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Biochemical Characterization of the Epithelial Dystroglycan Complex*

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Dystroglycan is a widely expressed extracellular matrix receptor that plays a critical role in basement membrane formation, epithelial development, and synaptogenesis. Dystroglycan was originally characterized in skeletal muscle as an integral component of the dystrophin glycoprotein complex, which is critical for muscle cell viability. Although the dystroglycan complex has been well characterized in skeletal muscle, there is little information on the structural composition of the dystroglycan complex outside skeletal muscle. Here we have biochemically characterized the dystroglycan complex in lung and kidney. We demonstrate that the presence of sarcoglycans and sarcospan in lung reflects association with dystroglycan in the smooth muscle. The smooth muscle dystroglycan complex in lung, composed of dystroglycan, dystrophin/utrophin, β-, δ-, ε-sarcoglycan, and sarcospan, can be biochemically separated from epithelial dystroglycan, which is not associated with any of the known sarcoglycans or sarcospan. Similarly, dystroglycan in kidney epithelial cells is not associated with any of the sarcoglycans or sarcospan. Thus, our data demonstrate that there are distinct dystroglycan complexes in non-skeletal muscle organs as follows: one from smooth muscle, which is associated with sarcoglycans forming a similar complex as in skeletal muscle, and one from epithelial cells.

α-Dystroglycan is a highly glycosylated peripheral membrane protein associated with the membrane-spanning β-dystroglycan. These two proteins were originally isolated from skeletal muscle as components of a large oligomeric complex further comprised of dystrophin, the syntrophin, and sarcoglycan complexes and the recently identified protein sarcospan (1–5). In skeletal muscle, α-dystroglycan binds to the extracellular matrix component laminin α2-chain (6), whereas the intracellular domain of β-dystroglycan binds to the cytoskeletal protein dystrophin (7). Thus, dystroglycan is thought to act as a transmembrane link between the extracellular matrix and the cytoskeleton, and this linkage seems to be crucial for maintaining normal function (4, 8, 9). In addition, a defect in any of the sarcoglycans results in specific loss of the sarcoglycan-sarcospan subcomplex, destabilization of α-dystroglycan, and eventually muscle cell death (4).

Many of the dystroglycan-associated proteins found in skeletal and cardiac muscle are also expressed in other tissues. For example, β- and δ-sarcoglycan, sarcospan, and the syntrophins are all at the RNA level expressed in tissues other than skeletal and cardiac muscle (3, 5, 10–13). In addition, several dystrophin isoforms are ubiquitously expressed, including the autosomal dystrophin homologue utrophin (14–17). By far, dystroglycan is the most widely expressed component. Dystroglycan is expressed at high levels in many cell types and is particularly prominent on the basal side of epithelial cells facing basement membranes (18–23). Moreover, α-dystroglycan binds laminin-1, agrin, and perlecan (24–28). β-Dystroglycan, in turn, also binds to the dystrophin isoforms Dp71, Dp116, and Dp260 (7, 29) and has been shown to be associated with utrophin (30). Thus, different dystroglycan complexes may form in different tissues implying that dystroglycan may have important roles outside skeletal muscle. Indeed, dystroglycan has been implicated as a laminin/agrin receptor involved in epithelial cell development, basement membrane formation, and synaptogenesis (19, 21, 31–33). Recently, it was also demonstrated that dystroglycan serves as a receptor for both lymphocytic choriomeningitis virus (34) and *Mycobacterium leprae* (35). The roles of the dystroglycan-associated proteins outside skeletal muscle, however, have remained elusive. To elucidate potential functional roles, a first important step includes the determination of the cellular localization of these proteins. Specifically, are the dystroglycan-associated proteins that are present in non-skeletal muscle organs derived from smooth muscle, epithelial cells, or both?

ε-Sarcoglycan, a homologue of α-sarcoglycan, was recently identified and shown to be expressed in a wide variety of tissues including epithelial cells (36, 37). ε-Sarcoglycan has also been identified as an integral component of the smooth muscle sarcoglycan complex[1] and could also be part of an epithelial dystroglycan complex, possibly along with β- and δ-sarcoglycan and sarcospan.

Although dystroglycan has been shown to play important roles outside skeletal muscle, the dystroglycan complex from other cell types is only partially characterized. In peripheral nerve, the molecular mass of α-dystroglycan is 120 kDa in contrast to 156 kDa in skeletal muscle (38, 39). The molecular weight of α-dystroglycan in epithelial cells has remained elusive. A 120-kDa band has been identified in lung (18), but it is unclear whether the 120-kDa dystroglycan is derived from epithelial cells or smooth muscle. Moreover, α-dystroglycan is believed to act as a laminin-1 receptor involved in the development of kidney epithelial cells (19). However, a direct association between kidney α-dystroglycan and laminin-1 has not been demonstrated.

Here, we demonstrate that the presence of the sarcoglycans and sarcospan in lung reflects association with dystroglycan in the smooth muscle within lung, and we show that epithelial

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[1] V. Straub and K. P. Campbell, unpublished data.

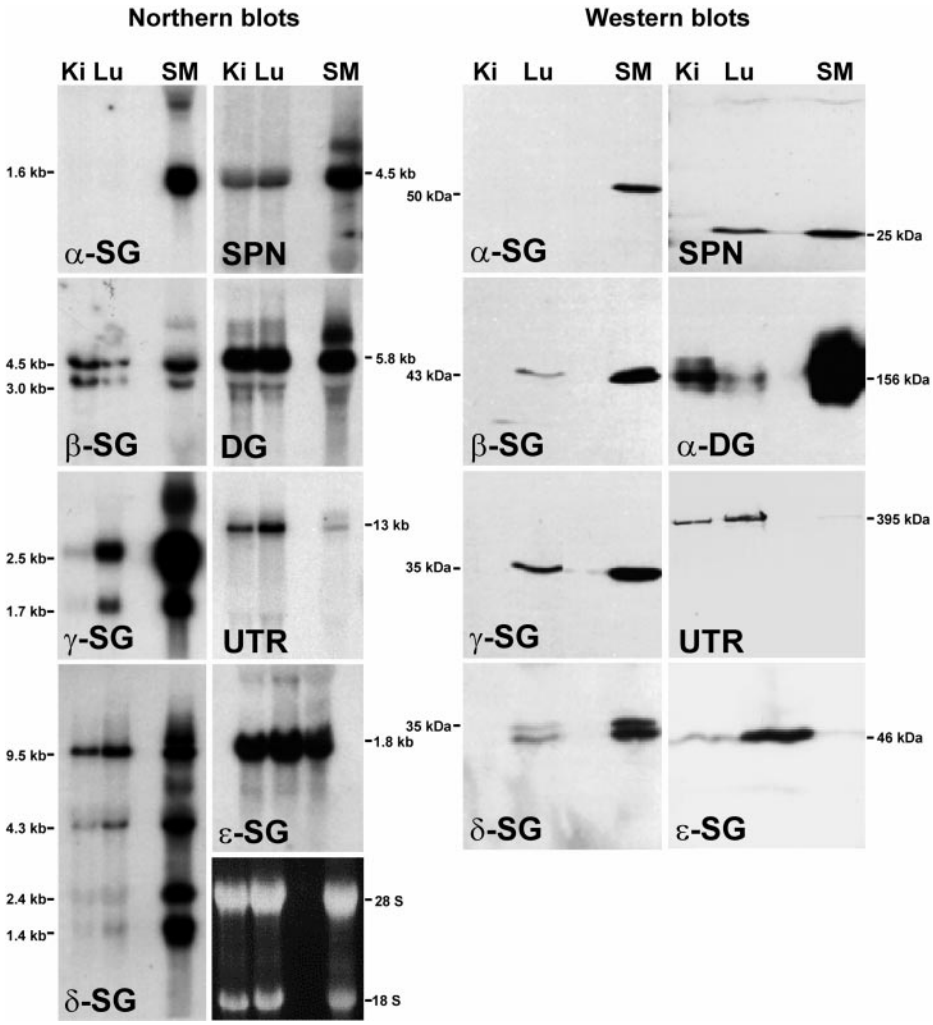

FIG. 1. **Expression of the sarcoglycans, sarcospan, dystroglycan, and utrophin in kidney, lung, and skeletal muscle.** Membranes with RNA from rabbit kidney (*Ki*), lung (*Lu*), and skeletal muscle (*SM*) were probed with cDNA probes against α-sarcoglycan (*α-SG*), β-sarcoglycan (*β-SG*), γ-sarcoglycan (*γ-SG*), δ-sarcoglycan (*δ-SG*), sarcospan (*SPN*), dystroglycan (*DG*), utrophin (*UTR*), and ε-sarcoglycan (*ε-SG*). Equal loading of RNA was verified by ethidium bromide visualization of ribosomal RNA. The identity of the signal detected above the 5.8-kb dystroglycan transcript in the skeletal muscle lane is not known. Five hundred μg of KCl-washed membranes from rabbit kidney and lung and 250 μg of KCl-washed membranes from rabbit skeletal muscle membranes (for ε-sarcoglycan blot, 500 μg of skeletal muscle KCl membranes was used) were separated on 3–12% polyacrylamide gels and transferred to nitrocellulose. Nitrocellulose filters were separately probed with antibodies against α-sarcoglycan (*α-SG*), β-sarcoglycan (*β-SG*), γ-sarcoglycan (*γ-SG*), δ-sarcoglycan (*δ-SG*), sarcospan (*SPN*), α-dystroglycan (*α-DG*), utrophin (*UTR*), and ε-sarcoglycan (*ε-SG*).

dystroglycan is not associated with any of the known sarcoglycans and can be separated from the smooth muscle dystroglycan complex. Dystroglycan, β-sarcoglycan, δ-sarcoglycan, ε-sarcoglycan, and sarcospan are all part of a smooth muscle complex, whereas neither α-, β-, γ-, and δ-sarcoglycan nor sarcospan are expressed in epithelial cells from lung and kidney. ε-Sarcoglycan, on the other hand, is expressed in epithelial cells (36) but is not complexed with dystroglycan, as revealed by sucrose gradient fractionation. We have also partially characterized dystroglycan from kidney, an organ mainly composed of epithelial cells. We found that the major part of α-dystroglycan from adult kidney has a molecular mass of 156 kDa, whereas the major part of α-dystroglycan from fetal kidney has a molecular mass of 120 kDa. Both forms were shown to bind laminin-1. Taken together, our results demonstrate a distinct smooth muscle dystroglycan complex and a distinct epithelial dystroglycan complex, and this information will be useful for further investigation of dystroglycan function.

EXPERIMENTAL PROCEDURES

Animals—New Zealand White rabbits were from Knapp Creek Farms (Amana, IA). Mice (C57BL/10) were bred at the University of Iowa from stocks originally obtained from Jackson Laboratories (Jackson Laboratories, Bar Harbor, ME). F1B control and BIO 14.6 cardiomyopathic hamsters were obtained from BioBreeders (Fitchburg, MA). All animals were kept in the animal care unit of the University of Iowa College of Medicine according to the animal care guidelines.

Northern Blot Analysis—Total RNA from rabbit kidney, lung, and skeletal muscle was extracted using RNAzol B (Tel-Test) according to the manufacturer's specifications. 20 μg of total RNA was electrophoresed on a 1.25% agarose gel containing 5% formaldehyde. Equal loading was verified by ethidium bromide visualization of the RNA, which was subsequently transferred to Hybond N membrane (Amersham Pharmacia Biotech). RNA was cross-linked to the membrane using a Stratagene UV cross-linker. Membranes were then prehybridized and hybridized using standard methods (40). Hybridization was performed with the following cDNA probes: a 1.1-kb[2] cDNA probe representing mouse α-sarcoglycan (41); a 1-kb cDNA probe representing hamster β-sarcoglycan (40); a 1-kb cDNA probe representing hamster γ-sarcoglycan (40); a 1-kb probe representing hamster δ-sarcoglycan (40); a 0.7-kb cDNA probe representing human sarcospan (5); a rabbit full-length clone of dystroglycan (18); a 1-kb cDNA clone corresponding to exon 70 through the beginning of 79 of mouse utrophin; an 850-base pair *Eco*RI/*Xho*I fragment derived from EST clone 1149778 corresponding to mouse ε-sarcoglycan (36). cDNA inserts were labeled with [α-^{32}P]dCTP to a specific activity of 2×10^8 cpm/μg DNA using Ready Prime kit (Amersham Pharmacia Biotech). Washes were carried out at 65 °C in 1× SSC, 1% SDS initially and then in 0.1× SSC, 0.1% SDS. Membranes were exposed for autoradiography.

Antibodies—Mouse monoclonal antibody IIH6 against α-dystroglycan (8) and rabbit polyclonal antibodies against α-sarcoglycan (rabbit 98) (42), δ-sarcoglycan (rabbit 215) (43), ε-sarcoglycan (rabbit 232) (41), sarcospan (rabbits 216 and 235) (5, 41), and utrophin (rabbit 56) (44) were previously described. An affinity purified rabbit antibody (rabbit 245) was produced against a COOH-terminal fusion protein of γ-sarcoglycan containing amino acids 167–291. Goat polyclonal antibodies against β-sarcoglycan (goat 26) (41) and goat 20 antiserum (18) were described previously. Monoclonal antibodies Ad1/20A6 against α-sarcoglycan, βSarc1/5B1 against β-sarcoglycan, and 35DAG/21B5 against γ-sarcoglycan were generated in collaboration with Louise V. B. Anderson (Newcastle General Hospital, Newcastle upon Tyne, UK). Monoclonal antibody 43 DAG/8D5

[2] The abbreviations used are: kb, kilobase pair; PAGE, polyacrylamide gel electrophoresis; WGA, wheat germ agglutinin.

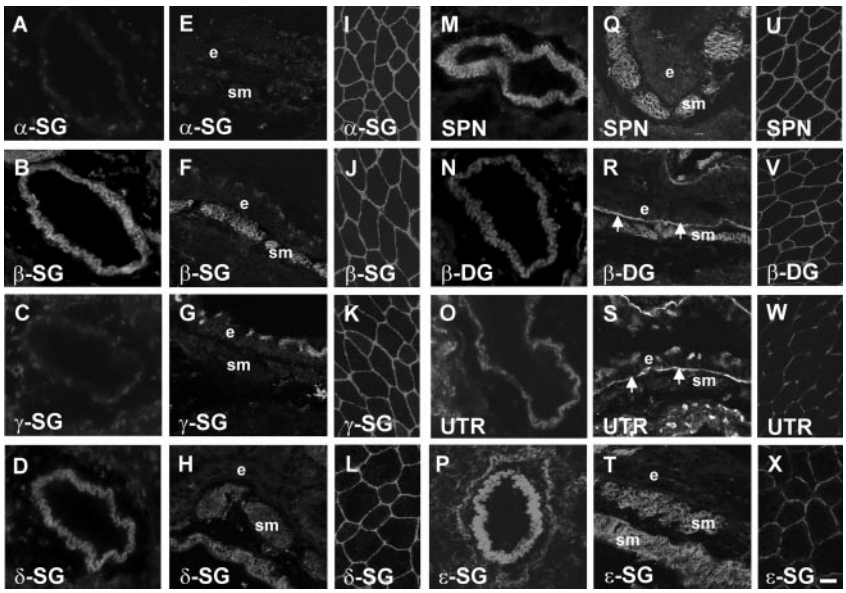

FIG. 2. **Immunofluorescence analysis of dystroglycan-associated proteins in lung.** Cryosections from mouse lungs (*A–H, M–O,* and *Q–S*), mouse skeletal muscle (*I—L* and *U–W*), hamster lung (P and *T*), and hamster skeletal muscle (*X*) were labeled by indirect immunofluorescence with antibodies against α-sarcoglycan (*A, E,* and *I*), β-sarcoglycan (*B, F,* and *J*), γ-sarcoglycan (*C, G,* and *K*), δ-sarcoglycan (*D, H,* and *L*), sarcospan (*M, Q,* and *U*), β-dystroglycan (*N, R,* and *V*), and utrophin (*O, S,* and *W*). The rabbit polyclonal antibody against human ε-sarcoglycan has a higher affinity for hamster ε-sarcoglycan than mouse ε-sarcoglycan. We therefore used hamster tissues to analyze ε-sarcoglycan expression (*P, T,* and *X*). *A–D* and *M–P* show stainings of the smooth muscle of arteries, whereas *E–H* and *Q–T* show stainings of smooth muscle and respiratory epithelium. *e*, epithelium; *sm*, smooth muscle. *Arrows* in *R* and *S* denote epithelial basement membranes. *Bar*, 50 μm.

against β-dystroglycan was also generated by Louise V. B. Anderson. Polyclonal antibodies against dystroglycan fusion protein B (18) were affinity purified from sheep OR12. Sheep OR12 was injected with fusion protein B and boosted with fusion protein D (18).

Immunoblot Analysis of Membrane-enriched Preparations—Preparation of KCl-washed membranes from adult rabbit kidney, lung, and skeletal muscle were described previously (45). Membranes were resolved by SDS-PAGE on 3–12% linear gradients and transferred to nitrocellulose membranes (46). Immunoblot staining was performed as described previously (45).

Sucrose Gradient Fractionation Analysis of Purified Dystroglycan Complexes—Tissues (1–5 g) were solubilized in 100 ml of 50 mM Tris-HCl, pH 7.4, 500 mM NaCl containing 1% digitonin in the presence of pepstatin A (0.6 μg/ml), aprotinin (0.5 μg/ml), leupeptin (0.5 μg/ml), phenylmethylsulfonyl fluoride (0.1 mM), benzamidine (0.75 mM), calpain inhibitor I (5 nM), and calpeptin (5 nM). The solubilized proteins were circulated overnight at 4 °C on an 8-ml wheat germ agglutinin (WGA)-agarose column (Vector Laboratories). The columns were washed with 40 ml of 50 mM Tris-HCl, pH 7.4, 500 mM NaCl containing 0.1% digitonin and eluted with 0.3 M *N*-acetylglucosamine in 50 mM Tris-HCl, 500 mM NaCl containing 0.1% digitonin. The WGA eluate was incubated with protein G (Amersham Pharmacia Biotech)-agarose for 1 h, to remove contaminating immunoglobulins. The protein G beads were spun down, and the supernatant was diluted to 100 mM NaCl with 50 mM Tris-HCl, pH 7.4, containing 0.1% digitonin and applied to a DEAE-cellulose column and washed with 50 mM Tris-HCl, pH 7.4, 100 mM NaCl containing 0.1% digitonin. The column was eluted with a gradient of 100–750 mM NaCl buffer containing 50 mM Tris-HCl, pH 7.4, and 0.1% digitonin, and 4-ml fractions were collected. The fractions containing dystroglycan were pooled and concentrated to 400 μl using Centricon-30 concentrators (Amicon). The samples were applied to a 5–30% sucrose gradient and centrifuged with a Beckman VTi65.1 vertical rotor at 200,000 × *g* for 3 h at 4 °C. The gradients were fractionated into 800-μl fractions, which were immunoblotted as described (45).

Laminin Preparation and Biotinylation—Mouse EHS laminin (laminin-1) was generously provided by Dr. Hynda K. Kleinman at the National Institutes of Health. Laminin-1 was biotinylated with NHS-biotin (Vector Laboratories) in a molar ratio of 1:750–1000 in reaction buffer 0.2 M $NaHCO_3$, pH 8.5, containing 0.5 M NaCl. The reaction was quenched, and unbound biotin was removed by dialysis against Tris-buffered saline (50 mM Tris, pH 7.5, 150 mM NaCl).

Laminin Overlay Assay—Dystroglycan containing sucrose gradient fractions were separated on 3–12% gradient gels and transferred to nitrocellulose membranes as described (45). The blots were blocked in laminin binding buffer (140 mM NaCl, 1 mM $MgCl_2$, 10 mM triethanolamine, pH 7.6) containing 5% non-fat dry milk and subsequently washed for 90 min in laminin binding buffer containing 0.05% Tween 20 (TLBB). Blots were incubated in TLBB containing 3% bovine serum albumin and biotinylated laminin-1 for 8 h and washed in TLBB for 20 min. After incubation with ABC reagents (Vector Laboratories), the blots were washed in TLBB for 20 min and developed in 4-chloro-1-naphthol and H_2O_2.

Immunofluorescence Analysis—For immunofluorescence analysis, 8-μm cryosections were prepared from C57BL/10 wild type mouse lungs, kidneys, and skeletal muscle, and F1B and BIO 14.6 hamster lungs, kidneys, and skeletal muscle. All procedures were performed at room temperature. Sections were blocked with 3% bovine serum albumin in phosphate-buffered saline for 30 min and then incubated with the primary antibodies for 90 min or overnight. After washing in phosphate-buffered saline, sections were incubated with Cy3-conjugated secondary antibodies (Vector Laboratories) for 90 min and then mounted with Vectashield mounting medium and observed under a Bio-Rad MRC-600 laser scanning confocal microscope.

RESULTS

Expression of the Sarcoglycans, Sarcospan, Dystroglycan, and Utrophin in Kidney and Lung—In order to analyze which proteins are associated with dystroglycan in kidney and lung, we first screened for the presence of sarcoglycan mRNAs in these organs. As expected, the 1.6-kb α-sarcoglycan transcript was only detected in skeletal muscle (Fig. 1). The previously characterized 4.4- and 3.0-kb transcripts representing β- sarcoglycan (10, 11) were present in both lung and kidney as well as the previously characterized δ-sarcoglycan mRNAs (40) of 9.5, 4.3, 2.3, and 1.4 kb (Fig. 1). In kidney and lung the 9.5-kb δ-sarcoglycan transcript appeared to be the most prominent. γ-Sarcoglycan mRNAs of 2.5 and 1.7 kb were faintly expressed in kidney, moderately in lung, and strongly in skeletal muscle (Fig. 1). Sarcospan mRNA has been shown to be expressed in several organs (5), and the 4.5-kb transcript could also be detected in kidney and lung (Fig. 1). In contrast to the sarcoglycan and sarcospan mRNAs, which are all more highly expressed in skeletal muscle than kidney and lung, an equal intensity of the 5.8-kb dystroglycan transcript was detected in the three organs (Fig. 1). The 1.8-kb ε-sarcoglycan mRNA was highly expressed in kidney and lung (Fig. 1). ε-Sarcoglycan was also detected in skeletal muscle but at a lower level. Similarly,

the 13-kb utrophin transcript was also detected in kidney and lung and at a lower level in skeletal muscle (Fig. 1).

Because it is possible that transcripts are present in cells without being translated, we next analyzed whether the sarcoglycan protein products were expressed in the kidney and lung. KCl-washed membranes from kidney, lung, and skeletal muscle were screened for protein expression. Although the β-, γ-, and δ-sarcoglycan transcripts were readily expressed in the kidney, we were not able to detect their corresponding polypeptides (Fig. 1). In the lung, however, the 43-kDa β-sarcoglycan, the 35-kDa γ-sarcoglycan, and the 35-kDa δ-sarcoglycans were clearly detectable (Fig. 1). Likewise, the 25-kDa sarcospan was not present in the kidney but was present in the lung (Fig. 1). In contrast, dystroglycan with a molecular mass of 156 kDa, the 46-kDa ε-sarcoglycan, and the 395-kDa utrophin were all readily detected in kidney and lung (Fig. 1).

Previous reports have shown that full-length dystrophin is present in the smooth muscle of kidney but not epithelial cells (16, 47). In the lung, dystrophin is exclusively expressed in smooth muscle (48). The adult lung is mainly composed of epithelial cells and smooth muscle, whereas the kidney is largely composed of epithelial cells. Thus, the expression data suggest that the presence of dystroglycan, β-sarcoglycan, γ/δ-sarcoglycan, ε-sarcoglycan, sarcospan, and utrophin/dystrophin in lung could represent the dystroglycan complex from smooth muscle. Likewise, the presence of dystroglycan, ε-sarcoglycan, and utrophin in the kidney could represent an epithelial complex. To test that hypothesis we used immunofluorescence analysis and sucrose gradient fractionation to determine whether proteins observed by Western blot were derived from epithelial cells, smooth muscle, or both.

Distribution of Dystroglycan-associated Components in Lung—By immunofluorescence analysis we found that α- and γ-sarcoglycan were absent in smooth muscle surrounding respiratory epithelium, whereas β-, δ- and ε-sarcoglycan and sarcospan were readily detected, along with utrophin and β-dystroglycan (Fig. 2). Similar results were found in the smooth muscle of arteries within the lung (Fig. 2). In addition, in other smooth muscle containing tissues similar observations have been made.[1] Although γ-sarcoglycan was readily detected in lung by Western blot analysis (see Fig. 1), we were never able to detect it by immunofluorescence in lung or in any other organ, and currently we do not have any explanation for this discrepancy. As we did not detect α-sarcoglycan in the lung, we can rule out contamination of striated muscle in the lung preparations. Dystroglycan is expressed in most epithelial cells underlying the basement membrane (23). This is also true for lung, as exemplified in Fig. 2*R*, where β-dystroglycan was detected in the basement membrane area of the respiratory epithelium. As expected, β-dystroglycan was also expressed in the surrounding smooth muscle. Also, utrophin was co-expressed with β-dystroglycan in the epithelial basement membrane region and smooth muscle (Fig. 2*S*). However, none of the sarcoglycans could be detected in the epithelial cells. These data indicate that there might be two dystroglycan complexes in lung, one associated with epithelial cells and one associated with smooth muscle.

Biochemical Characterization of Dystroglycan Complexes from Lung—We demonstrated the presence of two dystroglycan complexes in lung by partially purifying dystroglycan from lung. Purification of dystroglycan was achieved by WGA affinity chromatography followed by ion exchange chromatography and high speed centrifugation through sucrose gradients. Only proteins that bind with high affinity and specificity will be retained with dystroglycan during sucrose gradient fractionation. This purification method has been successfully used to purify the dystroglycan complex from skeletal and cardiac muscle (49). Dystroglycan was extracted from adult rabbit lung, and proteins from the sucrose gradient fractions were separated by SDS-PAGE. The resulting polyacrylamide gels were immunoblotted with various antibodies. Staining with monoclonal antibodies against β-dystroglycan revealed one peak of β-dystroglycan in fractions 6–8 and one peak in the last three fractions (Fig. 3). β-Dystroglycan in the last fractions was accompanied with dystrophin, utrophin, β-sarcoglycan, γ/δ-sarcoglycan, ε-sarcoglycan, and sarcospan (Fig. 3 and data not shown). A 156-kDa α-dystroglycan band was detected in fractions 3–8, suggesting that some α-dystroglycan is associated with β-dystroglycan seen in fractions 6–8 but also that some α-dystroglycan is not associated with β-dystroglycan (Fig. 3). Antibody IIH6 which shows a carbohydrate-dependent staining did not detect any α-dystroglycan in the last fractions. Interestingly, IIH6 does not detect smooth muscle α-dystroglycan.[1] However, it has been shown that smooth muscle α-dystroglycan has a molecular mass of 100 and 156 kDa.[1] In none of the earlier fractions (fractions 6–8) did we observe a co-migration of dystroglycan with other dystroglycan-associated proteins. Together, these data indicate that α- and β-dystroglycan seen in the earlier fractions may represent dystroglycan derived from epithelial cells, and dystroglycan seen in fractions 11–14 accompanied by dystroglycan-associated proteins may represent the smooth muscle dystroglycan complex.

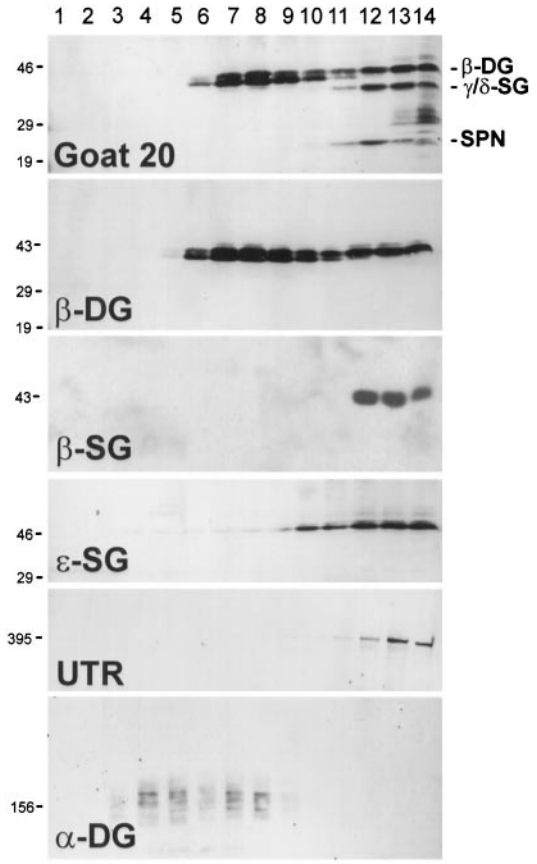

FIG. 3. **Presence of an epithelial and smooth muscle dystroglycan complex in lung.** Purified dystroglycan complex from rabbit lung was centrifuged through sucrose gradients. *Fractions 1–14* from the sucrose gradients were electrophoresed on 3–12% SDS-polyacrylamide gels. Nitrocellulose transfers of identical samples were stained with goat 20 antiserum and antibodies against β-dystroglycan (*β-DG*), β-sarcoglycan (*β-SG*), ε-sarcoglycan (*ε-SG*), utrophin (*UTR*), and α-dystroglycan (*α-DG*). Molecular masses are indicated in kDa.

This was further shown by sucrose gradient fractionation analysis of purified dystroglycan from the BIO 14.6 hamster lung. Recently, a mutation in the δ-sarcoglycan gene of the BIO

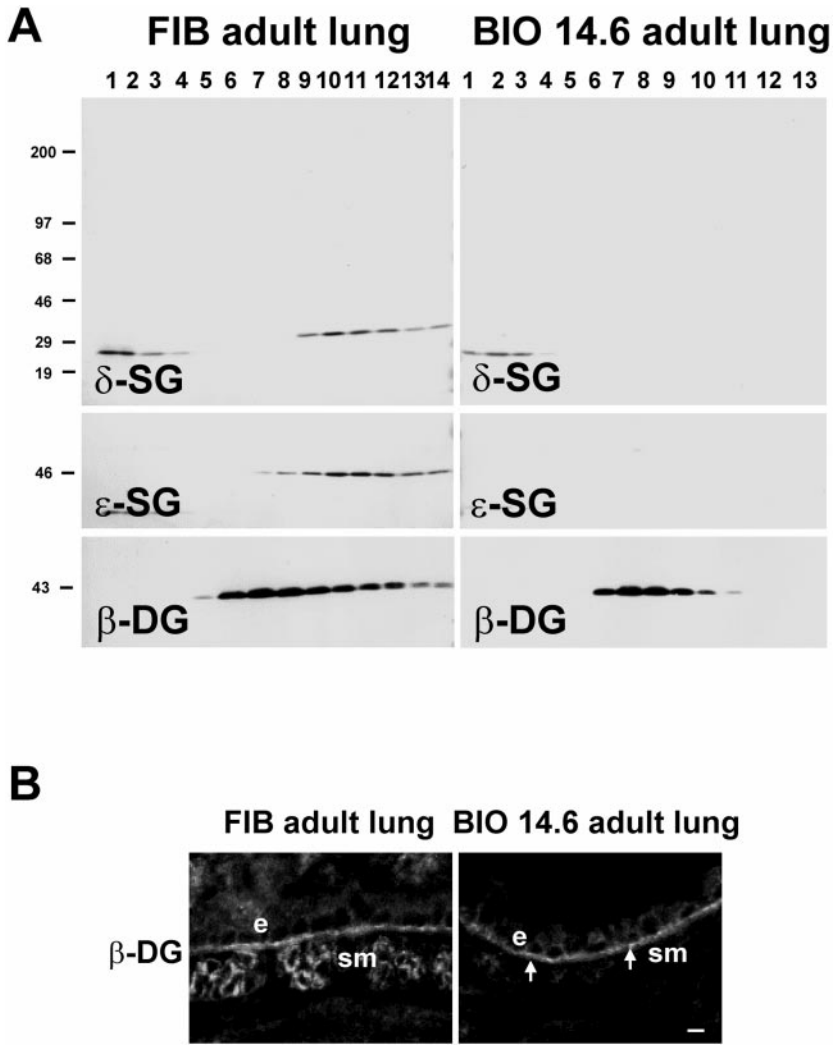

FIG. 4. **Absence of dystroglycan in smooth muscle but not epithelial cells of the δ-sarcoglycan-deficient BIO 14.6 hamster.** *A,* sucrose gradient fractions of purified dystroglycan complex from F1B and BIO 14.6 hamster lungs were resolved by 3–12% SDS-PAGE and transferred to nitrocellulose. The lanes represent fractions of the sucrose gradient fractionation. Control (*F1B*) and BIO 14.6 blots were stained with δ-sarcoglycan antibodies (δ-*SG*), ϵ-sarcoglycan antibodies (ϵ-*SG*), and β-dystroglycan antibodies (β-*DG*). Molecular standards in kDa are indicated at *left*. *B,* cryosections from F1B and BIO 14.6 hamster lungs were labeled by indirect immunofluorescence with antibodies against β-dystroglycan. *e*, epithelium; *sm*, smooth muscle. *Arrows* denote basement membrane. *Bar*, 50 μm.

14.6 cardiomyopathic hamster was identified to cause the disease in this animal strain (50, 51). As previously shown, the null mutation in the δ-sarcoglycan gene affects the distribution of α- and β-dystroglycan and the sarcoglycans in skeletal muscle of the BIO 14.6 hamster (40, 43, 52). As δ-sarcoglycan is absent from epithelial cells, the null mutation in the BIO 14.6 hamster should not affect dystroglycan in epithelial cells of the lung but would presumably affect the expression in lung smooth muscle. We demonstrate that this is indeed the case. The control hamster sucrose gradient profile showed that δ- and ϵ-sarcoglycan co-sedimented in fractions 9–14 (Fig. 4*A*). β-Dystroglycan co-sedimented with the smooth muscle sarcoglycans in fractions 9–14 (Fig. 4*A*). In addition, there were earlier fractions (fractions 6–8), with high levels of β-dystroglycan without association of the sarcoglycans (Fig. 4*A*). The epithelial β-dystroglycan signals in these fractions remained in the BIO 14.6 lung fractions, whereas smooth muscle β-dystroglycan in the later fractions was absent (Fig. 4*A*). As expected, no sarcoglycan staining could be detected in BIO 14.6 lung fractions (Fig. 4*A*). The presence of β-dystroglycan in lung epithelial cells of the BIO 14.6 hamster was further verified by immunofluorescence analysis. β-Dystroglycan remained in the basement membrane region of lung epithelial cells but was absent in lung smooth muscle of the BIO 14.6 hamster (Fig. 4*B*). Taken together these results suggest that within one tissue there are different dystroglycan complexes. To determine further the composition of the dystroglycan complex from epithelial cells, we next isolated the dystroglycan-containing complex from adult rabbit kidney.

Characterization of the Dystroglycan Complex from Adult Rabbit Kidney—The dystroglycan complex was extracted from adult rabbit kidney by WGA affinity chromatography followed by ion exchange on a DEAE column and sucrose gradient fractionation. Proteins from the sucrose gradient fractions were separated by SDS-PAGE. The resulting polyacrylamide gels were immunoblotted with antibodies to the components of the dystroglycan complex. Staining with monoclonal antibody IIH6 revealed a 156-kDa α-dystroglycan band, peaking in fractions 7 and 8 (Fig. 5). Immunoblotting with antiserum against purified skeletal muscle dystroglycan complex (goat 20) revealed α-dystroglycan as a broad smear between 100 and 156 kDa suggesting the existence of smaller molecular weight isoforms of α-dystroglycan (Fig. 5). Goat 20 antiserum was raised against the skeletal muscle dystroglycan complex (5) and recognizes dystrophin, α-dystroglycan, β-dystroglycan, the sarcoglycan and syntrophin complexes (Fig. 5). Staining with goat 20 antiserum also revealed a peak of β-dystroglycan in fractions 7 and 8 (Fig. 5). This was further verified using monoclonal antibody 43DAG/8D5 against β-dystroglycan (data not shown).

As previously indicated we did not detect α-, β-, γ-, δ-sarcoglycan or sarcospan in the kidney as shown by immunostaining using goat 20 antiserum and monoclonal antibodies against the sarcoglycans (Fig. 5 and data not shown). Furthermore, sucrose gradient fractionation separated ϵ-sarcoglycan and utrophin from dystroglycan. Western blotting with ϵ-sarcoglycan antibodies demonstrated that ϵ-sarcoglycan began to sediment in

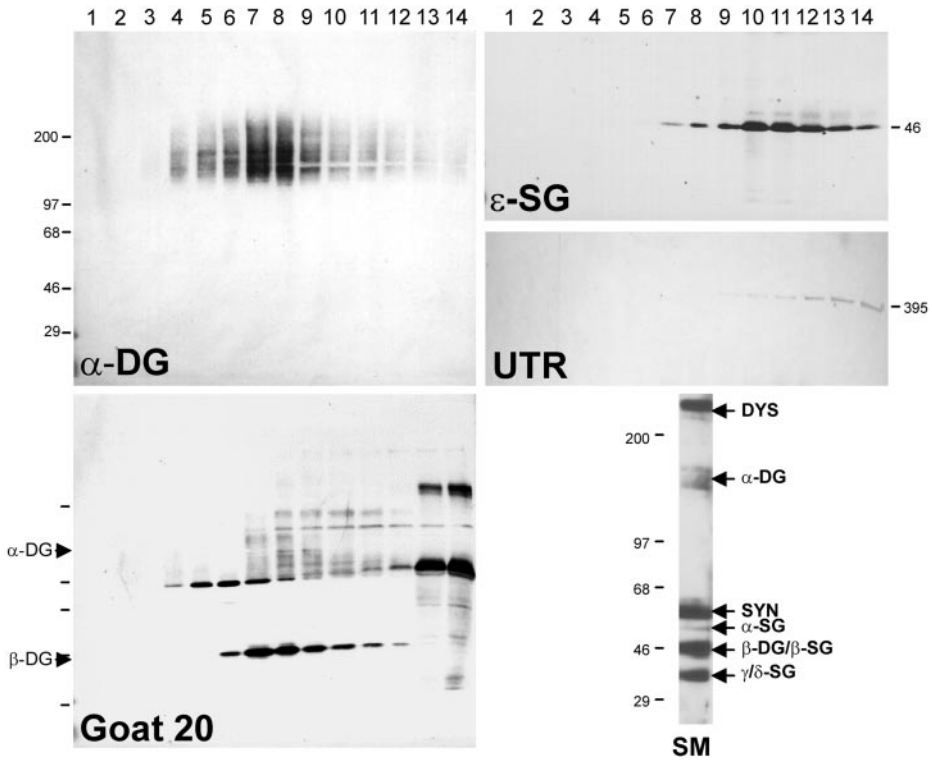

FIG. 5. **Biochemical characterization of kidney dystroglycan.** Sucrose gradient fractions of purified dystroglycan from adult rabbit kidney were resolved by 3–12% SDS-PAGE and transferred to nitrocellulose. Blots of identical samples were probed with α-dystroglycan (*α-DG*) antibodies, goat 20 antiserum, and ε-sarcoglycan (*ε-SG*) and utrophin (*UTR*) antibodies. The strong bands seen in fractions 13 and 14 on the goat 20 blot most likely represent band 3 from erythrocytes (revealed by using band 3-specific antibodies, data not shown). A blot of purified dystroglycan complex from rabbit skeletal muscle (*sm*) stained with goat 20 antiserum is shown for comparison. Molecular standards are indicated (kDa).

fraction 7 and peaked in fractions 10 and 11 (Fig. 5). As described, α- and β-dystroglycan peaked in fractions 7 and 8 (Fig. 5). Utrophin began to sediment in fraction 10 and peaked in fractions 12–14 (Fig. 5). Taken together, it appears that ε-sarcoglycan and utrophin are not integral components of a kidney dystroglycan complex, at least not in the adult kidney. In the fetal kidney, however, dystroglycan and ε-sarcoglycan are colocalized in epithelial cells (19, 36). Utrophin mRNA is also highly expressed in fetal kidney (53). Hence, we next isolated fetal kidney dystroglycan complex to determine whether dystroglycan, ε-sarcoglycan, and utrophin could be complexed at early stages of development.

Characterization of the Dystroglycan Complex from Fetal Rabbit Kidney—The dystroglycan complex was extracted from fetal rabbit kidneys. Centrifugation of the dystroglycan complex through sucrose density gradients followed by SDS-PAGE and immunoblotting with monoclonal antibody IIH6 revealed a major dystroglycan band of 120 kDa (Fig. 6). Also, dystroglycan antibodies from sheep OR 12 reacted with a 120-kDa α-dystroglycan band in addition to β-dystroglycan bands of 43 kDa (Fig. 6). At present it is unclear why two bands of β-dystroglycan around 43 kDa are detected. They could represent post-translational modification and/or processing events of β-dystroglycan. Stainings with antiserum from goat 20 demonstrated the presence of a possible 100-kDa form of α-dystroglycan (Fig. 6). In summary, it appears that α-dystroglycan in fetal rabbit kidney has a molecular mass of 120 kDa, and smaller isoforms may also exist.

As in the adult kidney, we did not detect α-, β-, γ-, δ-sarcoglycans or sarcospan in fetal kidney (Fig. 6). Also, sucrose gradient fractionation separated fetal kidney ε-sarcoglycan from fetal kidney dystroglycan, suggesting that ε-sarcoglycan is not an integral component of a fetal kidney dystroglycan complex (Fig. 6). Utrophin began to sediment in fractions 7 and 8. Then the expression declined in fractions 9 and 10 and peaked in fractions 12–14 (Fig. 6). Unlike ε-sarcoglycan, utrophin expression still overlaps with dystroglycan expression, raising the possibility that in the fetal kidney, utrophin could be part of a dystroglycan complex.

Kidney Dystroglycan Binds Laminin-1—The role of laminin-1 and dystroglycan in embryonic kidney development has been well established (19, 54), but it has never been formally shown that they are directly associated. The molecular mass of α-dystroglycan in the adult kidney is 156 kDa and in the fetal kidney α-dystroglycan is 120 kDa (Figs. 5–7). To address the question whether the two dystroglycan isoforms bind laminin-1, we performed a laminin-overlay assay using biotinylated laminin-1. As shown in Fig. 7, biotinylated laminin-1 bound the 156-kDa α-dystroglycan from adult kidney and the 120-kDa α-dystroglycan from fetal kidney.

DISCUSSION

The overall goal of this study was to biochemically characterize the dystroglycan complex in epithelial cells. Our results demonstrated the following: 1) dystroglycan present in epithelial cells is not associated with any of the known sarcoglycans; 2) the presence of β-sarcoglycan, δ-sarcoglycan, and sarcospan in non-skeletal muscle organs reflects association with dystroglycan in smooth muscle within these organs; and 3) α-dystroglycan from fetal and adult kidney may be differentially glycosylated, but both isoforms bind laminin-1.

Our statements are based on the following observations. We did not detect α-, β-, γ-, δ-sarcoglycan or sarcospan in kidney or lung epithelial cells. Similar results were obtained in esophagus, bladder, and small intestine (data not shown). Although we identified sarcoglycan and sarcospan transcripts in the kidney, we were never able to detect corresponding polypeptides. By immunofluorescence, we found expression of these proteins in smooth muscle of large blood vessels only (data not shown). β-Sarcoglycan, δ-sarcoglycan, and sarcospan present in lung were shown to be part of a smooth muscle dystroglycan complex as revealed by sucrose gradient fractionation. Two other dystroglycan-associated proteins are ε-sarcoglycan and utrophin. The former protein has been shown to be an integral component of smooth muscle sarcoglycan complex[1] and the latter an integral component of the dystroglycan complex found in neuromuscular junctions (55). Dystroglycan and ε-sarcoglycan are

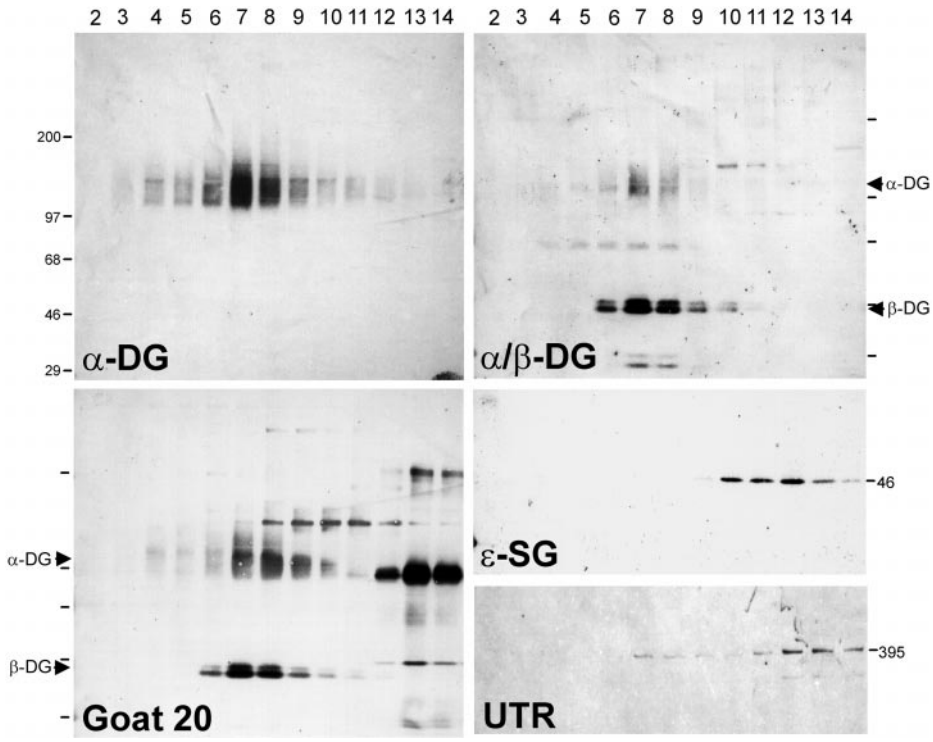

FIG. 6. **Dissociation of ϵ-sarcoglycan from fetal kidney dystroglycan.** Dystroglycan purified from fetal rabbit kidney was centrifuged through sucrose gradients. *Fractions 2–14* from the sucrose gradient were electrophoresed on 3–12% polyacrylamide gels. Nitrocellulose transfers of identical samples were stained with α-dystroglycan (*α-DG*) antibodies, goat 20 antiserum, FP-B-antibodies (*α/β-DG*), ϵ-sarcoglycan antibodies (*ϵ-SG*), and utrophin antibodies (*UTR*). The strong bands seen in fractions 13 and 14 on goat 20 blot most likely represent band 3 from erythrocytes (revealed by using band 3-specific antibodies, data not shown). Molecular standards are indicated (kDa).

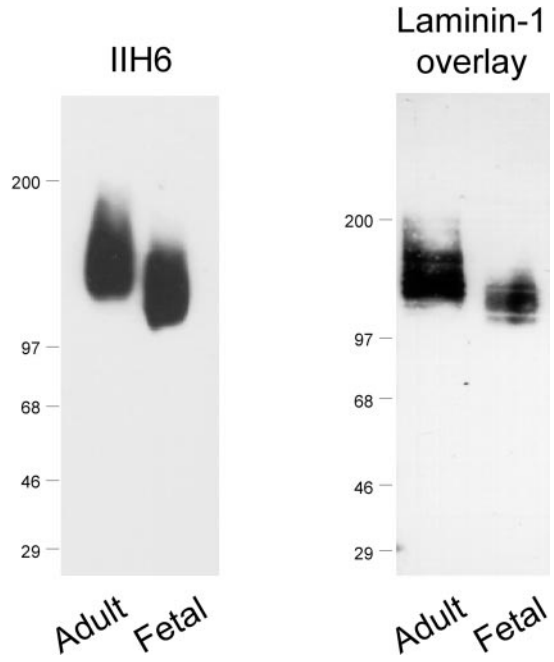

FIG. 7. **Adult and fetal kidney α-dystroglycan bind laminin-1.** Dystroglycan-rich sucrose gradient fractions from adult and fetal dystroglycan preparations were separated on 3–12% gradient gels and transferred to nitrocellulose and subsequently probed with IIH6 for a side-by-side comparison of the molecular weight of α-dystroglycan from adult and fetal kidney. Nitrocellulose transfers were also incubated with biotinylated laminin-1. Both the 156- and 120-kDa isoforms of kidney α-dystroglycan bind laminin-1. Molecular masses in kDa are indicated at *left*.

co-localized in embryonic kidney epithelium, and utrophin mRNA is also present in the embryonic kidney, hence these molecules could be associated with each other (19, 36, 53). Likewise, dystroglycan and utrophin are partially co-expressed in adult kidney epithelium (17, 19). However, in adult kidney, dystroglycan, ϵ-sarcoglycan, and utrophin dissociate from each other during sucrose gradient fractionation, indicating that they are not tightly associated in adult kidney epithelial cells. Moreover, dystroglycan and utrophin are co-expressed in epithelial cells of the lung (see Ref. 17, see also Fig. 2), but we were also unable to demonstrate an association of these two proteins in lung epithelial cells. Nevertheless, we were able to demonstrate an association of β-dystroglycan, β-, δ-, and ϵ-sarcoglycan, and utrophin in smooth muscle indicating that the extraction method applied here does not disrupt the interactions between the components of the smooth muscle dystroglycan complex. Weak interactions between β-dystroglycan, ϵ-sarcoglycan, and utrophin in epithelial cells could possibly become disrupted in our preparations, but also genetic evidence supports our interpretation: β-dystroglycan is retained in lung epithelial cells of utrophin null mice (17).

Also, dystroglycan and ϵ-sarcoglycan in the fetal kidney are not associated as revealed by sucrose gradient fractionation. Utrophin, however, may be associated with dystroglycan in fetal kidney. Although the major part of utrophin does not co-migrate with dystroglycan, as revealed by sucrose gradient fractionation, there is a weaker peak of utrophin in fractions 7 and 8, co-migrating with dystroglycan, preceding the peak of utrophin in the later fractions (see Fig. 2). It is interesting to note that as skeletal muscle matures utrophin is gradually replaced by dystrophin (56), and one possibility is that dystroglycan in mature non-muscle organs interacts with yet unidentified cytoskeletal proteins or some of the shorter dystrophin isoforms. For example, Dp140, a shorter dystrophin isoform, is expressed in a subset of both fetal and mature kidney epithelial cells (16, 47). However, we have not been able to demonstrate an interaction between Dp140 and β-dystroglycan.[3] Still, it would be interesting to generate mice lacking all possible dystrophin and utrophin isoforms to study the roles of these proteins in epithelial cells.

β-Dystrobrevin is another dystrophin-related protein that has been shown to be highly expressed in kidney, lung, and liver, but the subcellular localization of this protein is not known, and it has yet to be determined whether β-dystrobrevin

[3] M. Durbeej, D. Jung, and K. P. Campbell, unpublished data.

is directly or indirectly associated with β-dystroglycan (57–59). The syntrophins are dystrophin-binding proteins that also have been shown to be ubiquitously expressed (60), and very recently the β2-syntrophin was found to be associated with dystrobrevin in Madin-Darby canine kidney cells (61). Whether this is also seen in epithelial cells *in vivo* remains to be determined.

In this report, we have also characterized epithelial dystroglycan by analyzing dystroglycan from kidney. α-Dystroglycan from fetal kidney has a molecular mass of 120 kDa, whereas the adult kidney α-dystroglycan has 156 kDa. These data suggest that α-dystroglycan is differentially glycosylated in fetal *versus* mature tissue. The molecular weights of α-dystroglycan in other tissues also vary. For example, α-dystroglycan in mammalian skeletal muscle is 156 kDa (39), 140 kDa in cardiac muscle (62), and 120 kDa in brain and peripheral nerves (24, 38). There is evidence that α-dystroglycan is a sialylated mucin-type glycoprotein, and the structures of the sialylated *O*-linked oligosaccharides have been analyzed to some extent (63, 64), but the biological relevance of these differentially glycosylated isoforms is presently unclear. All dystroglycan isoforms appear to bind at least laminin-1 and laminin-2 (6, 18, 38), but the different glycosylation patterns in different tissues could reflect plasticity of the receptor function.

In summary, we have shown the presence of two different dystroglycan complexes in non-skeletal muscle organs: one from epithelial cells, which is not associated with any of the known sarcoglycans, and one from smooth muscle cells which is associated with β-, δ-, ϵ-sarcoglycan, sarcospan, and dystrophin/utrophin. There is yet the possibility that dystroglycan in epithelial cells is associated with hitherto unidentified molecules forming a similar complex as in skeletal and cardiac muscle, and this is currently being investigated.

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REFERENCES

1. Ervasti, J. M., Ohlendieck, K., Kahl, S. D., Gaver, M. G., and Campbell, K. P. (1990) *Nature* **345,** 315–319
2. Yoshida, M., and Ozawa, E. (1990) *J. Biochem. (Tokyo)* **108,** 748–752
3. Froehner, S. C., Adams, M. E., Peters, M. F., and Gee, S. H. (1997) *Soc. Gen. Physiol. Ser.* **52,** 197–207
4. Lim, L. E., and Campbell, K. P. (1998) *Curr. Opin. Neurol.* **11,** 443–452
5. Crosbie, R. H., Heighway, J., Venzke, D. P., Lee, J. C., and Campbell, K. P. (1997) *J. Biol. Chem.* **272,** 31221–31224
6. Sunada, Y., Bernier, S. M., Kozak, C. A., Yamada, Y., and Campbell, K. P. (1994) *J. Biol. Chem.* **269,** 13729–13732
7. Jung, D., Yang, B., Meyer, J., Chamberlain, J. S., and Campbell K. P. (1995) *J. Biol. Chem.* **270,** 27305–27310
8. Ervasti, J. M., and Campbell, K. P. (1993) *J. Cell Biol.* **122,** 809–823
9. Durbeej, M., Henry, M. D., and Campbell, K. P. (1998) *Curr. Opin. Cell Biol.* **10,** 594–601
10. Lim, L. E., Duclos, F., Broux, O., Bourg, N., Sunada, Y., Allamand, V., Meyer, J., Richard, I., Moomaw, C., Slaughter, C., Tomé, F. M. S., Fardeau, M., Lackson, C. E., Beckmann, J. S., and Campbell, K. P. (1995) *Nat. Genet.* **11,** 257–265
11. Bönnemann, C. G., Modi, R., Noguchi, S., Mizuno, Y., Yoshida, M., Gussoni, E., McNally, E. M., Duggan, C. A., Hoffman, E. P., Ozawa, E., and Kunkel, L. M. (1995) *Nat. Genet.* **11,** 266–273
12. Nigro, V., Piluso, G., Belsito, A., Politano, L., Puca, A. A., Papparella, S., Rossi, E., Viglietto, G., Esposito, M. G., Abbondanza, C., Medici, N., Molinari, A. M., Nigro, G., and Puca, G. A. (1996) *Hum. Mol. Genet.* **5,** 1179–1186
13. Jung, D., Duclos, F., Apostol, B., Straub, V., Lee J. C., Allamand V., Venzke, D. P., Sunada Y., Moomaw, C. R., Leveille, C. J., Slaughter, C. A., Crawford, T. O., McPherson, J. D., and Campbell, K. P. (1996) *J. Biol. Chem.* **271,** 32321–32329
14. Byers, T., Lidov, H. G., and Kunkel, L. M. (1993) *Nat. Genet.* **4,** 87–93
15. Schofield, J. N., Blake, D. J., Simmons, C., Morris, G. E., Tinsley, J. M., Davies, K. E., and Edwards, Y. H. (1994) *Hum. Mol. Genet.* **8,** 1309–1316
16. Durbeej, M., Jung, D., Hjalt, T., Campbell, K. P., and Ekblom, P. (1997) *Dev. Biol.* **181,** 156–167
17. Grady, R. M., Merlie, J. P., and Sanes, J. R. (1997) *J. Cell Biol.* **136,** 871–882
18. Ibraghimov-Beskrovnaya, O., Ervasti, J. M., Leveille, C. J., Slaughter, C. A., Sernett, S. W., and Campbell, K. P. (1992) *Nature* **355,** 696–702
19. Durbeej, M., Larsson, E., Ibraghimov-Beskrovnaya, O., Roberds, S. L., Campbell, K. P., and Ekblom P. (1995) *J. Cell Biol.* **130,** 79–91
20. Gorecki, D. C., Derry, J. M. J., and Barnard, E. A. (1994) *Hum. Mol. Gen.* **3,** 1589–1597
21. Williamson, R. A., Henry, M. D., Daniels, K. J., Hrstka, R. F., Lee, J. C., Sunada, Y., Ibraghimov-Beskrovnaya, O., and Campbell, K. P. (1997) *Hum. Mol. Genet.* **6,** 831–841
22. Blank, M., Koulen, P., and Kroger, S. (1997) *J. Comp. Neurol.* **389,** 668–678
23. Durbeej, M., Henry, M. D., Ferletta, M., Campbell, K. P., and Ekblom, P. (1998) *J. Histochem. Cytochem.* **46,** 449–457
24. Gee, S. H., Blacher, R. W., Douville, P. J., Provost, P. R., Yurchenco, P. D., and Carbonetto, S. (1993) *J. Biol. Chem.* **268,** 14972–14980
25. Ruegg, M. A., and Bixby, J. L (1998) *Trends Neurosci*. **21,** 22–27
26. Gesemannn, M., Brancaccio, A., Schumacher, B., and Ruegg, M. A. (1998) *J. Biol. Chem.* **273,** 600–605
27. Peng, H. B., Ali, A. A., Daggett, D. F., Rauvala, H., Hassel, J. R., and Smalheiser, N. R. (1998) *Cell. Adhes. Commun.* **5,** 475–489
28. Talts, J. F., Zeynep, A., Goring, W., Brancaccio, A., and Timpl, R. (1999) *EMBO J.* **18,** 863–870
29. Saito, F., Masaki, T., Kamakura, K., Anderson, L. V. B., Fujita, S., Fukuta-Ohi, H., Sunada, Y., Shimizu, T., and Matsumura, K. (1999) *J. Biol. Chem.* **274,** 8240–8246
30. James, M., Man, N. T., Wise, C. J., Jones, G. E., and Morris, G. E. (1996*) Cell Motil. Cytoskeleton* **23,** 163–174
31. Henry, M. D., and Campbell, K. P. (1998) *Cell* **95,** 859–870
32. Montanaro, F., Gee, S. H., Jacobson, C., Lindenbaum, M. H., Froehner, S. C., and Carbonetto, S. (1998) *J. Neurosci.* **18,** 1250–1260
33. Jacobson, C., Montanaro, F., Lindenbaum, M., Carbonetto, S., and Ferns, M. (1998) *J. Neurosci.* **18,** 6340–6348
34. Cao, W., Henry, M. D., Borrow, P., Yamada, H., Elder, J. H., Ravkov, E. V., Nichol, S. T., Compans, R. W., Campbell, K. P., and Oldstone, M. B. (1998) *Science* **11,** 2079–2081
35. Rambukkana, A., Yamada, H., Zanazzi, G., Mathus, T., Salzer, J. L., Yurchenco, P., Campbell, K. P., and Fischetti, V. A. (1998) *Science* **22,** 2076–2079
36. Ettinger, A. J., Feng, G., and Sanes, J. R. (1997) *J. Biol. Chem.* **272,** 32534–32538
37. McNally, E. M., Ly, C. T., and Kunkel, L. M. (1998) *FEBS Lett.* **422,** 27–32
38. Yamada, H., Shimizu, T., Tanaka, T., Campbell, K. P., and Matsumura, K. (1994) *FEBS Lett.* **352,** 49–53
39. Ervasti, J. M., and Campbell, K. P. (1991) *Cell* **66,** 1121–1131
40. Straub, V., Duclos, F., Venzke, D., Lee, J. C., Cutshall, S., Leveille, C. J., and Campbell, K. P. (1998) *Am. J. Pathol.* **153,** 1623–1630
41. Duclos, F., Straub, V., Moore, S. A., Venzke, D. P., Hrstka, R. F., Crosbie, R. H., Durbeej, M., Lebakken, C. S., Ettinger, A. J., Holt, K. H., Lim, L. E., Sanes, J. R., Davidson, B. L., Faulkner, J. A., Williamson, R., and Campbell, K. P. (1998) *J. Cell Biol.* **142,** 1461–1471
42. Roberds, S. L., Anderson, R. D., Ibraghimov-Beskrovnaya, O., and Campbell, K. P. (1993) *J. Biol. Chem.* **268,** 23739–23742
43. Holt, K. H., Lim, L. E., Straub, V., Venzke, D., Duclos, F., Anderson, R. D., Davidson, B. L., and Campbell, K. P. (1998) *Mol. Cell.* **1,** 841–848
44. Ohlendieck, K., Ervasti, J. M., Matsumura, K., Kahl, S. D., Leveille, C. J., and Campbell, K. P. (1991) *Neuron* **7,** 499–508
45. Ohlendieck, K., Ervasti, J. M., Snook, J. B., and Campbell, K. P. (1991) *J. Cell Biol.* **112,** 135–148
46. Towbin, H. T., Staehelin, T., and Gordon, J. (1979) *Proc. Natl. Acad. Sci. U. S. A.* **76,** 4350–4354
47. Lidow, H. G. W., and Kunkel, L. M. (1998) *Lab. Invest.* **78,** 1543–1551
48. Houzelstein, D., Lyons, G. E., Chamberlain, J. E., and Buckingham, M. E. (1992) *J. Cell Biol.* **119,** 811–821
49. Iwata, Y., Pan, Y., Hanada, H., Yoshida T., and Shigekawa M. (1996) *J. Mol. Cell Cardiol.* **28,** 2501–2509
50. Nigro, V., Okazaki, Y., Belsito, A., Piluso, G., Matsuda, Y., Politano, L., Nigro, G., Ventura, C., Abbondanza, C., Molinaro, A. M., Acampora, D., Nishimura, M., Hayashizaki, Y., and Puca, G. A. (1997) *Hum. Mol. Genet.* **6,** 601–617
51. Sakamoto, A., Ono, K., Abe, M., Jasmin, G., Eki, T., Murakami, Y., Masaki, T., Toyo-Oka, T., and Hanaoka, F. (1997) *Proc. Natl. Acad. Sci. U. S. A.* **94,** 13873–13878
52. Roberds, S. L., Ervasti, J. M., Anderson, R. D., Ohlendieck, K., Kahl, S. D., Zoloto, D., and Campbell, K. P. (1993) *J. Biol. Chem.* **268,** 11496–11499
53. Schofield, J., Houzelstein, D., Davies, K., Buckingham, M., and Edwards, Y. H. (1993) *Dev. Dyn.* **198,** 254–264
54. Klein, G., Langegger, M., Timpl, R., and Ekblom, P. (1988) *Cell* **55,** 331–341
55. Matsumura, K., Ervasti, J. M., Ohlendieck, K., Kahl, S. D., and Campbell, K. P. (1992) *Nature* **360,** 588–591
56. Clerk, A., Morris, G. E., Dubowitz, V., Davies, K. E., and Sewry, C. A. (1993) *Histochem. J.* **25,** 554–561
57. Blake, D. J., Nawrotzki, R., Loh, N. Y., Gorecki, D., Davies, K. E. (1998) *Proc. Natl. Acad. Sci. U. S. A.* **85,** 241–246
58. Peters, M. F., O'Brien, K. F., Sadoulet-Puccio, H. M., Kunkel, L. M., Adams, M. E., Froehner, S. C. (1998) *J. Biol. Chem.* **272,** 31561–31569
59. Puca, A. A., Nigro, V., Piluso, G., Belsito, A., Sampalo, S., Quaderi, N., Rossi, E., Iorio, G. D., Ballabio, A., and Franco, B. (1998) *FEBS Lett.* **425,** 7–13
60. Peters, M. F., Adams, M. A., and Froehner, S. C. (1997) *J. Cell Biol.* **138,** 81–93
61. Kachinsky, A. M., Froehner, S. C., and Milgram, S. L. (1999) *J. Cell Biol.* **145,** 391–402
62. Ervasti, J. M., Burwell, A. L., and Geissler, A. L. (1997) *J. Biol. Chem.* **272,** 22315–22321
63. Chiba, A., Matsumura, K., Yamada, H., Inazu, T., Shimizy, T., Kusunoki, S., Kanazawa, I., Kobata, A., and Endo, T. (1997) *J. Biol. Chem.* **272,** 2156–2162
64. Sasaki, T., Yamada, H., Matsumura, K., Shimizy, T., Kobata, A., and Endo, T. (1998) *Biochim. Biophys. Acta* **1425,** 599–606