CD44 exhibits a cell type dependent interaction with Triton X-100 insoluble, lipid rich, plasma membrane domains

Stephen J. Neame*, Caroline R. Uff, Humma Sheikh, Susan C. Wheatley and Clare M. Isacke†
Department of Biology, Imperial College of Science, Technology and Medicine, Prince Consort Road, London SW7 2BB, UK
*Present address: Eisai London Research Laboratories Ltd, Bernard Katz Building, University College London, Gower Street, London WC1E 6BT, UK
†Author for correspondence (e-mail: c.isacke@ic.ac.uk)

SUMMARY

CD44 is an abundant, widely expressed transmembrane glycoprotein which can act as a receptor for the extracellular matrix glycosaminoglycan, hyaluronan. Biochemical and morphological studies have demonstrated that in fibroblasts a significant proportion of the CD44 population is resistant to Triton X-100 extraction and that the detergent insoluble protein is co-localized with components of the cortical cytoskeleton. Surprisingly, this distribution is not abrogated upon deletion of the CD44 cytoplasmic tail indicating that mechanisms other than a direct interaction with the cytoskeleton can regulate CD44. In this manuscript, the mechanisms underlying this detergent-insoluble association are further investigated. There was no evidence that the Triton X-100 insolubility of CD44 resulted from homotypic aggregation, an association with hyaluronan or from a direct, or indirect, association with the cytoskeleton. Instead, evidence is presented that the detergent insolvability of fibroblast CD44 at 4°C results from an association of the CD44 transmembrane domain with Triton X-100 resistant, lipid rich, plasma membrane domains. The proportion of the CD44 found in these Triton X-100 insoluble structures is dependent upon cell type and cannot be altered by changing cell motility or extracellular matrix associations. These studies provide evidence for a novel mechanism regulating this adhesion protein in the plasma membrane.

Key words: CD44, detergent solubility, transmembrane domain, plasma membrane

INTRODUCTION

Currently there is intense interest in the mechanisms by which adhesion proteins are involved in both outside-in signalling, in which interactions of the adhesion protein with the extracellular milieu influence intracellular events, and inside-out signalling in which intracellular events leads to modulation of the adhesion protein extracellular activity. CD44 is a hyaluronan receptor which has been implicated to play a role in a variety of physiological processes including cell adhesion, cell migration, developmental tissue morphogenesis and metastatic spread (reviewed by Lesley et al., 1993; Herrlich et al., 1993; Knudson and Knudson, 1993). Recent studies on the regulation of CD44 have begun to elucidate how this single gene is involved in such a diverse range of cellular events.

Structural modification of CD44 occurs at the level of alternative splicing whereby up to 10 variant exons can be inserted in multiple combinations into a single site in the extracellular domain (reviewed by Lesley et al., 1993; Milde et al., 1994b). It has yet to be determined how alternative splicing modifies CD44 function, although at least in some cases, the expression of these additional exons confers a metastatic phenotype to non-metastatic cells (Günthert et al., 1991; Rudy et al., 1993). Alternative splicing is cell type dependent in that the majority of lymphoid and fibroblast lines constitutively express the 80-100 kDa haematopoietic form of CD44 (CD44H) which does not contain any alternatively spliced exons while many epithelial cells, tumours and activated lymphocytes express one or more higher molecular mass CD44 isoforms (Arch et al., 1992; Koopman et al., 1993; Mackay et al., 1994; Jackson et al., 1995). Both the core CD44 protein and the alternatively spliced exons are also subject to N- and O-linked glycosylation and the variable addition of glycosaminoglycan side chains (Camp et al., 1991; Jalkanen et al., 1988; Faassen et al., 1992; Haggerty et al., 1992; Jackson et al., 1995). Again, the extent of this glycosylation is cell type dependent (Camp et al., 1991) and these modifications can regulate function, for example by increasing the affinity for non-hyaluronan ligands such as collagen and fibronectin (Faassen et al., 1992; Jalkanen and Jalkanen, 1992) and heparin-binding growth factors (Bennett et al., 1995). It has yet to be determined whether such variation has a role to play in the observed cell type dependence of the CD44H isoform to act as a hyaluronan receptor (Lesley et al., 1990; Liao et al., 1993; Lesley et al., 1993; Murakami et al., 1994).

The highly conserved 71 amino acid CD44 cytoplasmic domain (reviewed by Isacke, 1994; Milde et al., 1994a) has also been implicated in the regulation of CD44 function. For example, truncated tailless molecules have a reduced or abolished ability to bind hyaluronan (Lesley et al., 1992;
MATERIALS AND METHODS

Reagents

The human specific anti-CD44 mAb, E1/2, the species cross reactive anti-CD44 mAb, IM7, and the anti-annexin II rabbit polyclonal antisem have been described previously (Isacke et al., 1992, 1993). To express these proteins in MDCK and Swiss 3T3 cells has been previously described (Neame and Isacke, 1993; see Fig. 3) leading to the suggestion that any association of CD44 with the detergent-insoluble cytoskeleton must be indirect. One possibility is that since in these experiments wild-type (WT) and tailless (T-) human CD44 are expressed in rodent fibroblasts which already contain endogenous CD44, the inability of T-CD44 to be released into the Triton X-100 soluble fraction (Neame and Isacke, 1993) and the blots developed using the enhanced chemiluminescence (ECL) kit (Amersham) and exposure to X-ray film (Fuji XR) at room temperature.

RESULTS

Mechanism to retain CD44 in a fibroblast Triton X-100 insoluble fraction

We have previously demonstrated that removal of the CD44 cytoplasmic tail does not increase the amount of this protein released into the Triton X-100 soluble fraction (Neame and Isacke, 1993; see Fig. 3) leading to the suggestion that any association of CD44 with the detergent-insoluble cytoskeleton must be indirect. One possibility is that since in these experiments wild-type (WT) and tailless (T-) human CD44 are expressed in rodent fibroblasts which already contain endogenous CD44, the inability of T-CD44 to be released into the detergent soluble phase could result from an association with endogenous WT protein which in turn is directly associated with the cytoskeleton. To address this, clonal cell lines of Swiss 3T3 cells expressing transfected WT or T- human CD44 were induced to patch by the addition of a human specific anti-CD44 mAb followed by a polyclonal second layer Ab. When the cells are kept at 4°C, patching is inhibited and there is a complete co-localization of transfected and endogenous CD44...
Cell type dependent regulation of CD44

When parallel cultures are incubated at 37°C, the majority of transfected WT or T- human CD44 is found patched on the cell surface whereas there is little movement of the endogenous murine CD44. In these cultures the minor amount of overlap in antibody staining is not sufficient to account for the approximately 50% of transfected human CD44 which is resistant to Triton X-100 extraction.

Parallel experiments were conducted using human CD44 transfected BW5147 T lymphoma cells. BW5147 cells express high levels of endogenous murine CD44 of which >95% is soluble in 0.5% Triton X-100 (see Fig. 8). In these cells there is no evidence for co-localization of either WT or T- transfected CD44 with endogenous CD44 after incubation at 37°C (data not shown). These experiments indicate that CD44 does not form multimeric homotypic complexes in the membrane.

A second explanation for the retention of CD44 in the detergent-insoluble fibroblast fraction is through the binding of extracellular matrix components by the CD44 extracellular domain. It is well established that the CD44 ligand, hyaluronan, is synthesised and deposited by fibroblasts in culture (Chen et al., 1989; Heldin et al., 1989) raising the possibility that aggregation of CD44 with hyaluronan gives rise to detergent-insoluble CD44:hyaluronan complexes. In order to address this, cultures of human fibroblasts were treated with hyaluronidase which removes all cell associated hyaluronan as detected with a bovine proteoglycan probe (Green et al., 1988; data not shown). Prior removal of this major CD44 ligand (Fig. 2) or inclusion of hyaluronan in the extraction buffer (Fig. 4) had no significant effect on Triton X-100 solubility of CD44. Recently it has been demonstrated that the detergent insolubility of another transmembrane glycoprotein, syndecan-1, is mediated by its heparan sulphate side chains and that inclusion of heparan sulphate in the detergent extraction buffer increases the Triton X-100 solubility (Miettinen and Jalkanen, 1994). In similar experiments, no increased CD44 solubility was detected when either heparan sulphate or chondroitin sulphate were included in the detergent extraction buffer (Fig. 4).

If fibroblast CD44 is associated with the cytoskeleton, whether directly or indirectly via a linker protein, disruption of the cytoskeleton might be expected to increase the detergent solubility. Lacy and Underhill (1987) demonstrated that cytochalasin B treatment reduced the levels of hyaluronan bound by detergent extracted cells. It was suggested that this was due to a disruption of an actin:CD44 interaction leading to increased levels of detergent soluble CD44. Treatment of fibroblasts with this drug causes the cells to radically alter their morphology such that the cytoplasm retracts around the nucleus leaving branched processes radiating from this central mass (data not shown; Morris and Tannenbaum, 1980). When cytochalasin B treated and untreated cells are extracted with Triton X-100 no change in the detergent solubility of endogenous CD44 or of WT and T- transfected CD44 is observed (Fig. 3). Similar results have recently been reported using cytochalasin D (Perschl et al., 1995a). As CD44 was not examined directly in the original studies (Lacy and Underhill, 1987) this discrepancy in results may well be due to the fact that the redistribution of CD44 after cytochalasin treatment reduces its hyaluronan binding capacity rather than its detergent solubility. Stabilization of the actin network by culturing F1084 fibroblasts (Fig. 4), Swiss 3T3 fibroblasts or HT-29 cells (data not shown) in the presence of phalloidin...
Molecular size markers are in kDa. Using the ECL reagent and exposed to X-ray film for 10 minutes, followed by an HRP-conjugated anti-rat Ig (d). Blots were developed with the ECL reagent and exposed to X-ray film for 10 minutes. Molecular size markers are in kDa.

Fig. 3. Disruption of the actin cytoskeleton does not alter the detergent extraction profile of CD44. F0184 human fibroblasts (a), Swiss 3T3 cells expressing WT CD44 (b), T- CD44 (c) and Swiss 3T3 cells transfected with the pSRα vector alone (d) were cultured for 24 hours and then for a further 3 hours with 10 µl/ml DMSO (−) or 10 µg/ml cytochalasin B (+). Cells were then extracted with 0.4% Triton X-100 for 15 minutes on ice and the distribution of CD44 between the detergent insoluble cell pellet (P) and soluble supernatant (S) was assayed by immunoblotting using mAb E1/2 followed by an HRP-conjugated anti-mouse Ig (a to c) or mAb IM7 followed by an HRP-conjugated anti-rat Ig (d). Blots were developed using the ECL reagent and exposed to X-ray film for 10 minutes. Again had no effect on the solubility of CD44, nor did nocodazole-induced disruption of microtubules (Fig. 4). Together these studies indicate that the ability of CD44 to be retained in the detergent insoluble cytoskeletal fraction of fibroblasts at 4°C does not result from: (a) a direct interaction of the cytoplasmic domain with intracellular components; (b) the formation of multimeric homotypic complexes in the plasma membrane; (c) an association of the extracellular domain with its major characterized ligand, hyaluronan; or (d) an indirect interaction with microfilaments or microtubules.

Finally, to determine whether the highly conserved transmembrane domain has a role in regulating CD44 solubility, F1084 or Swiss 3T3 fibroblasts were subjected to a number of different extraction conditions (Fig. 5). As previously reported (Neame and Isacke, 1993), extraction with Triton X-100 at 4°C results in approximately 50% of the CD44 being detergent soluble and 50% being retained with the insoluble cell matrix. Similar results were obtained for other species and cell types.

Table 1. The association of CD44 with Triton X-100 detergent insoluble material is cell type dependent

<table>
<thead>
<tr>
<th>Cell line</th>
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<td>Differentiated embryonal carcinoma</td>
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<td>Fibroblast</td>
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Semi-confluent cell cultures were extracted on ice for 15 minutes in buffer containing 0.5% Triton X-100 under altered culture and extraction conditions. Semi-confluent dishes of F1084 fibroblasts cultured for 48 hours were then left untreated (a-d-h) or treated for 3 hours with 25 µM phalloidin (b) or 20 µM nocodazole (c). Cells were then extracted for 15 minutes on ice in extraction buffer containing 0.5% Triton X-100 containing no additional additives (a-c), 25 µM phalloidin (d), 20 µM nocodazole (e), 0.5 mg/ml hyaluronan (f), 0.5 mg/ml chondroitin sulphate (g) or 0.5 mg/ml heparan sulphate (h). The distribution of CD44 between the detergent insoluble pool and soluble pool was assayed by immunoblotting using mAb E1/2 followed by an HRP-conjugated anti-rat Ig. Blots were developed with the ECL reagent and exposed to X-ray film for 1 minute.

Under these conditions greater than 95% of the transferrin receptor is extracted indicating essentially complete solubilization of plasma and endosomal membranes. No change in CD44 solubility is observed when higher concentrations of sodium chloride are present in the extraction buffer, if Ca2+ or Mg2+ are removed or if Triton X-114 substitutes for Triton X-100. By contrast, treatment of the cells with either Triton X-100 or Triton X-114 at 37°C or with n-octylglucoside at 4°C results in greater than 95% of the CD44 being sequestered into the detergent soluble pool. Comparable results were obtained with Swiss 3T3 fibroblasts (Fig. 5b) and with transfected WT and T- CD44 (data not shown). The behaviour of CD44 in these different detergent extraction conditions is reminiscent of...
that reported for glycosylphosphatidylinositol (GPI) anchored proteins which are found associated with membrane subdomains enriched in glycolipids (Brown and Rose, 1992). Furthermore, a similar extraction pattern is observed with annexin II (Fig. 5b) which again is found associated with Triton X-100 insoluble, lipid rich domains (Lisanti et al., 1994).

**A cell type dependence of CD44 Triton X-100 solubility**

In fibroblasts, 30-70% of CD44 is recovered in a non-ionic detergent insoluble fraction (Tarone et al., 1984; Lacy and Underhill, 1987; Carter and Wayner, 1988; Figs 2,3,4). As reported previously, we have been unable to demonstrate any association of CD44 with a detergent insoluble fraction in polarized MDCK epithelial cells (Neame and Isacke, 1992, 1993). We describe here experiments to investigate this cell type dependence. First, these previous results are not an artefact of the cell lines originally employed as this difference between fibroblastic and epithelioid cells extends to a wide range of cell lines originating from a number of different species (Table 1). The only exceptions are the cervical epithelioid HeLa cell line which has a profile characteristic of fibroblasts with approximately 25% of the CD44 retained in the detergent insoluble fraction, and the SV40 T antigen expressing COS-1 monkey fibroblast line which has no detectable CD44 retained in the Triton X-100 insoluble cell fraction. Two lymphoid cell lines were also examined: AKR1 cells, which are CD44-negative; and BW5147 cells, which express large amounts of endogenous CD44 (Trowbridge et al., 1982). Like epithelial cells, greater than 95% of the AKR transfected CD44 and BW5147 endogenous CD44 is detergent soluble.

In all of the fibroblast cell lines examined, the predominant CD44 species expressed is the 80-100 kDa CD44H isoform (Figs 2 to 5) which contains none of the alternatively spliced exons. By contrast the majority of the epithelioid cell lines express one or more CD44 splice variants (for example HC11 cells (Wheatley and Isacke, 1995); MDCK cells, Fig. 6; HT-29 cells, Fig. 8). However, there is no evidence that the detergent solubility of CD44 in epithelial cells is related to the presence of the alternatively spliced exons as no difference is observed in the detergent solubility of the different isoforms over a range of detergent concentrations, and transfected CD44H in epithelial cells exhibits an identical extraction profile to the endogenous epithelial CD44 (Fig. 6; Neame and Isacke, 1993).

The distinctly different detergent solubility of CD44 expressed in fibroblastic cells compared to that expressed in epithelial/lymphoid cells suggests the possibility of different functions for CD44 in these functionally distinct cell types. The most obvious differences between these cell types is that fibroblasts have a motile solitary nature involving cell:extracellular matrix interactions as opposed to the more stationary cell:cell oriented interactions of the epithelial cell types. To address these issues, the migration of cells was manipulated. By time lapse video microscopy it can be seen that exponentially growing MDCK cells exhibit limited motility which becomes completely restricted as the cells assume confluence (data not shown). A comparison of sparse and stationary cultures reveals no difference in the detergent solubility of either transfected or endogenous CD44 (Fig. 6A). To exert a more dramatic change, MDCK cells were cultured in the presence of scatter factor (reviewed by Furlong, 1992). Scatter factor/hepatocyte growth factor obtained either in purified form or as a component of MRC-5 fibroblast conditioned medium induces the breakdown of epithelial junctions and the migration of cells across the tissue culture plates (Fig. 6B). However, there is no increase in the detergent insolubility of endogenous CD44 (Fig. 6C) or transfected human CD44H (Fig. 6D) after 24 hours. Similarly, culturing cells for between 1 and 9 hours (data not shown) or for periods of time longer than 24 hours (Fig. 6C) in the presence of scatter factor has no effect. As an alternative approach, fibroblasts were cultured in semi-solid medium to prevent their migration on a tissue culture substratum. A comparison of cells extracted from methylcellulose after 24 or 48 hours with cells growing on plates revealed no differences in the detergent solubility of CD44 (Fig. 7).

To address the issue as to whether an association with extracellular matrix induces the association of CD44 with the Triton X-100 insoluble fraction, the interaction of CD44 with hyaluronan was examined. We have already demonstrated that removal of fibroblast associated hyaluronan does not increase the amount of detergent soluble CD44 (Fig. 2). An alternative approach was to determine whether there was an increase in the amount of Triton X-100 insoluble CD44 in BW5147 cells...
cultured on a hyaluronan-coated substratum or in the presence of soluble hyaluronan. These cells are competent to bind hyaluronan in solution and adhere to hyaluronan-coated plates (Lesley et al., 1990) but the presence of hyaluronan does not increase the detergent insolubility of BW5147 CD44 (Fig. 8A). Similarly, if MDCK cells are plated onto a hyaluronan-coated substratum, there is no change in the detergent extraction of the WT transfected human CD44 (a,b) or pSRα vector alone (e,f) were plated into 6 cm tissue culture dishes and either cultured for 24 hours (a,c,e) or for 7 days (b,d,f) to obtain sparse and fully polarized cultures, respectively. Transfected CD44 was detected using mAb E1/2 (a,d); endogenous CD44 was detected using mAb IM7 (e,i). (B) MDCK cells were cultured for 24 hours and then for a further 24 hours alone (a), in the presence of MRC-5 conditioned medium (b) or in the presence of 5 ng/ml purified scatter factor (c). Bar, 100 μm. (C) 1x10⁶ MDCK cells were plated onto 6 cm tissue culture plates and cultured for 24 hours. The medium was then removed and replaced either with MRC-5 fibroblast conditioned medium for 0 (a), 1 (b), 2 (c) or 3 (d) days. CD44 was detected using mAb IM7. (D) 1x10⁶ MDCK cells expressing WT human CD44 were cultured for 24 hours and then for a further 24 hours in the presence of 0 (a), 2 (b), 10 (c) or 50 (d) ng/ml scatter factor. Transfected CD44 was detected using mAb E1/2.

Fig. 7. Expression and distribution of CD44 in fibroblasts is not modulated by loss of matrix attachment. 1x10⁶ Swiss 3T3 fibroblasts (a-d) or NIH 3T3 fibroblasts (e,f) were cultured for 48 hours on tissue culture plates (a,c,e) or suspended in methylcellulose (b,d,f). Cells were extracted on ice with 0.5% Triton X-100 for 15 minutes and the distribution of CD44 between the detergent insoluble cell pellet (P) and the soluble supernatant (S) was assessed by immunoblotting using mAb IM7 followed by an HRP-anti-rat Ig. Blots were developed using the ECL reagent and exposed to X-ray film for 40 seconds. Molecular size markers are in kDa.

DISCUSSION

It is often considered that adhesion protein function is influenced by interactions with intracellular components as well as interaction with extracellular ligand. One frequently cited method to examine transmembrane protein associations with the underlying cytoskeleton is extraction of cells at 4°C with non-ionic detergents. Proteins which are not solubilized are considered to be cytoskeletonally associated. In the case of fibroblasts, 30-70% of CD44 is found to be insoluble in Triton X-100. This together with membrane motility and in vitro studies was taken as supporting evidence that CD44 is associ-
Cells were cultured on hyaluronan coated plates, with soluble hyaluronan or in the presence of hyaluronan producing cells. Subsequently cells were extracted with 0.5% Triton X-100 for 15 minutes on ice and the distribution of CD44 between the detergent insoluble pellet (P) and the detergent soluble supernatant (S) was assessed by immunoblotting. Blots were developed using the ECL reagent and exposed to film for 45 seconds. Molecular size markers are in kDa. (A) \(1 \times 10^5\) BW5147 cells were cultured in 24-well plates previously coated with 5 mg/ml hyaluronan (a), in the presence of 0.5 mg/ml soluble hyaluronan (b) or in the absence of hyaluronan (c) for 2 hours. CD44 was detected using mAb IM7. (B) \(1 \times 10^5\) MDCK cells expressing WT human CD44 were cultured on 35 mm plates previously coated with 5 mg/ml hyaluronan (a) or uncoated dishes (b) for 24 hours. Transfected human CD44 was detected using mAb E1/2. (C) \(1 \times 10^5\) HT-29 cells were cultured alone (a) or co-cultured with \(1 \times 10^5\) Swiss 3T3 cells for 48 (b) or 72 (c) hours. Human CD44 was detected using mAb E1/2.

![Fig. 8. Addition of hyaluronan does not induce an association of epithelial or lymphoid CD44 with the detergent insoluble fraction.](image)

**Fig. 8.** Addition of hyaluronan does not induce an association of epithelial or lymphoid CD44 with the detergent insoluble fraction. Cells were cultured on hyaluronan coated plates, with soluble hyaluronan or in the presence of hyaluronan producing cells. Subsequently cells were extracted with 0.5% Triton X-100 for 15 minutes on ice and the distribution of CD44 between the detergent insoluble pellet (P) and the detergent insoluble supernatant (S) was assessed by immunoblotting. Blots were developed using the ECL reagent and exposed to film for 45 seconds. Molecular size markers are in kDa. (A) \(1 \times 10^5\) BW5147 cells were cultured in 24-well plates previously coated with 5 mg/ml hyaluronan (a), in the presence of 0.5 mg/ml soluble hyaluronan (b) or in the absence of hyaluronan (c) for 2 hours. CD44 was detected using mAb IM7. (B) \(1 \times 10^5\) MDCK cells expressing WT human CD44 were cultured on 35 mm plates previously coated with 5 mg/ml hyaluronan (a) or uncoated dishes (b) for 24 hours. Transfected human CD44 was detected using mAb E1/2. (C) \(1 \times 10^5\) HT-29 cells were cultured alone (a) or co-cultured with \(1 \times 10^5\) Swiss 3T3 cells for 48 (b) or 72 (c) hours. Human CD44 was detected using mAb E1/2.

It has been well documented that fibroblasts synthesize and secrete hyaluronan, and hyaluronan can be detected associated with the fibroblasts employed here using a bovine proteoglycan probe (Wheatley and Isacke, 1995). Moreover, the observation that addition of hyaluronan to some CD44-positive lymphoid cells results in cell aggregation (Lesley et al., 1990) demonstrates that this ligand has multiple CD44 binding sites. However, experiments shown here suggest that such a mechanism is not responsible for the detergent insoluble pool of CD44 in fibroblasts. Fibroblast associated hyaluronan can be removed with hyaluronidase but this removal does not increase the amount of Triton X-100 soluble CD44 (Fig. 2). BW5147 cells which have the capacity to bind hyaluronan (Lesley et al., 1990) can be cultured either on hyaluronan coated plates or in the presence of soluble hyaluronan. Neither treatment increases the proportion of detergent insoluble CD44 (Fig. 8). Similar experiments employing MDCK and HT-29 epithelial cell lines including the co-culture with hyaluronan producing Swiss 3T3 cells similarly has no effect (Fig. 8). Finally, the observation that the presence of hyaluronan, heparan sulphate or chondroitin sulphate in the extraction buffer does not increase the solubility of fibroblast CD44 (Fig. 4) argues against a role for glycosaminoglycan side chains in mediating CD44 insolubility. (c) That the transmembrane domain of CD44 is associated with a transmembrane cytoskeletonally-associated linker protein. Chimera experiments in which the transmembrane domain of CD44 has been replaced with that from the CD45 molecule or the CD3 \(\zeta\) chain of the T cell receptor have demonstrated that it is the transmembrane domain of CD44 which is responsible for retaining a proportion of the protein in the fibroblast Triton X-100 insoluble fraction (Perschl et al., 1995a). The evidence indicating that the Triton X-100 insolubility of fibroblast CD44 does not result from the CD44 transmembrane domain interacting with a cytoskeletonally-associated linker protein is that Triton X-100 insoluble CD44 migrates in sucrose density gradients to the upper part of the gradient indicating an association with more buoyant material whereas Triton X-100 soluble CD44 is found predominantly in the 40% sucrose cushion (Perschl et al., 1995a). Plasma membrane domains which have identical buoyancy characteristics to these CD44 containing domains are stable under the same detergent conditions in which CD44 is insoluble, and are destabilized by detergent conditions that solubilize CD44 (Fig. 5; Brown and Rose, 1992). In similar experiments sucrose gradient buoyancy has been used to isolate a caveoli rich fraction which contains GPI linked proteins, transmembrane proteins and plasma membrane associated cytosolic proteins (Lisanti et al., 1994). One of these latter proteins, annexin II, also shows increased solubility in \(n\)-octylglucoside and has a similar detergent solubility profile to that of CD44 (Fig. 5b).

Taken together these data indicate that the transmembrane domain of CD44 ensures its inclusion in Triton X-100 insoluble, lipid rich, plasma membrane complexes. However, although there are striking similarities between the detergent extraction of GPI linked proteins such as PLAP from the apical surface of MDCK cells (Brown and Rose, 1992) and CD44 from fibroblasts, several issues remain unresolved: (a) CD44, unlike PLAP, is a transmembrane protein and therefore the association with plasma membrane lipids must be functionally distinct. It remains to be determined whether as is the case for PLAP, CD44 associated membrane structures are...
enriched in glycosphingolipids. Certainly CD44 cannot be associated with the same glycosphingolipid enriched complexes as PLAP because both PLAP and T-CD44 are expressed on the apical plasma membrane of MDCK cells and yet there is no evidence of T-CD44 Triton X-100 insolubility in these cells (Brown and Rose, 1992; Neame and Isacke, 1993). This indicates that T-CD44 and PLAP are in distinct apical plasma membrane subdomains. Furthermore in these experiments we cannot determine whether the retention of CD44 in Triton X-100 insoluble complexes results from an association with lipids and/or with other transmembrane proteins concentrated in such domains. (b) It is not known why there is always a significant proportion of Triton X-100 soluble CD44 in fibroblasts in addition to the 30-70% detergent insoluble fraction. This is not due to saturation by CD44 of a detergent-insoluble complex as transfection of CD44 into fibroblasts does not change the proportion of detergent insoluble endogenous material and moreover, the behaviour of transfected CD44 mirrors that of endogenous CD44. Jacobson et al. (1984a,b) found that CD44 was more rapidly redistributed in the leading edge of fibroblasts than in the trailing edge and proposed that CD44 associated with cellular components in a dynamic manner with the association being weaker at the leading edge. Together, these data would be consistent with a model whereby the association of CD44 with the detergent-insoluble complexes in fibroblasts is transient. (c) The ability of CD44 to associate with Triton X-100 insoluble, lipid rich, complexes is cell type dependent (Table 1). No CD44 is found associated with a Triton X-100 insoluble fraction in epithelial cells and yet such cells contain Triton X-100 insoluble lipid complexes (Brown and Rose, 1992). This suggests that there is a significant difference between the localization of CD44 in membrane subdomains between fibroblasts and epithelial/lymphoid cells and raises the question as to how this difference in distribution might be regulated and whether it reflects different functions for CD44 in different cell types. The most obvious differences between fibroblasts and epithelial/lymphoid cells is that the former have a migratory phenotype and are primarily involved in cell:matrix interactions whereas the latter are non-motile cells involved in cell:cell interactions. However, although cell motility and interaction with the extracellular matrix can be manipulated, such manipulations do not alter the Triton X-100 solubility of CD44 (Figs 2,6,7,8). In conclusion, the data presented here argues for a mechanism whereby CD44 associates with Triton X-100 insoluble, lipid rich, complexes in a transient, cell type and temperature dependent manner.

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