Band 4.1-like proteins of the bovine lens

Effects of differentiation, distribution and extraction characteristics

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Bovine lens epithelium, cortex and nucleus were screened for the presence of red-cell-membrane band 4.1-like proteins by using an immunoblot method. Lens epithelial cells were found to contain proteins of M_r 78 000 and higher ($\sim 150\,000$) that cross-reacted with anti-(protein 4.1) sera. Fibre cells of the superficial cortex were also found to contain these two proteins, as well as an additional protein of approx. 80 000 M_r . In contrast, deep layers of the cortex and the lens nucleus contained no detectable cross-reactive protein at these M_r values. Treatment of a crude membrane fraction prepared from superficial bovine cortices with a low-ionic-strength buffer resulted in release of the high- M_r band 4.1-like protein. The 80 000- and 78 000- M_r proteins remained with the membrane fraction in low-ionic-strength buffer, but were released into solution by high-ionic-strength-buffer treatment. We have also demonstrated that the human red-blood-cell membrane, like lens epithelial cells and fibre cells, also contains a high- M_r band 4.1-like protein that is released from membranes by low-ionic-strength-buffer treatment.

The membrane skeleton of the human red blood cell (RBC) is composed of a meshwork of proteins which laminate the cytoplasmic face of the plasma membrane. This lattice of proteins has an important role in stabilizing the fragile lipid bilayer and in determining the shape and mechanical properties of the RBC [for recent reviews of RBC membrane structure and function see Cohen (1983) and Goodman & Shiffer (1983)].

Proteins that take part in the formation of the RBC membrane skeleton include spectrin, actin and band 4.1 protein (see the above-cited reviews). Spectrin is a dimer composed of subunits of 240 kDa (α -spectrin) and 220 kDa (β -spectrin). Spectrin dimers are capable of associating in a 'head-to-head' manner to form tetrameric spectrin (Shotton *et al.*, 1979), which is thought to be the predominant form of spectrin in the normal RBC membrane (Ralston *et al.*, 1977; Ungewickell &

Gratzer, 1978; Middaugh & Ji, 1980). The 'tail' end of the spectrin dimer has been shown to bind to F-actin (Brenner & Korn, 1979; Cohen *et al.*, 1980). This interaction is strengthened by protein 4.1 (Ungewickell *et al.*, 1979; Fowler & Taylor, 1980; Cohen & Korsgren, 1980), which also binds to spectrin dimers close to the site of spectrin-actin interaction (Tyler *et al.*, 1980). It is believed that protein 4.1 also enhances spectrin-actin interaction in the intact RBC membrane (Cohen & Foley, 1980).

The spectrin-actin-4.1 lattice of the RBC is linked to the plasma membrane through the binding of the β -subunit of spectrin to a protein called ankyrin (Bennett, 1978; Bennett & Stenbuck, 1979a), which in turn binds to band 3, the RBC anion channel protein (Bennett & Stenbuck, 1979b, 1980; Hargraves *et al.*, 1980). It has also been suggested that protein 4.1 might serve as a second membrane attachment site for the spectrin-actin-4.1 lattice, since 4.1 is retained on membranes under low-ionic-strength conditions, which result in the release of spectrin and actin (Goodman & Shiffer, 1983; Tyler *et al.*, 1979). Association of protein 4.1 with the membrane may occur through an interaction with the glyco-

Abbreviations used: RBC, red blood cell; SDS, sodium dodecyl sulphate; 1D, one-dimensional; 4.1, band 4.1 protein; HMW-4.1-like protein, band 4.1-like protein of relatively high M_r ; DNAase, deoxyribonuclease.

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phorins, the transmembrane sialoglycoproteins of the RBC membrane (Anderson & Louvrien, 1984).

Protein 4.1 can be resolved by discontinuous 1D SDS/polyacrylamide-gel electrophoresis into proteins of 80 and 78 kDa that have been designated 4.1a and 4.1b respectively (Mueller & Morrison, 1977). These proteins are similar in sequence as judged by peptide mapping (Goodman *et al.*, 1982) and may be functionally equivalent (Goodman & Shiffer, 1983). Protein 4.1b is the predominant species in young RBCs (Sauberman *et al.*, 1979), but equal amounts of 4.1a and 4.1b are found in mature cells (Goodman *et al.*, 1982), suggesting that 4.1a may be derived from 4.1b by a post-translational modification that results in slightly reduced mobility on SDS-containing gels. Antibodies prepared against protein 4.1 have also detected the presence of other minor low- M_r 4.1-like proteins in the RBC membrane which may represent membrane-bound proteolytic fragments of proteins 4.1a and b (Cohen *et al.*, 1982).

Fairly recently, analogues of RBC spectrin have been detected in a wide variety of cell types (for review, see Lazarides & Nelson, 1982), including the avian (Repasky *et al.*, 1982) and the mammalian lens (Lehto & Virtanen, 1983; Aster *et al.*, 1984). Proteins serologically related to protein 4.1 have also been detected in several non-erythroid cell types (Spiegel *et al.*, 1982; Cohen *et al.*, 1982; Goodman *et al.*, 1984). We have recently detected proteins antigenically related to protein 4.1 in the lenses of several species of mammals (Aster *et al.*, 1984). The lenses of all species screened showed the presence of a cross-reactive doublet of M_r close to that of the RBC 4.1 doublet. All species except the human also showed the presence of a cross-reactive protein of a significantly higher M_r than that of the 4.1a/b doublet. This observation has been confirmed and extended by Granger & Lazarides (1984), who have localized the 4.1-like proteins of lens epithelial and fibre cells to the plasma membrane.

The bovine lens provides an excellent tissue for the study of non-erythroid spectrin and protein 4.1 analogues, since it is non-vascular (eliminating RBC contamination) and readily available. The anterior surface of the lens is covered by a monolayer of epithelial cells that differentiate into the elongated, anuclear fibre cells that make up the bulk of the lens cortex. Pure epithelial and fibre cells are easily isolated, making it possible to study changes in cytoskeletal proteins that accompany fibre-cell morphogenesis. Fibre cells are initially laid down in the superficial cortex, but then are gradually displaced towards the centre of the lens as additional cells are deposited, a process that continues throughout life. As fibre cells are internalized, further changes in cell morphology

related to the maturation and eventual aging of these cells also occur. It is possible to study changes in cell composition that are associated with these processes by successively isolating and comparing the content of layers of fibre cells from the superficial cortex through the deep cortex and the lens nucleus (for a review of lens morphology, see Maisel *et al.*, 1981). We undertook the work described here to begin to investigate the effect of fibre-cell differentiation and location on the 4.1-like proteins of the lens, and also to determine conditions that might be used to release these proteins from lens membranes.

Materials and methods

Materials

Bovine eyes were obtained fresh from 1–2-year old animals at a local slaughterhouse. Human blood was obtained by venipuncture from normal volunteers with their informed consent and used immediately. Chemicals used in solutions, Sephadex G-25, goat anti-rabbit IgG linked to horseradish peroxidase, 4-chloro-1-naphthol, DNAase I and S_1 nuclease were obtained from Sigma (St. Louis, MO, U.S.A.). Nitrocellulose filters (0.45 μ m pore size) were obtained from Millipore (Lexington, MA, U.S.A.). Ultrafiltration filters were obtained from Amicon (Danvers, MA, U.S.A.).

Preparation of lens samples

Eyes were stored on ice and processed within 2 h of excision. Lenses were removed with a razor blade and transferred to Petri dishes containing cold 50 mM-Tris buffer (pH 7.5)/2 mM-MgCl₂/0.1% β -mercaptoethanol/1 mM-phenylmethanesulphonyl fluoride (homogenization buffer). Attached zonules and iris were removed under the dissecting microscope, and cleaned lenses were then transferred to a second Petri dish filled with buffer. The lens capsule was then gently removed. Most lens epithelial cells were found to remain bound to the removed capsules. These capsules were free of fibre cells, as judged by careful inspection under the dissecting microscope.

To study the 4.1-like proteins of the lens epithelium, 10–15 capsules were added to 0.5 ml of a solubilization buffer containing 10 mM-Tris, pH 6.8, 1% SDS, 0.1% β -mercaptoethanol, 0.01% EDTA and 1 mM-phenylmethanesulphonyl fluoride. DNAase I and S_1 nuclease were then added (20 μ g of each), and the resultant mixture was incubated at 37°C for 20 min. Insoluble capsular material was then removed by centrifugation at 50 000 g for 15 min. SDS, β -mercaptoethanol and glycerol were then added to the remaining superna-

tant to give concentrations of 3% (w/v), 5% (v/v) and 10% (v/v) respectively. Samples were stored at -20°C .

To study the effect of fibre-cell location on lens 4.1-like proteins, cleaned decapsulated lenses were dissected into superficial, intermediate and deep-cortical fractions of approximately equal weight. The lens nucleus was not subdivided. Samples were then mixed with 30 vol. of homogenization buffer and disrupted in a low-clearance Dounce homogenizer. A crude membrane fraction was then collected by centrifugation at $30000g$ for 10 min, resuspended in homogenization buffer and pelleted as described above. Samples were then solubilized in 3% SDS/5% β -mercaptoethanol/10% glycerol (0.5 ml/100 mg pellet wet weight) and stored at -20°C .

Preparation of RBC membranes

RBC membranes were prepared by a slight modification of the procedure used by Dodge *et al.* (1963). Whole blood was centrifuged at $2000g$ for 5 min. Plasma and the buffy coat were then removed by aspiration, and the RBCs were then washed thrice with cold 154 mM-NaCl. Packed cells were then lysed by the addition of 40 vol. of 5 mM-sodium phosphate buffer, pH 7.6, containing 1 mM-EDTA. The lysate was incubated with stirring for 20 min at 4°C , and membranes were then collected by centrifugation at $48000g$ for 10 min. Membranes were then washed with lysing buffer until white.

Extraction of proteins from lens and RBC membranes

A crude membrane fraction was prepared from 10 bovine superficial cortices and washed twice with homogenization buffer. In this step, and in all subsequent steps, centrifugation was carried out at $48000g$ for 10 min. The membrane fraction was then washed once with cold 4 mM-Tris/4 mM-EDTA buffer, pH 7.6, and was then suspended in 10 vol. of 0.2 mM-Tris, pH 7.6, containing 0.2 mM-EDTA and 0.2 mM-di-isopropyl fluorophosphate, that had been preheated to 37°C in a water bath. After a 30 min incubation, the membrane fraction was separated from the supernatant by centrifugation. Proteins released from the membrane fraction were then precipitated from the supernatant by the addition of $(\text{NH}_4)_2\text{SO}_4$ to 90% saturation, followed by incubation for 2 h at 4°C . Precipitated proteins were collected by centrifugation and solubilized with SDS as described above. The remaining membrane fraction was then re-extracted with low-ionic-strength buffer, washed once with 4 mM-Tris/4 mM-EDTA, and then washed once with 50 mM-Tris buffer, pH 7.6, containing 1 mM-EDTA. The membrane fraction was then suspended in about 6.0 ml of the latter

buffer, and solid guanidinium chloride and di-isopropyl fluorophosphate were added to give final concentrations of 1 M and 0.2 mM respectively. This mixture was then incubated with stirring at 4°C overnight, and the membrane fraction was again separated from released proteins by centrifugation. The pellet was washed once with 50 mM-Tris/1 mM-EDTA, and was then solubilized with SDS for electrophoretic analysis. The high-salt extract was desalted on a column (0.8 cm $\times$ 20 cm) of Sephadex G-25 column equilibrated with 5 mM-sodium phosphate buffer, pH 7.6, containing 20 mM-KCl, 1 mM-EDTA, 0.5 mM-dithiothreitol. Fractions containing protein, as detected by monitoring A_{280} , were then pooled and concentrated in an Amicon ultrafiltration chamber on a YM 10 filter.

RBC membranes were extracted with a low-ionic-strength buffer as described by Tyler *et al.* (1979), except that the membrane/buffer ratio was 1:10 rather than 1:30 (v/v). Proteins released from membranes by this treatment were then concentrated by $(\text{NH}_4)_2\text{SO}_4$ precipitation as described for lens extracts. RBC protein 4.1 was extracted from membranes and purified to homogeneity by the method of Tyler *et al.* (1979).

Preparation of antibodies

Polyclonal antibody to RBC 4.1 was produced in New Zealand White rabbits and affinity-purified on an Affi-gel 10-protein 4.1 column as described by Cohen *et al.* (1982). Cross-reaction of this antibody with lens and RBC 4.1-like proteins was described previously (Aster *et al.*, 1984).

Detection of 4.1-like proteins

Discontinuous 1D electrophoresis was performed on 7.5% (w/v) polyacrylamide gels containing SDS as described by Laemmli (1970). The total display of proteins was revealed by staining gels with Coomassie Blue R dye. Proteins in unstained gels run in parallel were transferred to nitrocellulose paper by the method of Towbin *et al.* (1979), except that the time of transfer was increased to 20 h. Proteins antigenically related to protein 4.1 were then specifically stained with anti-4.1 sera or affinity-purified anti-4.1 antibody as described previously (Aster *et al.*, 1984).

Results and discussion

To begin to look at the effect of differentiation on the 4.1-like proteins of the lens, we examined the anti-4.1 staining pattern of lens epithelial and fibre cells (Fig. 1). Epithelial cells were found to contain cross-reactive proteins of M_r 78 000, and of significantly higher M_r , a protein henceforth referred to as the 'HMW-4.1-like protein'. Fibre cells were found to contain both of these proteins,

Fig. 1. 4.1-Like proteins of bovine lens epithelial and fibre cells

Lens epithelial cells (lane 1; $\sim 200 \mu\text{g}$ of protein) and fibre-cell membranes (lane 2; $\sim 65 \mu\text{g}$ of protein) were analysed by SDS/polyacrylamide-gel electrophoresis. (a) Shows the Coomassie Blue-staining pattern of a representative gel. The positions of some landmark cytoskeletal proteins are indicated by their respective molecular masses: spectrin, 240 kDa; vimentin, 57 kDa; and actin, 42 kDa. (b) Shows the corresponding immunoblot stained with anti-4.1 serum. The arrow denotes a 4.1-like protein of relatively high M_r (~ 150000). A fibre-cell-specific 4.1-like protein is indicated by its molecular mass (80 kDa).

and also an additional protein of 80 kDa that appeared to be fibre-cell-specific. In this experiment, and in all other experiments relying on staining of blots with anti-4.1 serum for the detection of proteins, no cross-reaction was observed when pre-immune serum was substituted for immune serum (results not shown).

Cessation of protein synthesis in the reticulocyte and subsequent maturation of the RBC in the peripheral blood is associated with the appearance of an increased ratio of protein 4.1a (80 kDa) to protein 4.1b (78 kDa), suggesting that 4.1a is derived from 4.1b by a post-translational modification that slightly decreases electrophoretic mobility on polyacrylamide gels containing SDS (Sauberman *et al.*, 1979). It is thus interesting that superficial lens fibre cells, whose protein synthesis decreases markedly on completion of maturation (Reeder & Bell, 1965), also accumulate a 4.1-like protein of 80 kDa that appears to be absent or greatly decreased in their progenitor cell, the lens epithelial cell. Given the situation in the RBC, the most parsimonious explanation for the appearance of the protein of 80 kDa in fibre cells would be for

this protein to be derived from the lens protein of 78 kDa by a post-translational modification similar to that which results in conversion of RBC 4.1b to 4.1a. The persistence of fibre cells and their proteins for long time periods (Young & Fulhorst, 1966; Wannemacher & Spector, 1968) may act to facilitate this modification.

We have also investigated the distribution of the 4.1-like proteins within the lens by isolating successively deeper layers of fibre cells and screening for the presence of 4.1-like proteins (Fig. 2). The membranes of the superficial cortical fibre cells of a single lens were again observed to contain 4.1-like proteins of 80 kDa and of high M_r (Fig. 2b, lane 1); the components of the 80 kDa/78 kDa doublet were not well resolved on this particular blot. In contrast, membrane fractions of the intermediate cortex, the deep cortex, and the lens nucleus (lanes 2–4 respectively) showed no anti-4.1 staining at these M_r values, but did show cross-reactive bands in the lower M_r (≤ 30000) region of the blot. It is possible that these bands found in the deeper, and thus older (Kuwabara, 1975), layers of fibre cells represent membrane-bound degradation

Fig. 2. Effect of fibre-cell location on the 4.1-like proteins of the lens cortex

SDS/polyacrylamide-gel electrophoretic analysis was performed on crude membrane fractions prepared from superficial cortex (lane 1), intermediate cortex (lane 2), deep cortex (lane 3), and the lens nucleus (lane 4). (a) Shows the Coomassie Blue-staining pattern of these fractions; the positions of spectrin (240 kDa), vimentin (57 kDa), and actin (42 kDa) are indicated. (b) Shows the anti-4.1 staining pattern. Cross-reactive proteins of high M_r (arrow) and of ~80 kDa are observed in the superficial cortical membrane fraction.

products of the 4.1-like proteins observed in the superficial cortex, but direct evidence for this explanation is presently lacking.

We do not believe that the disappearance of the 4.1-like proteins from older lens membrane fractions could have been caused by proteolysis occurring during sample preparation for several reasons. First, eyes were obtained within a few minutes of death, were immediately chilled on ice, and were processed in cold buffers. Secondly, inclusion of the proteinase inhibitors phenylmethanesulphonyl fluoride or di-isopropyl fluorophosphate (J. C. Aster, unpublished work) in the buffers used had no effect on the immunoblot pattern that was obtained. Finally, analysis of membranes from an unfractionated cortex gave an immunoblot pattern that was a composite of those obtained when superficial and deep cortices were analysed separately (Aster *et al.*, 1984), thereby ruling out the possibility that a proteinase confined to the deep cortical and nuclear fractions might have become activated during sample preparation. Rather, the loss of immunoreactive proteins of higher M_r from older membrane fractions may be the result of proteolysis occurring *in vivo*.

It has previously been demonstrated that the concentration of several other lens cytoskeletal proteins, including spectrin, actin (Nasser *et al.*,

1980) and tubulin (Kuwabara, 1968) decrease as fibre cells become increasingly internalized with age. Decreases in membrane-associated spectrin, actin and vimentin with increasing cell age are also evident in Fig. 2(a). Regardless of the precise mechanism of degradation, it is interesting that the fibre cell maintains its shape and membrane integrity in the face of the loss of many cytoskeletal components (for review, see Maisel *et al.*, 1981), especially since removal of the membrane skeleton from the RBC membrane causes rapid vesiculation of the remaining lipid bilayer. Unusual properties that may act to preserve membrane stability in older fibre cells include an elaborate network of intercellular articulations (Cohen, 1965) that contain a high density of lens gap junctions (Phillipson *et al.*, 1975), and the presence of a high concentration of cholesterol in the membrane, which may act to increase membrane rigidity (Feldman & Feldman, 1965; Cotlier *et al.*, 1978).

One important characteristic of extrinsic membrane proteins is that they can be dissociated from membranes by mildly perturbing physical treatments. Since protein 4.1 interactions in the RBC are disrupted by changes in the ionic strength of the suspending buffer (Tyler *et al.*, 1979), we investigated the effect of high- and low-ionic strength buffers on the association of the lens 4.1-

like proteins with a crude lens membrane fraction (Fig. 3). We found that the cortical 4.1-like doublet was retained in the membrane fraction after low-ionic-strength buffer treatment (Fig. 3b, lane 4), but was readily released into solution by subsequent high-ionic-strength-buffer treatment (lane 7). In contrast, the HMW-4.1-like protein (Fig. 3b, lane 5), as well as significant amounts of lens

Fig. 3. Extraction characteristics of the 4.1-like proteins of the superficial lens cortex.

SDS/polyacrylamide-gel electrophoretic patterns are shown for the following samples: RBC membranes (lane 1; $\sim 40 \mu\text{g}$ of protein); purified protein 4.1 (lane 2; $\sim 2.5 \mu\text{g}$ of protein); lens superficial cortical membranes (lane 3); lens membranes extracted with low-ionic-strength buffer (lane 4); the low-ionic-strength supernatant (lane 5); lens membranes further extracted with high-ionic-strength buffer (lane 6); and the high-ionic-strength supernatant (lane 7). (a) Shows a gel stained with Coomassie Blue. The positions of α -spectrin (240 kDa), protein 4.1 (80 kDa) and actin (42 kDa) are indicated. (b) Shows the staining pattern obtained with anti-4.1 serum.

spectrin and actin (Fig. 3a, lane 5), was efficiently released from the membrane fraction under low-ionic-strength conditions. The dissimilar extraction characteristics of these two types of 4.1-related proteins argue indirectly for differences in the manner in which these proteins interact with other membrane components. It should be noted that, in the initial report describing the lens HMW-4.1-like protein, the M_r of this protein, as estimated from immunoblots, was given as 125 000 (Aster *et al.*, 1984). This appears to be an underestimation of the M_r , since recent purification of this protein has allowed us to more accurately estimate its M_r on SDS-containing gels to be ~ 150 000 (J. C. Aster, unpublished work).

Like the lens 80 kDa/78 kDa doublet, proteins 4.1a and 4.1b are also retained on the RBC membrane under conditions of low ionic strength that result in the extraction of most spectrin and actin from the membrane. The work of Anderson & Louvrien (1984) suggests that binding of these proteins to the RBC membrane occurs through an interaction with one or more of the glycoporphins, the transmembrane sialoglycoproteins of the RBC, thus implicating protein 4.1 as a possible site of linkage between the membrane skeleton and the plasma membrane. Several studies (Alcala *et al.*, 1975; Roy *et al.*, 1979; Garadi *et al.*, 1982) have demonstrated the presence of glycoproteins in the fibre-cell membrane which represent potential glycoporphin-like sites for the binding of the lens 4.1-like doublet.

The detection of a lens HMW-4.1-like protein lead us to examine the possibility that a similar protein might be present in the RBC membrane. Anti-4.1 serum stained RBC proteins 4.1a and 4.1b, and also cross-reacted with many minor, membrane-bound bands of slightly higher and lower M_r (Fig. 4b, lane 2); this is similar to the pattern of staining previously noted by Cohen *et al.* (1982). In addition, a minor cross-reactive protein of M_r close to that of the lens HMW-4.1-like protein was also observed. This protein was released from RBC membranes with high efficiency by low-ionic-strength-buffer treatment, as judged by the absence of staining in the treated membranes (lane 3) and the increased staining intensity, relative to proteins 4.1a and 4.1b, at high M_r in the low-ionic-strength supernatant (lane 4). In contrast, most 4.1a and 4.1b were retained on the extracted membranes, as evidenced by their more intense Coomassie Blue staining in the treated membranes (Fig. 4a, lane 3) than in the resultant supernatant (lane 4). Some non-4.1 proteins, especially spectrin (the high- M_r doublet seen in Fig. 4b, lane 4), were also stained with the antiserum that was used. This probably resulted from the large amount of protein loaded on this gel

Fig. 4. Detection of a minor, HMW-4.1-like protein in the human RBC membrane

SDS/polyacrylamide-gel-electrophoretic patterns are shown in (a) and (b) for the following samples: lens superficial cortical membranes (lane 1; $\sim 65 \mu\text{g}$ of protein); human RBC membranes (lane 2; $\sim 80 \mu\text{g}$ of protein); low-ionic-strength-buffer-extracted RBC membranes (lane 3; $\sim 80 \mu\text{g}$ of protein); and the low-ionic-strength supernatant (lane 4; $\sim 60 \mu\text{g}$ of protein). (a) Shows a gel stained with Coomassie Blue. The positions of lens spectrin (240 kDa) and RBC protein 4.1 (denoted by the arrows) are indicated. (b) Shows a blot stained with anti-4.1 serum. The position of a RBC HMW-4.1-like protein is indicated in lanes 2 and 4 with an arrow. (c) Shows the pattern obtained when a RBC low-ionic-strength extract was stained with affinity-purified anti-4.1 antibody (lane 1; $2 \mu\text{g}$ of antibody) or antibody pre-incubated with a vast excess of purified protein 4.1 (lane 2).

and/or the presence of low levels of non-4.1 antibodies in our antisera. To demonstrate the antigenic relatedness of the RBC HMW-4.1-like protein to protein 4.1 more convincingly, we affinity-purified anti-4.1 antibody from pooled immune sera and then used this reagent to stain the proteins extracted from the RBC membrane by low-ionic-strength-buffer treatment. Affinity-purified antibody also cross-reacted with the RBC HMW-4.1-like protein and resulted in greatly decreased background staining of non-4.1 proteins (Fig. 4c, lane 1). Pre-incubation of this antibody with excess purified protein 4.1 markedly inhibited staining (lane 2), suggesting that this protein interacts with the antigen-binding site of anti-4.1 antibody.

Similar HMW-4.1-like proteins have also been detected in the RBCs of several other mammals (Granger & Lazarides, 1984; J. C. Aster, unpublished work), indicating that this protein is a conserved, albeit minor, component of RBC membranes generally. This protein was probably overlooked in past studies because of its low concentration in the membrane. The molecular interactions of the lens and the RBC HMW-4.1-like proteins within their respective membranes remain to be determined. The relatively high concentration of this protein in the mammalian lens suggests that it may play a specialized role in this particular cell type.

Although the function of the protein 4.1a/b and spectrin analogues of the lens is presently uncertain, it is probably significant that all spectrins thus far characterized can bind F-actin, and that the interaction of non-erythroid spectrin with F-actin is enhanced by RBC protein 4.1 (Burns *et al.*, 1984), indicating that the ability to interact with protein 4.1 has been conserved during the divergence of tissue-specific spectrins. Lens spectrin (Repasky *et al.*, 1982; Lehto & Virtanen, 1983), actin (Kibbelaar *et al.*, 1979; Ireland *et al.*, 1983) and 4.1-like proteins (Granger & Lazarides, 1984) have all been shown to co-localize to the plasma membrane, suggesting that the lens membrane, like that of the RBC, may be underlaid by a reticulum composed of these three proteins. As in the RBC, the lens 4.1a and 4.1b analogues could function, in part, to enhance the interaction of spectrin and actin within such a reticulum. Our data are also consistent with the possibility that the lens 4.1a and 4.1b analogues may serve as a point of linkage between the cytoskeleton and the plasma membrane of the lens fibre cell.

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