F2 hc, and have proved that it is associated covalently with 4F2 hc by a disulfide-bond via cysteine at position 103 of the latter. The 4F2 hc has been indicated to be expressed on the cell surface as a monomer on its own, while 4F2 lc is transported to the plasma membrane only in the presence of 4F2 hc. 4F2 hc is expressed on the epithelial cell surface of most embryonic tissues in vivo, and the analysis on cultured cells has indicated further that 4F2 hc is expressed selectively at cell-cell adhesion sites generated by cadherins. The present results thus reveal a critical role of 4F2 hc in the control of intracellular trafficking as well as the cell surface topology of the 4F2 heterodimer, and provide a new clue to delineate the mechanisms for its involvement in diverse cellular functions. We also present the results predicting the presence of additional 4F2 lc(s) that is distinct from LAT1 in some normal epithelial tissues such as kidney and intestine.
Antibodies and cell lines--Anti-4F2 he monoclonal antibody (mAbs), 14.37, has been reported previously (3). To raise anti-4F2 mAbs for multiple purposes, Armenian hamsters were immunized with pooled SDS-PAGE gel slices corresponding to the 80 kDa 4F2 he or 37 kDa lc from the P3U1 cell lysates that had been immunoprecipitated with 14.37 mAb. Hybridoma supernates were screened by two independent assays: immunoprecipitation of l25I-labeled cell lysates immunoprecipitated with 14.37 mAb. Hybridoma supernates were screened by two independent assays; immunoprecipitation of l25I-labeled cell lysates, and immunoblotting of the cell lysates immunoprecipitated with 14.37 mAb. By these procedures, two additional anti-4F2 he mAbs were obtained: 10.10 mAb, capable of efficiently immunoprecipitating the 4F2 heterodimer from P3U1 cells as well as the 4F2 he expressed by eDNA transfection, and 10.4 mAb, effective for the detection of 80 kDa 4F2 he by immunoblotting. The 10.10 mAb could be used for immunoprecipitation, immunostaining and immunohistochemistry, while the 10.4 mAb for immunoblotting. Another mAb, 10.7, was also capable of immunoprecipitating the 4F2 heterodimer from P3U1 cells. It specifically reacted to the 37 kDa band by the immunoblotting, and was indicated to recognize the 4F2 he (see also text). The 10.7 mAb has been shown to stain the cells only after the permeabilization, suggesting that its epitope is in the cytoplasmic region. Anti-E-cadherin (ECCD-2) was purchased from Takara Co., Ltd., Kyoto, Japan, and anti-N-cadherin (NCD-2) was provided by Dr. Takeichi, Kyoto University, Kyoto, Japan. Anti-Myc antibody (9E10) was purified in our laboratory. L cells and those stably transfected with E-cadherin (EL) and N-cadherin eDNA (NL) were also provided by Dr. Takeichi. All cell lines were maintained in Dulbecco's modified Minimal Essential Medium supplemented with 10% fetal calf serum.

cDNA transfection and expression cloning-- cDNA library of BAL 17.2 mouse B lymphoma cells was constructed in the expression vector pPISC (Iwai et al., unpublished), and COS cells were transfected with the cDNA library (10 μg/5x10^6 cells) by electroporation. Cells were harvested by trypsinization three days after the transfection, and fixed and permeabilized by Fix and Parn (Caltag, So. San Francisco, CA), as instructed by manufacturer. Cells were stained with 10.7 mAb followed by fluorescein isothiocyanate (FITC) -conjugated anti-hamster IgG (Caltag, So. San Francisco, CA). Positively stained cells were collected by cell sorting with FACS Vantage (Becton Dickinson, Mountain View, CA). Episomal plasmids were directly recovered from such collected cells by the method described by Davis et al. (18).

Briefly, the sorted cells were treated with buffer containing 100 mM EDTA, 10 mM Tris-Cl pH 8.0, 0.1% SDS and 100 μg/ml of proteinase K at 55 °C for overnight followed by phenol/chloroform extraction and ethanol precipitation. Samples were suspended in 2 μl water and transformed into bacteria. Plasmids were purified from liquid culture of bacteria and again transfected into COS cells. After 3 cycles of the procedure, a single plasmid clone, p10.7, was isolated and sequenced. The cDNA transfection into COS and HeLa cells was done using electroporation and CaPO4 method respectively.

Plasmid Construction -- 4F2 he cDNA tagged with Myc epitope at the C-terminus was constructed by subcloning synthetic oligonucleotides encoding the epitope tag and a part of cDNA into 3' end of the cDNA. Single residue mutants of 4F2 he (cysteine at position 103 being substituted for serine, C103S, and cysteine at 325 for serine, C325S) were constructed by the two-step PCR and confirmed by DNA sequencing. For cRNA synthesis, cDNAs of both 4F2 he (3) and 4F2 he were subcloned into pSP73 vector (Promega, Madison, WI). After linearizing the plasmids with Xho-I, cRNAs were synthesized by using mMESSAGE, mMACHINE SP6 kit (Ambion, Austin, TX), as instructed by the manufacturer.

Immunoprecipitation, immunoblotting and Northern blotting-- Cells either unlabeled or surface labeled with biotin using biotin-XX succinimidyl ester (Molecular Probes, Eugene, OR) were lysed with a lysis buffer (1% NP-40, 50 mM Tris-Cl pH 7.4, 0.15 M NaCl, 10 mM EDTA, PMSF, leupeptin, antipain, chymostatin trypsin inhibitor), incubated with antibodies (2-5 μg) at 4 °C for 3 hours, and then precipitated with protein.
A-Sepharose 4B (Amersham-Pharmacia Biotech, Uppsala, Sweden) at 4 °C for 30 min. Lysates were electrophoresed in SDS-PAGE, blotted on polyvinylidene difluoride (PVDF) membranes, incubated with antibodies followed by horseradish peroxidase (HRP) -conjugated second antibodies or with avidin biotin complex (ABC) reagent (Vector, Burlingame, CA) for biotinylated samples, and developed using a Supersignal Western blotting detection system (Pierce, Rockford, IL). Northern blotting was done as described previously (3).

Immunofluorescence staining and immunohistochemistry—Cells were cultured on the cover slips, rinsed with TBS+ (Tris buffered saline, pH 7.4, supplemented with 10 mM CaCl2), fixed with 3 % formaldehyde in TBS+ and blocked with 2 % BSA in TBS+. For double staining, the cells were incubated with anti-4F2 h c mAb (10.10) and anti-E-cadherin or anti-Myc for 1 hour at room temperature, and then with biotin-conjugated goat anti-hamster IgG (Caltag, So. San Francisco, CA) and FITC-conjugated rabbit anti-rat IgG or anti-mouse IgG (Caltag, So. San Francisco, CA) for 1 hour at room temperature followed by Texas red-avidin (Biomeda, Foster City, CA). The samples were dried, mounted in ProLong antifade kit (Molecular Probe, Eugene, OR), and analyzed with a confocal laser microscopy (Olympas, Osaka, Japan). Immunohistochemistry was performed as described before (19). Briefly, whole embryos (E14) were fixed with 4 % paraformaldehyde at 4 °C for 30 min. Frozen sections at 10-16 mm thickness were preblocked, incubated with 10.10 mAb, and then with biotin-conjugated goat anti-hamster IgG followed by ABC kit.

Measurement of amino acid uptake in Xenopus oocytes—Amino acid uptake was measured as described (6) with slight modifications. Briefly, five to seven Xenopus oocytes per condition were washed twice in amino acid-free uptake solution (100 mM choline chloride, 2 mM KCl, 1 mM MgCl2, 1 mM CaCl2, and 10 mM Hepes pH 7.5). Oocytes injected with cRNAs or water as a control were incubated with 200 µl uptake solution containing 50 µM radiolabeled amino acids at 370 KBq/ml for 30 min at 25 °C.

Amino acid competition experiments were performed by adding 5 mM inhibitors to the uptake solutions. After incubation, oocytes were washed 5 times with 1 ml ice-cold wash solution (80 µM choline chloride, 20 mM L-arginine, 20 mM L-leucine, 2 mM KCl, 1 mM MgCl2, 1 mM CaCl2, and 10 mM Hepes pH 7.5). Each oocyte was then transferred to a vial, dissolved with 200 µl 10 % SDS followed by addition of 3 ml scintillation solution for scintillation counting.
RESULTS

4F2 heavy chain is associated covalently with a system L amino acid transporter by disulfide bond.-- An anti-mouse 4F2 monoclonal antibody (mAb), 10.7, was produced, that was capable of immunoprecipitating a band at 120 kDa in nonreducing condition and two bands at 80 kDa and 37 kDa positions in reducing condition from the surface-labeled P3U1 cell lysates (data not shown). When the immunoprecipitate of P3U1 lysate with either anti-4F2 he (14.37) or 10.7 mAb was blotted with 10.7, a 37 kDa band was detected (Fig. 1, A, left), and conversely, immunoprecipitation with 10.7 resulted in the coprecipitation of 80 kDa 4F2 he (Fig. 1, A, right). The results indicate that 10.7 mAb recognizes the light chain of 4F2 heterodimer. We then isolated a 4F2 lc cDNA, P3U1, by expression cloning using the mAb as described in the Experimental Procedures. When COS cells were transfected with either 4F2 he (p14.37, ref. 3) or p10.7 cdNA, a 80 kDa or 37 kDa band was immunoprecipitated respectively only with the corresponding mAb as expected. On the other hand, when COS cells were cotransfected with both cDNAs, anti-4F2 he mAb could immunoprecipitate the 37 kDa band reactive to 10.7 mAb in addition to the 80 kDa 4F2 he band (Fig. 1, B). Since 4F2 he has only two cysteines at positions 103 and 325 in the extracellular region (5), a single residue mutation for each cysteine to serine was introduced, C103S and C325S, and cotransfected with p10.7 cdNA. As also shown in Fig. 1, B, the C103S mutation of 4F2 he abrogated the coprecipitation of the 37 kDa band by anti-4F2 he mAb, while the C325S mutation did not. These results have proved that the cdNA indeed encodes the 4F2 he.

The p10.7 cdNA consists of 3456 bp and contains an ORF (nt. 27 to 1565) encoding 512 residues (DDBJ accession number AB17189). The deduced amino acid sequence is highly homologous (98% identity) to the recently reported rat LAT1 (17). The hydrophobicity profile shown in Fig. 2, A predicts at least 11 and possibly 12 helical transmembrane domains. As shown in Fig. 2, B, injection of 4F2 lc cRNA alone into Xenopus oocytes induced negligible Na+-independent uptake of Leu or Arg, while 4F2 hc cRNA induced Arg uptake as reported previously (8, 9). When both cRNAs were coinjected, potent Na+-independent Leu uptake was induced while Arg-uptake tended to be suppressed as compared with that induced by 4F2 hc cRNA alone. The Leu uptake in the double cRNA transfectants was almost completely inhibited by Ile, Val, His, Phe as well as by 2-(+)-endoamino-bicycloheptane-2-carboxylic acid (BCH), a specific inhibitor of system L transport. A kinetic study revealed that the Na+-independent Leu uptake was saturable and high affinity, the Km being calculated to be around 25 μM (Fig. 2, B).

4F2 he guides the 4F2 lc to plasma membrane, which is independent of disulfide linkage.-- We then examined the intracellular trafficking of each protein. COS cells transfected with either 4F2 lc or hc cdNA alone, or with both, were surface labeled with biotin, lysed, and immunoprecipitated with anti-4F2 he or lc mAb followed by the detection of biotinylated proteins with ABC system. As controls, aliquots of the same cell lysates (one fourth) were immunoprecipitated similarly and blotted with the corresponding mAbs. The level of biotinylated 4F2 lc was found to be marginal as compared with the total 4F2 lc in the lc single transfectants, while the vast majority of 4F2 lc was estimated to be expressed on the cell surface in the hc/lc double transfectants (Fig. 3, A). In contrast, comparable levels of biotinylated 4F2 hc were detected in both hc single and hc/lc double transfectants (Fig. 3, A). Biotinylated 4F2 hc in the former was detected as a monomer without covalently-associated molecule as 4F2 hc (C103S) (Fig. 3, B), eliminating the possibility that mouse 4F2 he was associated with the endogenous 4F2 lc in COS cells and expressed on the cell surface. 4F2 hc (C103S), that failed to form disulfide-linkage with 4F2 lc, however, was capable of inducing cell surface expression of 4F2 lc as efficiently as wild type 4F2 hc (Fig. 4, A). These results were confirmed by immunofluorescence staining. When 4F2 hc or lc cdNA was singly transfected into HeLa cells, 4F2 hc was expressed on the cell surface, while 4F2 lc was remained mostly in the cytosol particularly in the Golgi area (Fig. 4, a vs. b). With the cotransfection of 4F2 hc and lc cdNAs, on the other hand, 4F2 lc was...
expressed on the cell surface with the same pattern as 4F2 hc (Fig. 4, c vs. d). An essentially similar effect was obtained by the cotransfection with 4F2 hc (C103S) cDNA as well (Fig. 4, e vs. f). These results indicate that 4F2 hc functions as a "guidance molecule" for 4F2 lc to the plasma membrane, for which the covalent linkage by a disulfide bond is not essential.

4F2 hc and 4F2 lc are coordinately induced in normal lymphocytes following activation. In normal mouse lymphocytes, the 4F2 lc transcript is induced rapidly following the mitogenic stimulation in vitro with Con A in a coordinated manner with that of 4F2 hc (Fig. 5, A). Also both transcripts are expressed in all leukemic cell lines examined and with similar relative intensities (data not shown). In Fig. 5, B, expression profiles of 4F2 hc and 4F2 lc transcripts in normal adult organs are shown. Although both mRNAs are expressed rather ubiquitously, the level of 4F2 lc mRNA appears to be disproportionately low as compared with that of 4F2 hc in kidney, small intestine, and liver (see below).

4F2 hc is sorted specifically to the cell-cell adhesion sites generated by cadherins—The expression pattern of 4F2 on the cells was then investigated. In OTF9 embryonic carcinoma cells, endogenous 4F2 hc was found to be located selectively at the cell-cell adhesion sites (Fig. 6, b). Since the distribution was found to be nearly identical to that of E-cadherin (Fig. 6, a), we intended to examine directly the effect of cadherins on 4F2 hc distribution at the cell surface using fibroblastic L cells and those stably transfected with E-cadherin (EL) or N-cadherin (NL). In L cells, 4F2 hc was stained diffusely on the surface and E-cadherin was undetectable (Fig. 6, c, d). In EL and NL cells, which exhibited significant cell-cell adhesion, 4F2 hc was concentrated at the cell-cell adhesion sites and colocalized with the cadherins (Fig. 6, e, f and g, h). Immunoprecipitation analysis revealed that 4F2 hc was associated with 4F2 lc in all these cells (data not shown). These results thus suggest that 4F2 hc is sorted specifically to the cell-cell adherent membrane sites, most likely together with 4F2 lc, once the stable cell adhesion is generated by cadherins.

Expression of 4F2 hc in various normal tissues: Localization at cell-cell adhesion sites in polarized epithelial cells and implication for multiple 4F2 lc(s).—Finally, we examined the cellular localization of 4F2 hc in various normal tissues. Immunohistochemistry of mouse embryos has indicated that 4F2 hc is expressed on the surface of epithelial cells of most tissues, including epidermis (Fig. 7, a), the choroid plexus in the brain (b), retina (c), as well as intestinal (d), renal (e), and thymic epithelium (f). In polarized epithelial cells such as in intestine and kidney, 4F2 hc expression is restricted apparently at the lateral adhesion sites (Fig. 7, d, e), consistent with the colocalized expression with E-cadherin in cell lines. We wished to know whether 4F2 hc on the cell surface of these tissues is associated with the 4F2 lc. Thus far, the only available anti-4F2 lc antibody, 10.7 mAAb, worked poorly in immunohistochemistry. Therefore immunoblotting analysis was performed. The 4F2 hc is expressed in all embryonic and adult tissues examined (Fig. 8, A). The 4F2 lc, however, is detected barely in the kidney, intestine and adult liver, while it is expressed strongly in others including brain, testis, and spleen as well as fetal liver (Fig. 8, A). Nonetheless, the 80 kDa 4F2 hc in kidney and intestine is detected still as a 120 kDa complex in nonreducing condition (Fig. 8, B), strongly implying that the 4F2 hc forms complex with 4F2 lc(s) that is distinct from the LAT1 in these polarized epithelial tissues.
DISCUSSION

In the present study, we have generated a monoclonal antibody to the mouse 4F2 lc and cloned its cDNA. The deduced amino acid sequence has revealed that 4F2 lc consists of 512 residues with at least 11 or possibly 12 helical transmembrane domains, calculated MW being 56 kDa. Our unpublished results indicate that both N- and C-terminal ends of the coding region are retained in the 4F2 lc, and thus much faster migration of 4F2 lc in SDS-PAGE at 37 kDa position appears to be due to the intrinsic structural features (Yang and Minato, unpublished observations). 4F2 lc is highly homologous (98 % identity) to the very recently reported rat LAT1 (17) and thus considered to be its mouse counterpart. Rat LAT1 has been shown to induce system L amino acid transport in Xenopus oocytes in the presence of 4F2 he. Based on the functional dependence on 4F2 hc, LAT1 is suggested to be a 4F2 lc. Our present results have confirmed that the mouse 4F2 lc can mediate amino acid transport with typical features of high affinity system L when expressed in Xenopus oocytes together with the 4F2 hc. We have indicated further that the 4F2 lc is associated covalently with 4F2 hc in the cells by a disulfide-bond via cysteine at position 103, which is conserved in the 4F2 hc of mouse, rat and human (4, 5). Thus, it has been proved that 4F2 hc is associated covalently with a system L amino acid transporter.

We then addressed the molecular basis for the functional dependence of 4F2 lc on 4F2 hc. Present results have indicated that 4F2 hc alone is expressed efficiently on the cell surface as monomer. In contrast, 4F2 lc is expressed minimally at the plasma membrane in mammalian cells, remaining mostly in the Golgi area, and requires 4F2 hc to be sorted to the cell surface. The results thus indicate that one of the functions of 4F2 hc is to guide 4F2 lc to the plasma membrane. Rather unexpectedly, the guidance effect is independent of disulfide-linkage, implying the involvement of noncovalent steric association. A similar mechanism has been proposed for the heterodimeric P-type cation-exchange ATPases, in which a β-subunit is responsible for the correct intracellular trafficking of α/β heterodimeric holoenzymes from ER to the cell surface (20).

Amino acid permeases in lower eukaryocytes are expressed usually as monomeric proteins (21). Membrane expression of permeases in yeast, however, is shown to be controlled by a unique ER-resident protein, SHR3, without which the transport of permeases from ER to plasma membrane is impaired selectively (22). In this aspect, it is noted that a mutation of BAT (M467T), most commonly detected in the patients of type I cysteineuria, results in the defective expression of BAT protein on the cell surface (23). The BAT is reported also to be associated with an as yet undefined 30 kDa protein in the kidney cells (24). It thus seems possible that 4F2 hc / BAT family, often called "transport-related" proteins, represents specific "guidance molecules" for selected amino acid transporters to the plasma membrane in mammalian cells. At present, it remains to be seen whether 4F2 hc has additional functions as an integral part of the transport carrier.

In normal mouse embryos, 4F2 hc has been shown to be expressed prominently in the epithelial cells of most tissue, in addition to the vascular and lymphohematopoietic cells. In the polarized epithelial cells such as in kidney and intestine, 4F2 hc expression appears to be restricted at the lateral sites, which primarily depended on cadherins (25). Indeed, immunofluorescence analysis of OTF9 embryonic carcinoma cells in culture has indicated clearly that the 4F2 hc is expressed selectively at the cell-cell adhesion sites and colocalizes with E-cadherin. Furthermore, in L cells stably transfected with E- or N-cadherin cDNA, 4F2 hc is expressed at the cell-cell adhesion sites while it is expressed diffusely on the surface of L cells without cadherin, indicating that the membrane topology of 4F2 hc is regulated by cadherins. Our unpublished results have indicated that E-cadherin is coimmunoprecipitated with 4F2 heterodimer from OTF9 cells lysed with mild detergents, suggesting that 4F2 complex is included in the membrane domain generated by E-cadherin (Suga and Minato, unpublished observation). Similar cell-cell adhesion-dependent restriction of the cell surface topology has been reported for Na+/K+-ATPase and Cl⁻/HCO₃⁻ channel (26, 27).

Our present results have implicated also the presence of multiple 4F2 lc (s) that are associated covalently with 4F2 hc. In cells of most tissues including lymphoid cells and
most cancer cells, both 4F2 he and lc are detected at comparable levels. In the intestine and kidney, however, 4F2 lc is detected barely by the 10.7 mAb. Nonetheless, the 4F2 he is present mostly as a 120 kDa complex rather than a 80 kDa monomer form also in these tissues, suggesting the presence of distinct 4F2 lc (s). Molecular heterogeneity in the system L transport activity has been previously demonstrated (28). Since 4F2 he in these polarized epithelial cells is localized selectively on the lateral, but not apical, surface, the yet undefined 4F2 lc (s) may be expected to exhibit unique functions, directional solute transports for instance.

The 4F2 antigen has been suggested to be involved in a wide variety of cellular functions, including cellular growth (12, 13), virus-induced cell aggregation and fusion (14, 15), and affinity regulation of β1-integrins (16). It has been reported also that anti-4F2 he antibodies affect the Ca2+-influx in sarcolemmal vesicles and parathyroid cell lines (29, 30). Although system L transport of the 4F2 heterodimer should play certainly important roles in proliferative cells including tumor cells in order to meet critical nutritional requirements, the relation of it to many other suspected functions remains obscure. Further studies on the guidance mechanisms of 4F2 he for 4F2 lc (s) and possibly other proteins, with or without covalent linkage, to the cell surface as well as the analysis on the mechanisms for regulation of membrane topology of 4F2 heterodimer by cell adhesion molecules might provide new clues to delineate the multiple functions of 4F2 antigen in various cell types.

Acknowledgments-- We are grateful to Drs. M. Takeichi and Y. Minami for valuable discussion and providing cells, and Dr. M. Maeda for the technical assistance. Proofreading of the manuscript by Dr. L. Reid is also appreciated. This work was supported by grants from the Ministry of Education and Science, Japanese Government.

REFERENCES

FOOTNOTES

Abbreviations used in the paper—ABC, avidin biotin complex; BAT, broad-specificity amino acid transporter; ConA, concanavalin A; ER, endoplasmic reticulum; FITC, fluorescein isothiocyanate; 4F2 hc and lc, 4F2 heavy chain and light chain; LAT1, L-type amino acid transporter 1; mAb, monoclonal antibody; MW, molecular weight.

The cDNA sequence data reported in the manuscript has been submitted to the GenBank databases under accession number AB17189.

FIGURE LEGENDS

Fig. 1. A monoclonal antibody (10.7) specific to 4F2 lc. A. P3U1 cell lysate was immunoprecipitated with control hamster IgG, anti-4F2 hc (10.10) or 10.7 mAb, electrophoresed in SDS-PAGE, and immunoblotted with another anti-4F2 hc (10.4) or with 10.7 mAb. B. COS cells were transfected with p10.7, 4F2 hc cDNA (p14.37), or with p10.7 combined with p14.37, p14.37 (C1035), or p14.37 (C3255) in a pSRα expression vector by electroporation. The cells were harvested three days after the transfection, lysed in a lysis buffer containing 1% NP-40, and immunoprecipitated followed by immunoblotting at the indicated combinations of mAbs.

Fig. 2. 4F2 lc is a multimembrane-spanning protein and mediates system L amino acid transport in the presence of 4F2 hc in Xenopus oocytes. A. Hydrophobicity profile of the deduced amino acid sequence of 4F2 lc cDNA (Kyte-Doolittle program). B. (Left panel) Oocytes were injected with cRNA (2.5 ng/egg) of 4F2 lc (lc), 4F2 hc (hc), or both (lc + hc), and assayed for the uptake of indicated amino acids 3 days after the injection. Control oocytes (-) received water. The uptake of amino acids was measured by incubating oocytes with 50 μM indicated radiolabeled amino acids for 30 min at 25 °C in the uptake solution containing 100 mM choline chloride in place of NaCl. Means and SD of 5 to 7 oocytes are indicated. (Center panel) The Na+-independent L-leucine uptake was determined as above in the presence of 5mM indicated inhibitors using the oocytes that had been injected with both 4F2 lc and 4F2 hc cRNAs (2.5 ng each) 3 days before. Means and SD of 5 to 7 oocytes are indicated. (Right panel) Oocytes that had been injected with both 4F2 lc and 4F2 hc cRNAs (2.5 ng each) 3 days before were incubated with varying concentrations of L-leucine for 30 min and the uptake was measured as above. The mean baseline uptake of control oocytes was subtracted from that of cRNA-injected oocytes at each concentration. Inset: Eadie-Hofstee plot of the data.
Fig. 3. 4F2 he guides the intracellular trafficking of the 4F2 lc to the plasma membrane independently of a disulfide-linkage. A. COS cells were transfected with 4F2 lc cDNA, 4F2 he cDNA, or with 4F2 lc together with 4F2 he or 4F2 he (C103S) cDNA by electroporation. The cells were harvested three days later, surface biotinylated, lysed in a lysis buffer, immunoprecipitated with anti-4F2 lc or he mAb, and electrophoresed in SDS-PAGE. The biotinylated proteins were detected with ABC kit. One fourth of each sample was similarly electrophoresed and immunoblotted with corresponding mAbs to estimate the total amounts of expressed proteins. B. COS cells were transfected as above. After the surface biotinylation, the lysates were immunoprecipitated with anti-4F2 he, and electrophoresed in SDS-PAGE at either reducing (left panel) or non-reducing (right panel) condition. The bitinylated proteins were detected with ABC kit. (left panel) open arrow head; 4F2 he, closed arrow head; coprecipitated 4F2 lc (right panel) open arrow head; 4F2 he in complex form, closed arrow head; 4F2 he monomer.

Fig. 4. 4F2 he guides the intracellular trafficking of 4F2 lc to the plasma membrane independently of disulfide-linkage -- Immunostaining analysis. HeLa cells were transfected with 4F2 he cDNA (a), 4F2 lc cDNA tagged with Myc epitope at the C-terminus (b), 4F2 he and Myc-4F2 lc cDNAs (c, d), or with 4F2 he (C103S) and Myc-4F2 lc cDNAs (e, f) by CaPO4 method. Three days later, the cells were fixed and stained with biotin-conjugated anti-4F2 he followed by Texas red-avidin (a), or anti-Myc mAb followed by FITC-conjugated anti-mouse IgG (b). For the double transfectants, the cells were double-stained with anti-4F2 he (c, e) and anti-Myc (d, f) as above. The pictures (c) and (d) as well as (e) and (f) represent the same fields respectively.

Fig. 5. Comparable expression profiles of the 4F2 he and 4F2 lc transcripts in normal tissues. A. Normal adult BALB/c spleen cells were cultured in the presence of ConA (2 μg/ml) for varying periods. Total RNA was extracted from the cells, and blotted using 32P-labeled cDNA probes of 4F2 he, 4F2 lc, and β-actin.

Fig. 6. 4F2 he is expressed selectively at the cell-cell adherent sites and colocalizes with cadherins. OTF9 cells (a, b), L cells (c, d), EL cells (e, f), and NL cells (g, h) were cultured on cover slips, fixed, and double-stained with anti-E-cadherin (a, c, e) or anti-N-cadherin (g) and biotin conjugated anti-4F2 he (b, d, f, h) followed by FITC-conjugated anti-rat IgG and Texas red-avidin. The stained cells were analyzed with a confocal laser microscopy.

Fig. 7. Expression of 4F2 he in normal embryonic tissues. Sections of fixed whole embryos (E14) of BALB/c mice were stained with anti-4F2 he mAb (10.10) followed by biotin-conjugated anti-hamster IgG and detected with a ABC kit. a; skin, (x200) b; choroid plexus in brain, c; retina, d; intestine, e; kidney, f; thymus (x400).

Fig. 8. Dissociated expression of the 4F2 he and 4F2 lc (LAT1) in kidney, intestine and liver -- Implication for the multiple 4F2 lc (s). A. Various organs from E18 embryos (E18) and adult (A) mice were homogenized, extracted in a lysis buffer, electrophoresed in SDS-PAGE in reducing condition, and immunoblotted with anti-4F2 he (10.4) and anti-4F2 lc (10.7) mAbs. B. Tissue extracts of indicated organs from adult mice were electrophoresed in SDS-PAGE in either non-reducing or reducing condition, and immunoblotted with anti-4F2 he mAb.
**A**

Hours after Con A stimulation

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**B**

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- 4F2 hc
  - 80 kDa
- 4F2 lc
  - 37 kDa