Non-Muscle α-Dystroglycan Is Involved in Epithelial Development

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Abstract. The dystroglycan complex is a transmembrane linkage between the cytoskeleton and the basement membrane in muscle. One of the components of the complex, α-dystroglycan binds both laminin of muscle (laminin-2) and agrin of muscle basement membranes. Dystroglycan has been detected in nonmuscle tissues as well, but the physiological role in nonmuscle tissues has remained unknown. Here we show that dystroglycan during mouse development in nonmuscle tissues is expressed in epithelium. In situ hybridization revealed strong expression of dystroglycan mRNA in all studied epithelial sheets, but not in endothelium or mesenchyme. Conversion of mesenchyme to epithelium occurs during kidney development, and the embryonic kidney was used to study the role of α-dystroglycan for epithelial differentiation. During in vitro culture of the metanephric mesenchyme, the first morphological signs of epithelial differentiation can be seen on day two. Northern blots revealed a clear increase in dystroglycan mRNA on day two of in vitro development. A similar increase of expression on day two was previously shown for laminin α1 chain. Immunofluorescence showed that dystroglycan is strictly located on the basal side of developing kidney epithelial cells. Monoclonal antibodies known to block binding of α-dystroglycan to laminin-1 perturbed development of epithelium in kidney organ culture, whereas control antibodies did not do so. We suggest that the dystroglycan complex acts as a receptor for basement membrane components during epithelial morphogenesis. It is likely that this involves binding of α-dystroglycan to E3 fragment of laminin-1.

THE formation of organized epithelial sheets with polarized cells is an important event during organogenesis (Simons et al., 1992). There is good evidence that cell–cell adhesion mediated by cadherins (Gumbiner, 1992; Larue et al., 1994) and adhesion of cells to basement membranes (Klein et al., 1988) are crucial for initiation of epithelial cell polarity. Integrins act as major cell surface receptors for basement membrane components such as laminins (Hynes, 1992; Timpl and Brown, 1994), and few if any other well-defined non-integrin receptors for basement membrane components have been identified from epithelial cells.

The developing kidney is an excellent model to study the development of epithelial cells and the role of basement membrane proteins. Laminin-1 is a basement membrane glycoprotein composed of three large polypeptides, the α1, β1 and γ1 chains (Burgeson et al., 1994). In the developing kidney the β1 and γ1 chains are widely expressed, whereas laminin α1 chain expression is specific for epithelial cells and appears at onset of epithelial cell polarization (Ekblom et al., 1990). Antisera reacting with the E8 or E3 fragment of laminin-1 were found to inhibit development of mesenchyme into epithelium in organ cultures of embryonic kidneys, suggesting that these domains of laminin-1 are important for the development of epithelial cell polarity (Klein et al., 1988). Much is now known about the interaction between E8 fragment and the cell surface, but it has remained unclear how E3 acts.

Several cell types bind to the E8 fragment of laminin-1 by a specific receptor on the cell surface, the α6β1 integrin (Sonnenberg et al., 1990) and antibodies against the α6 subunit of the integrin interfere with kidney tubule development in vitro (Sorokin et al., 1990). The E8 fragment of laminin-1 may thus act as an autocrine stimulator of kidney tubule development by binding to a cell surface receptor containing the α6 integrin subunit. Since kidney tubule development partially can be inhibited also by antibodies to the E3 fragment (Klein et al., 1988; Sorokin et al., 1992) additional receptors for laminin-1 which bind to the E3 fragment should exist. The E3 fragment is a major heparin binding domain of laminin (Ott et al., 1982). The E3 fragment can support adhesion of many cell types in vitro (Sonnenberg et al., 1990; Sorokin et al., 1992) and it would thus for many reasons be important to identify receptors

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for the E3 fragment of laminin-1. A single chain 67–70-kD protein (von der Mark and Kühl, 1985) has been suggested to act as a laminin receptor. However, it was reported to bind to fragment P1 of laminin-1 rather than fragment E3 (Graf et al., 1987) and antibodies to that P1 of laminin-1 did not perturb kidney tubule development in vitro (Klein et al., 1988). However, many non-integrin receptors for laminin may exist (Mecham, 1991).

In muscle, the dystroglycan complex apparently forms an important transmembrane linkage between laminin-2 and intracellular dystrophin. The 156 kD protein α-dystroglycan binds to transmembrane proteins and to laminin. α-Dystroglycan binds to E3-like G-domains of laminin-2 and this is inhibited by heparin (Ervasti and Campbell, 1993; Gee et al., 1993). Genetic evidence suggest that the linkage between laminin-2 and dystrophin is required for muscle integrity (Campbell, 1995). Absence of dystrophin (Hoffman et al., 1987) and a reduction of dystrophin-associated glycoproteins (Ervasti et al., 1990; Ohlendieck and Campbell, 1991) or deficiency of laminin-α2 chain (Sunada et al., 1994; Xu et al., 1994*a*,*b*; Tomé et al., 1994) lead to severe muscular dystrophy. Interestingly, α-dystroglycan also binds to the E3 fragment of laminin-1 in overlay assays (Ibraghimov-Beskrovnaya et al., 1992; Gee et al., 1993). Laminin-1 is expressed by epithelial cells rather than muscle cells in the embryo (Klein et al., 1990).

α-Dystroglycan has been found in non-muscle tissues (Ibraghimov-Beskrovnaya et al., 1992, 1993). The biological role of non-muscle α-dystroglycan is not clear, but several lines of research have raised the possibility that it could be a binding protein for the E3 fragment of laminin-1 in epithelial cells. Since the cellular origin of non-muscle dystroglycan was not known, we studied the distribution of dystroglycan mRNA in the developing embryo. It was of particular interest to investigate the distribution and role of dystroglycan in the embryonic kidney, where the E3 fragment was shown to be involved in development.

We now report a striking coexpression of dystroglycan mRNA with laminin α1 chain mRNA rather than with laminin α2 chain mRNA during kidney development. Dystroglycan and laminin α1 chain mRNAs are strictly confined to epithelial cells and the polypeptides are co-localized in the basement membranes of the ureter and the developing kidney tubules. A similar expression of dystroglycan mRNA in epithelial cells was seen in other non-muscle tissues also. Moreover, development of epithelial cells in organ culture of embryonic kidney could be inhibited by an antibody that blocks the interaction between laminin-1 and α-dystroglycan.

Materials and Methods

Tissues and Organ Culture

National Medical Research Institute (NMRI) hybrid mouse embryos were used. 12-d-old embryonic kidneys were isolated for whole organ culture and cultured in I-MEM (GIBCO BRL, Gaithersburg, MD) (Grobstein, 1955) supplemented with heat inactivated 10% fetal calf serum and 1% L-glutamine. In cultures where the volume of the added antibody was 25% of culture medium concentrated amino acids and vitamins for minimum essential medium (Flow Laboratories, Inc., McLean, VA) were supplemented to maintain physiological culture conditions (Kadoya et al., 1995).

Transfilter cultures were prepared using 11-d-old metanephric mesenchyme which was microsurgically separated from the ureter bud and cocultured with a heterologous inducer, the spinal cord (Grobstein, 1956; Saxén et al., 1968). We used I-MEM medium (Gibco BRL) and cultured as previously described (Klein et al., 1988). For the Northern blots, freshly isolated mesenchymes and mesenchyme induced for 24, 48, and 120 h in vitro were analyzed. The inducer spinal cord was removed from the filter and discarded.

Antibodies

mAbs (IIH6, VIA41, both IgM isotypes) have previously been described (Ervasti and Campbell, 1991, 1993). Antibody IIH6 has been previously shown to block binding of α-dystroglycan to laminin and agrin (Ervasti and Campbell, 1993; Campanelli et al., 1994; Gee et al., 1994). VIA41 binds to α-dystroglycan but does not intefere with binding to laminin (Ervasti and Campbell, 1993). Hybridoma supernatant containing these antibodies were concentrated from 10 ml to ~0.6 ml using a Centriprep concentrator (Amicon, Beverly, MA) and used in perturbation experiments with different amounts, as indicated, of concentrated antibody. Purified mAbs IIH6 against α-dystroglycan (Ervasti and Campbell, 1991) and XIXC2 against dystrophin (Ervasti et al., 1990) were also used in organ cultures at concentrations indicated. The mAbs (both IgM isotypes) were purified by size-exclusion chromatography to homogeneity as determined by SDS-PAGE. Purified GoH3, a rat mAb recognizing the α6 integrin subunit (Sonnenberg et al., 1990; Sorokin et al., 1990) was purchased from Central Laboratory of the Netherlands Red Cross Blood Transfusion Service (Amsterdam, The Netherlands; Lot No.1566-06-01). Purified rat IgG was purchased from Sigma Immunochemicals (St. Louis, MO).

Functional Assays

Whole organ cultures were incubated in the presence of varying amounts of concentrated antibodies and purified antibodies. Control incubations were performed without antibodies. Commercial mAb GoH3 contained BSA and therefore BSA was also added in some control experiments. In control cultures both the ureter epithelium and the epithelium derived from mesenchyme will form within a 2-d culture period (Grobstein, 1955). At 48 h of culture the explants were examined under stereomicroscope and the number of condensates were counted independently by two persons. Samples were collected and processed for histological examination. Tissues for histological examination were fixed in Bouin solution, embedded in paraffin and 5-μm sections were stained with hematoxylin and eosin.

Northern Blot Analysis

Tissues were frozen in liquid nitrogen, and total RNA was isolated (Chirgwin et al., 1979). 10 μg total RNA was denatured with glyoxal and electrophoresed on a 1% agarose gel. After transfer to Zeta-Probe GT membrane (Bio-Rad, Hercules, CA), RNA was fixed by UV cross-linking with a Stratalinker (Stratagene Corp., La Jolla, CA) under conditions recommended by the manufacturer. Membranes were pre-hybridized for 30 min at 65°C in solution containing 0.25 M Na_2HPO_4 and 7% SDS. Hybridization was performed in the same solution for 18–24 h at 65°C with the following cDNA probes: a 1.5-kb EcoRI fragment of a cDNA clone R43-B, corresponding to rabbit dystroglycan (Ibraghimov-Beskrovnaya et al., 1992) and a 1.1-kb human G3PDH cDNA probe (Clontech, Palo Alto, CA) as control. cDNA inserts were labeled with ^{32}P to a specific activity about 2×10^8 cpm/μg DNA by oligolabeling kit (Pharmacia, Biotech Inc., Piscataway, NJ). After hybridization filters were washed 2 × 1 h in 20 mM Na_2HPO_4, 5% SDS at 65°C and 2 × 1 h in 20 mM Na_2HPO_4, 1% SDS at the same temperature. Membranes were exposed to Hyperfilm MP films (Amersham Corp., Arlington Heights, IL) at −70°C in the presence of intensifying screens. Band intensities of the Northern blot were analyzed by a Bio-Rad GS-670 imaging densitometer and the profile analysis program of the Macintosh/Molecular Analyst 1.1 (Bio-Rad).

In Situ Hybridization

In situ hybridization was performed according to Ernfors et al. (1990) as described (Durbeej et al., 1993). To detect dystroglycan mRNA we used a synthetic 45-mer oligonucleotide probe 5′-CAC TCG AAA TGA GCG CCC GAC AAC AGC CGT ACC GTC TGG AAT GCC-3′, complementary to nucleotides 1523–1568 of mouse dystroglycan mRNA (O.

Ibraghimov-Beskrovnaya, unpublished data) and a 50-mer 5′-GCC TTC CTT TTA ATG TAT TCT GTC TTG ACT GTG TGC CAC TTG CCA TCA CT-3′ complementary to nucleotides 8452–8501 of mouse laminin α1 chain mRNA (Sasaki et al., 1988; Kadoya et al., 1995). The oligonucleotides were selected with the Oligo™ 4.0 software (National Biosciences Inc., Plymouth, MN). Control sections hybridized with the same amount of labelled probe plus unlabeled probe in excess did not show any specific binding.

Immunofluorescence

Tissues were frozen in tissue Tek (Miles, Naperville, IL). Unfixed cryostat sections (7 μm) were washed in PBS, blocked in 3% BSA in PBS for 20 min and incubated with affinity purified sheep polyclonal antibodies specific for fusion protein B (anti-FP-B) which recognizes both α- and β-dystroglycan (Ibraghimov-Beskrovnaya et al., 1992), affinity purified sheep polyclonal antibody specific for β-dystroglycan (sheep 43-DAG; Ohlendieck and Campbell, 1991) and rat monoclonal antibodies against fragment E3 of mouse laminin-1 (mAb 200; Sorokin et al., 1992). Anti-FP-B and sheep 43-DAG were diluted 1:5 and mAb 200 was used undiluted. Bound antibodies were visualized using donkey anti–sheep IgG or goat anti–rat IgG conjugated with FITC secondary antibodies (Jackson ImmunoResearch Laboratories, West Grove, PA). Slides were examined under a Zeiss microscope equipped with epifluorescence optics.

Results

Northern Blot Analysis of Dystroglycan mRNA in the Developing Kidney

The transmembrane 43-kD β-dystroglycan and extracellular 156-kD α-dystroglycan are encoded by a single messenger RNA of 5.8 kb (Ibraghimov-Beskrovnaya et al., 1992). This 5.8-kb transcript was expressed during kidney development. In 11-d-old embryonic kidneys, dystroglycan mRNA was expressed at a low level but the expression increased during embryonic development, reaching the highest level in late embryonic and newborn kidney. In adult kidneys the expression was low (Fig. 1). The pattern corresponds very well with the expression of α1 chain of laminin-1 during in vivo kidney development (Ekblom et al., 1990).

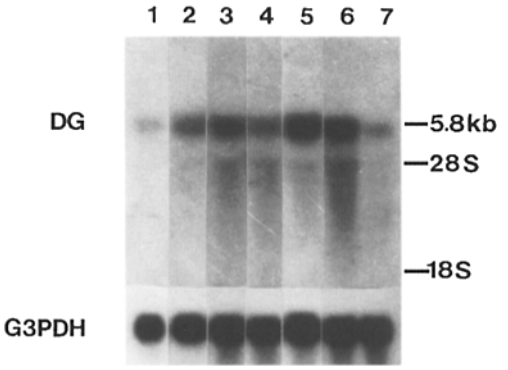


Figure 1. Northern blot analysis of dystroglycan mRNA expression during mouse kidney development in vivo. 10 μg of total RNA was used in each lane. RNA was isolated from 11-d-old embryonic kidneys (lane *1*), 14-d-old (lane *2*), 15-d-old (lane *3*), 16-d-old (lane *4*), 18-d-old (lane *5*) embryonic kidneys, newborn kidneys (lane *6*), and adult kidneys (lane *7*). The filter was hybridized with a cDNA probe corresponding to dystroglycan (*DG*). The highest level of dystroglycan mRNA was seen in late embryonic and newborn kidney. To show that equal amount of RNA was loaded in each lane, the same filter was hybridized with a G3PDH cDNA probe. Exposure times were 14 d for dystroglycan and 2 d for G3PDH.

Dystroglycan during In Vitro Conversion of Mesenchyme to Epithelium

The in vitro model system for mesenchyme conversion to epithelium (Grobstein, 1956) made it possible to study whether dystroglycan mRNA increases at induction of epithelial development or later when overt epithelial morphogenesis begins. In the organ culture model, the mesenchyme becomes induced at 24 h of culture. At 30–36 h the first signs of epithelial polarization can be seen, and at 48–72 h polarized epithelial tubules surrounded by a basement membrane are formed (see Klein et al., 1988). In uninduced mesenchyme the signal for dystroglycan mRNA was low. Between 0 and 24 h of culture there was some increase of expression but expression levels were still low. A strong signal was seen on day two of development (Fig. 2, *A* and *B*). Dystroglycan expression thus increased coordinately with expression of laminin α1 chain rather than

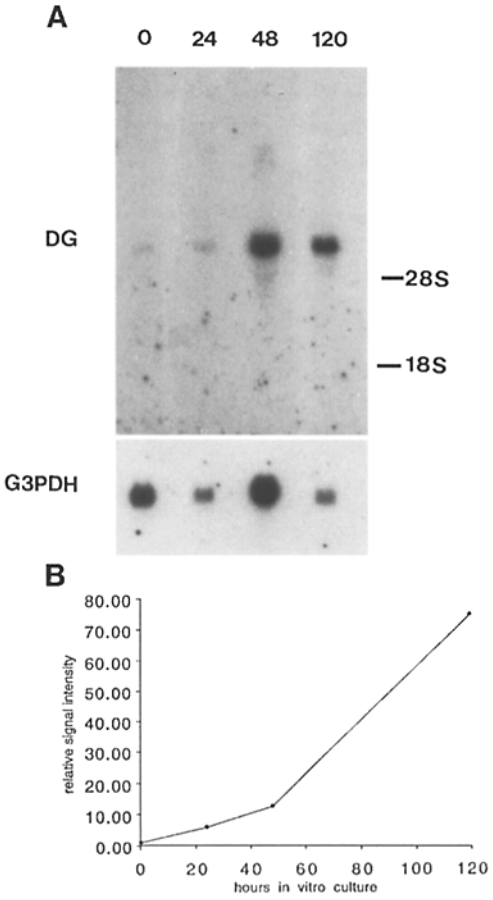


Figure 2. Increased expression of dystroglycan mRNA during in vitro conversion of metanephric mesenchyme to epithelium. (*A*) Northern hybridization for dystroglycan (*DG*) mRNA of 10 μg total RNA from uninduced kidney mesenchyme of 11-d-old mice (lane *0*), and mesenchyme cocultured with spinal cord for 24 h (lane *24*), 48 h (lane *48*) and 120 h (lane *120*). The same filter was hybridized with a G3PDH cDNA probe. Exposure times were 14 d for DG and 2d for G3PDH. (*B*) Densitometric analysis of the filter. The data were normalized to G3PDH signal intensities.

with expression of laminin β1, γ1 chains or nidogen (Ekblom et al., 1990, 1994).

Localization of Dystroglycan mRNA in the Developing Kidney

Since several cell types differentiate simultaneously and some of the laminins and laminin-associated proteins are expressed by mesenchyme (Ekblom et al., 1994; Vuolteenaho et al., 1994), we performed in situ hybridization to identify the cells producing dystroglycan mRNA. Initially, metanephric kidney rudiments are composed of a small epithelial ureter bud surrounded by undifferentiated stem cells. Interactions between the epithelial ureter bud and mesenchyme initiate the development of the metanephric kidney. The mesenchyme stimulates the ureter to grow and branch and to differentiate into new epithelium. The ureter, in turn, induces a part of the mesenchyme to convert into new epithelium. The first sign of this conversion is the formation of mesenchymal condensates around the tips of the ureter. The condensates subsequently form comma-shaped bodies and then S-shaped bodies. The S-shaped epithelia elongate and join the ureter to form the mature nephron. The newly induced areas are in the most cortical areas of the developing kidney, while the more differentiated areas are found in the medullary part (see Ekblom et al., 1990).

In 12-d-old embryonic kidneys both dystroglycan (Fig. 3, *A* and *B*) and laminin α1 chain mRNAs (Fig. 3, *C* and *D*) were expressed in the ureter epithelium that had begun to branch into the mesenchyme and had formed a T-shaped structure. Some faint expression of dystroglycan above background was also noted in the surrounding mesenchyme (Fig. 3, *A* and *B*). In none of the developing kidneys of later stages could we detect dystroglycan mRNA in the most cortical areas except for weak expression in the epithelial ureter. The uninduced mesenchymal stem cells and the induced but not yet differentiated mesenchyme, which both are found in the most cortical areas, expressed very little if any dystroglycan mRNA (Fig. 4). Strong expression of dystroglycan mRNA was first seen in the comma-shaped bodies, where the induced cells become polarized (Fig. 4, *A* and *B*). Strong expression was also seen in S-shaped tubules, as shown here for 14-d-old (Fig. 4, *A* and *B*) and 18-d-old (Fig. 4, *C* and *D*) embryonic kidneys. Strong expression of dystroglycan mRNA was also seen in the elongating tubules that extend from the cortex deep into the medulla which will develop into the

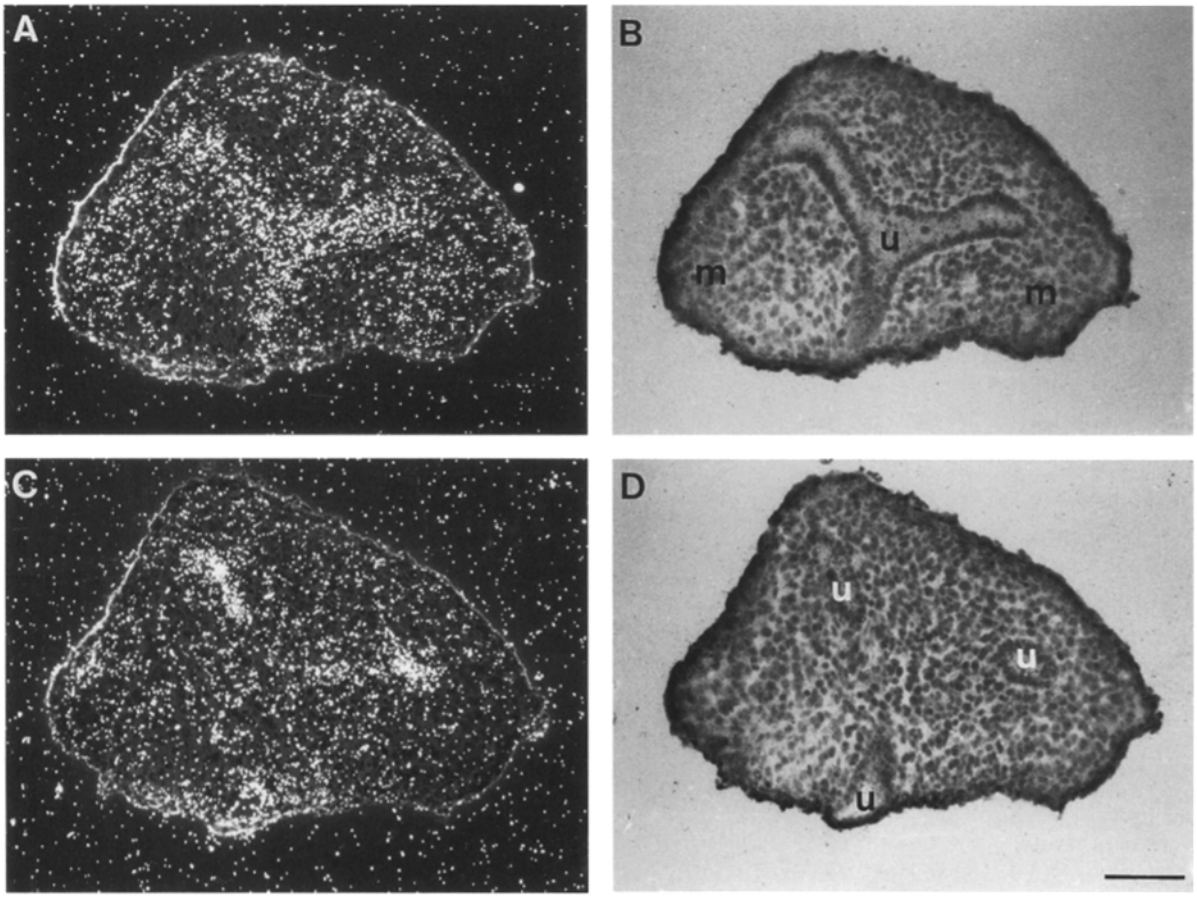


Figure 3. Coexpression of dystroglycan and laminin α1 chain mRNAs in 12-d-old embryonic kidneys. Adjacent sections of 12-d-old embryonic kidneys were analyzed by in situ hybridization with ^{35}S-labeled synthetic oligonucleotide probes against mouse dystroglycan mRNA (*A* and *B*) and against mouse laminin-1 α1 chain mRNA (*C* and *D*). Darkfield (*A* and *C*) and brightfield (*B* and *D*) micrographs are shown. Both dystroglycan (*DG*) and laminin α1 mRNAs are expressed in the T-shaped ureter (*u*) epithelium. For DG mRNA, some expression above background is also seen in the surrounding mesenchyme (*m*). Bar, 93 μm.

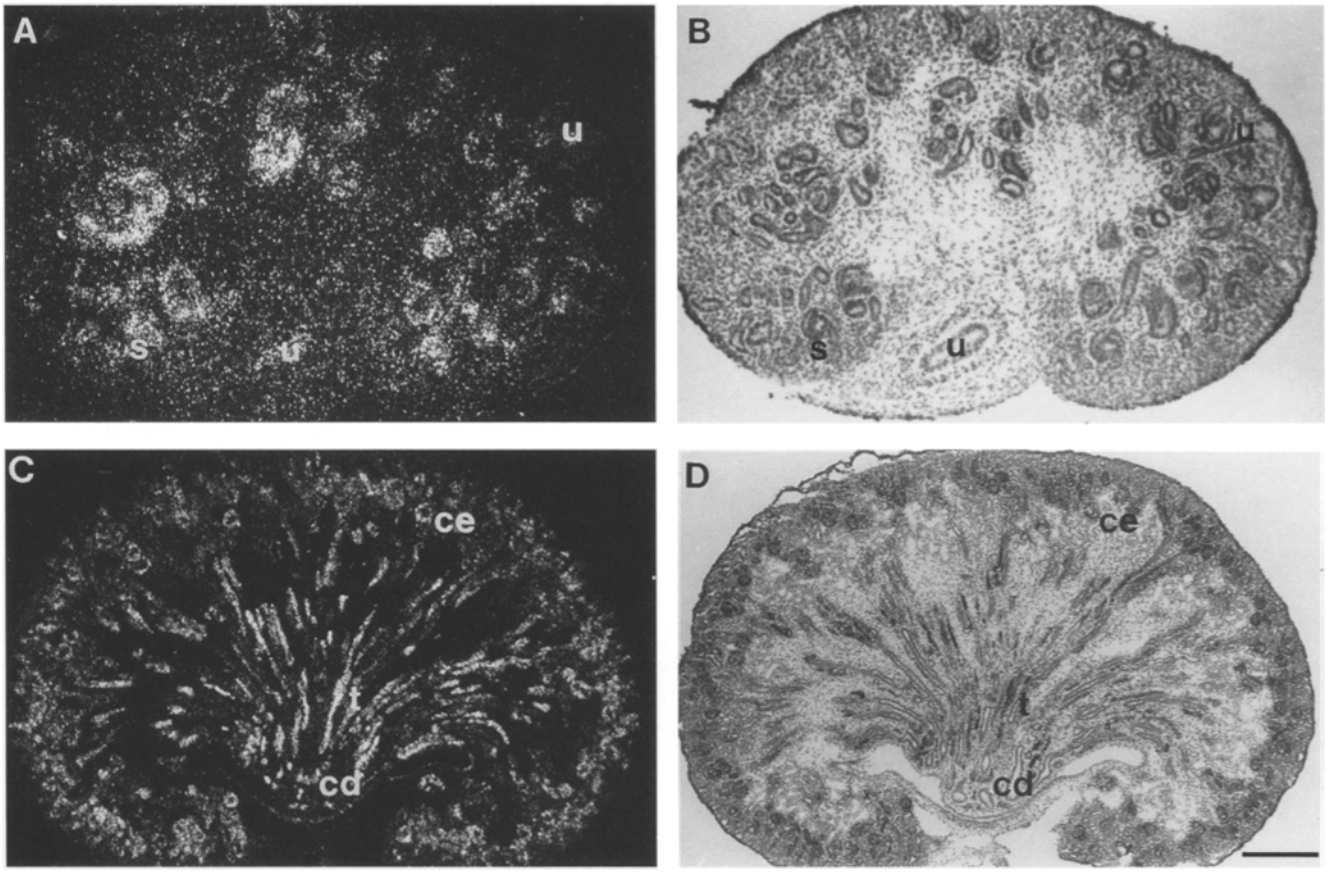


Figure 4. Detection of dystroglycan mRNA in epithelial cells of embryonic mouse kidney. Sections of 14-d-old embryonic (*A* and *B*) and 18-d-old embryonic (*C* and *D*) mouse kidneys were analyzed by in situ hybridization with an oligonucleotide probe against dystroglycan (*DG*) mRNA. The autoradiographs are shown in darkfield (*A* and *C*) and brightfield (*B* and *D*). In 14-d-old embryonic kidney (*A* and *B*) strong signals are seen in the newly formed epithelium; in the comma-shaped and S-shaped bodies (*s*). Expression is also seen in the ureter (*u*). Corresponding to the data of 14-d-old embryonic kidney strong signals are seen in comma-shaped and S-shaped bodies in the cortex of 18-d-old embryonic mouse kidney (*C* and *D*). Note that no signal can be seen in the uppermost cortex, containing uninduced and induced mesenchyme. A strong signal of DG mRNA is also seen in the medulla, in the elongating straight tubules (*t*) that extend from the cortex. These tubules are derived by elongation of the S-shaped tubules seen in 14-d-old embryonic kidneys. Moreover, the capsular epithelium (*ce*) of the glomeruli expresses DG mRNA. Note that no signal can be seen in the ureter derived collecting ducts (*cd*). Bar: (*A* and *B*), 80 μm ; (*C* and *D*) 160 μm.

loops of Henle. In contrast to the signal in the elongating tubules, no signal was detected in the adjacent ureter cell lineage, the cells of the collecting ducts. Expression of dystroglycan mRNA was also noted in the epithelium of Bowman's capsule (Fig. 4, *C* and *D*). The results suggest that dystroglycan expression during in vivo development is turned on locally where epithelial cell polarization occurs, coordinately with expression of α1 chain of laminin-1 (Ekblom et al., 1990).

Expression of Dystroglycan Polypeptides in the Developing Kidney

Antibodies (anti-FP-B) recognizing both α- and β-dystroglycan reacted with the basal cell surface and the basement membrane of the ureter and the developing tubules as shown here for 14-d-old (Fig. 5, *A* and *D*) and 18-d-old (Fig. 5 *B*) embryonic kidneys. Strong staining was seen in the basement membranes of the elongating tubules, whereas very little α- and β-dystroglycan could be detected in the basement membranes of the collecting ducts (Fig. 5 *B*). A similar staining was obtained with an antibody recognizing β-dystroglycan (data not shown). Staining of adjacent sections of 14-d embryonic kidneys with mAb 200 recognizing E3 fragment of α1 chain of laminin-1 (Fig. 5 *C*) and anti-FP-B (Fig. 5 *D*) revealed a codistribution of E3 fragment and α-/β-dystroglycan. Both α1 chain of laminin-1 and α-/β-dystroglycan were expressed in the basement membrane region of the ureter and developing tubules and none of the polypeptides could be detected in the mesenchyme (Fig. 5, *C* and *D*).

Localization of Dystroglycan mRNA in the Mouse Embryo

To test whether dystroglycan mRNA was expressed in the epithelium of other non-muscle organs, we performed in situ hybridization on sections of 13-d-old whole embryos. We found that dystroglycan mRNA was indeed expressed in the epithelium of several non-muscle organs: in epithelium of choroid plexus (Fig. 6, *A* and *B*); lung epithelium (Fig. 6, *C* and *D*); gut epithelium (Fig. 6, *E* and *F*); urogen-

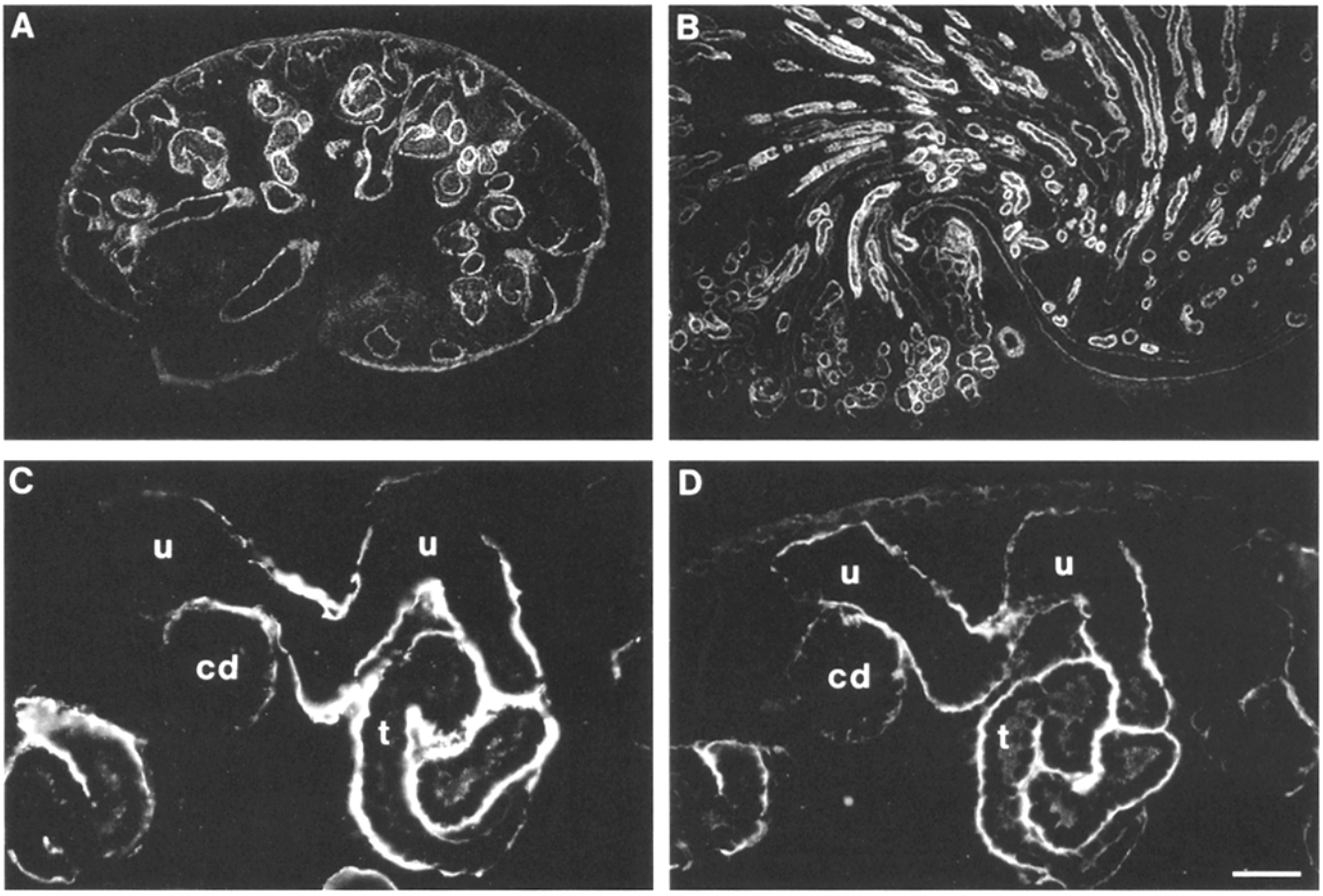


Figure 5. Immunolocalization of α/β-dystroglycan during in vivo development of mouse kidney. Sections of 14-d-old (*A* and *D*) and 18-d-old (*B*) embryonic kidneys were stained with anti-FP-B and antibodies were detected by indirect immunofluorescence. In 14- and 18-d-old kidneys (*A*, *B*, and *D*) expression of α-/β-dystroglycan is confined to the basement membranes of the ureter, developing tubules and in elongating straight tubules. Note that very little α-/β-dystroglycan is expressed in the basement membranes of the collecting ducts. Adjacent sections of 14-d-old kidneys were stained with mAb 200 (*C*) and anti-FP-B (*D*). Note co-localization of α1 chain of laminin-1 and α-/β-dystroglycan in the basement membrane of the ureter (*u*), in the newly formed basement membrane around the condensate (*cd*) and in the basement membrane of the developing tubules (*t*). Bar, (*A* and *B*) 78 μm; (*C* and *D*) 39 μm.

ital epithelium (Fig. 6, *G* and *H*) and tooth epithelium (Fig. 6, *I* and *J*). As expected it was also found in muscle cells, as shown here for skeletal muscle in the tail region (Fig. 6, *K* and *L*) and cardiomyocytes (Fig. 6, *M* and *N*).

Perturbation of Kidney Development with Antibodies Blocking Laminin–Dystroglycan Interaction

Hybridoma supernatant of mAb IIH6 concentrated approximately 1.5 times, which is known to block α-dystroglycan binding to laminin, perturbed kidney development in 10 out of 10 cases. In contrast, similarly concentrated hybridoma supernatant of mAb VIA41, which does not block laminin/α-dystroglycan interactions, did not influence kidney development in vitro (Table I). At higher concentrations mAb IIH6 supernatant drastically altered kidney development, and a slight effect was seen with VIA41 also (Table I). The blocking antibody IIH6 is an IgM and we therefore tested whether another IgM against intracellularly located dystrophin (mAb XIXC2) could perturb development. Purified mAb IIH6 at 50 μg/ml had no apparent effect on kidney development (Fig. 7 *B*, Table I). At 100 μg/ml of IIH6 the kidneys had fewer and more diffuse condensates (Fig. 7 *D*) than kidneys incubated in the presence of 100 μg/ml XIXC2 (Fig. 7 *C*) or without antibodies (Fig. 7 *A*, Table I). XIXC2 at 200 μg/ml did not alter development in any detectable way (Fig. 7 *E*) but a severe inhibition of kidney tubule formation was seen at 200 μg/ml of IIH6 (Fig. 7 *F*, Table I).

Histological analysis verified that neither 50 μg/ml of IIH6 or XIXC2 altered formation of kidney epithelial cells in vitro (Fig. 8, *A* and *B*). In contrast, a drastically diminshed amount of kidney epithelium was seen in cultures treated with 100 μg/ml IIH6 (Fig. 8 *C*) but not with 100 μg/ml of XIXC2 against dystrophin (Fig. 8 *D*). Some previously used blocking antibodies which perturb kidney development such as GoH3 against integrin α6 subunit and antiserum against nidogen-binding site of laminin-1 seem to increase cell death (Sorokin et al., 1990; Ekblom et al., 1994), but no such effects were seen with IIH6. Instead, we noted an abnormally large stromal cell compartment with well developed fibroblasts in the center of the explant, as shown here for explants treated with 100 μg/ml of IIH6 (Fig. 8 *C*). Histological sections of explants treated with

Table I. Development of Condensates in Treated Kidney Explants

Hybridoma	Number of explants	Number of condensates (percent of controls)
—	20	100
IIH6 (1.5× concentrated)	10	54–63
VIA4$_1$ (1.5× concentrated)	10	103
IIH6 (4× concentrated)	10	25
VIA41 (4× concentrated)	10	48–54
Purified antibody		
—	18	100
IIH6, 50 μg/ml	8	99–108
XIXC2, 50 μg/ml	8	89–93
IIH6, 100 μg/ml	8	68–74
XIXC2, 100 μg/ml	10	122–132
IIH6, 200 μg/ml	10	35–47
XIXC2, 200 μg/ml	7	108–109
GoH3, 100 μg/ml	10	42–44
GoH3, 100 μg/ml+ IIH6, 200 μg/ml	10	13–25
Rat IgG, 300 μg/ml	10	97–105

The variations in the given percent values indicate observer variation.

200 μg/ml of IIH6 were fragmented, indicating severe loss of tissue cohesion (data not shown). It has been shown previously that antibodies (GoH3) to the α subunit of another laminin receptor, the integrin α6β1, also perturb kidney development in vitro (Sorokin et al., 1990). We therefore tested whether the blocking antibodies IIH6 and GoH3 have an additive effect on kidney tubule development. IIH6 at 200 μg/ml severely inhibited kidney development (Figs. 7 *F* and 9 *A*), GoH3 at 100 μg/ml also perturbed development (Fig. 9 *B*, Table I). When IIH6 at 200 μg/ml and GoH3 at 100 μg/ml were added together a dramatic inhibition was seen (Fig. 9 *C*, Table I). Rat IgG at 300 μg/ml had no effect on kidney development (Fig. 9 *D*, Table I).

Discussion

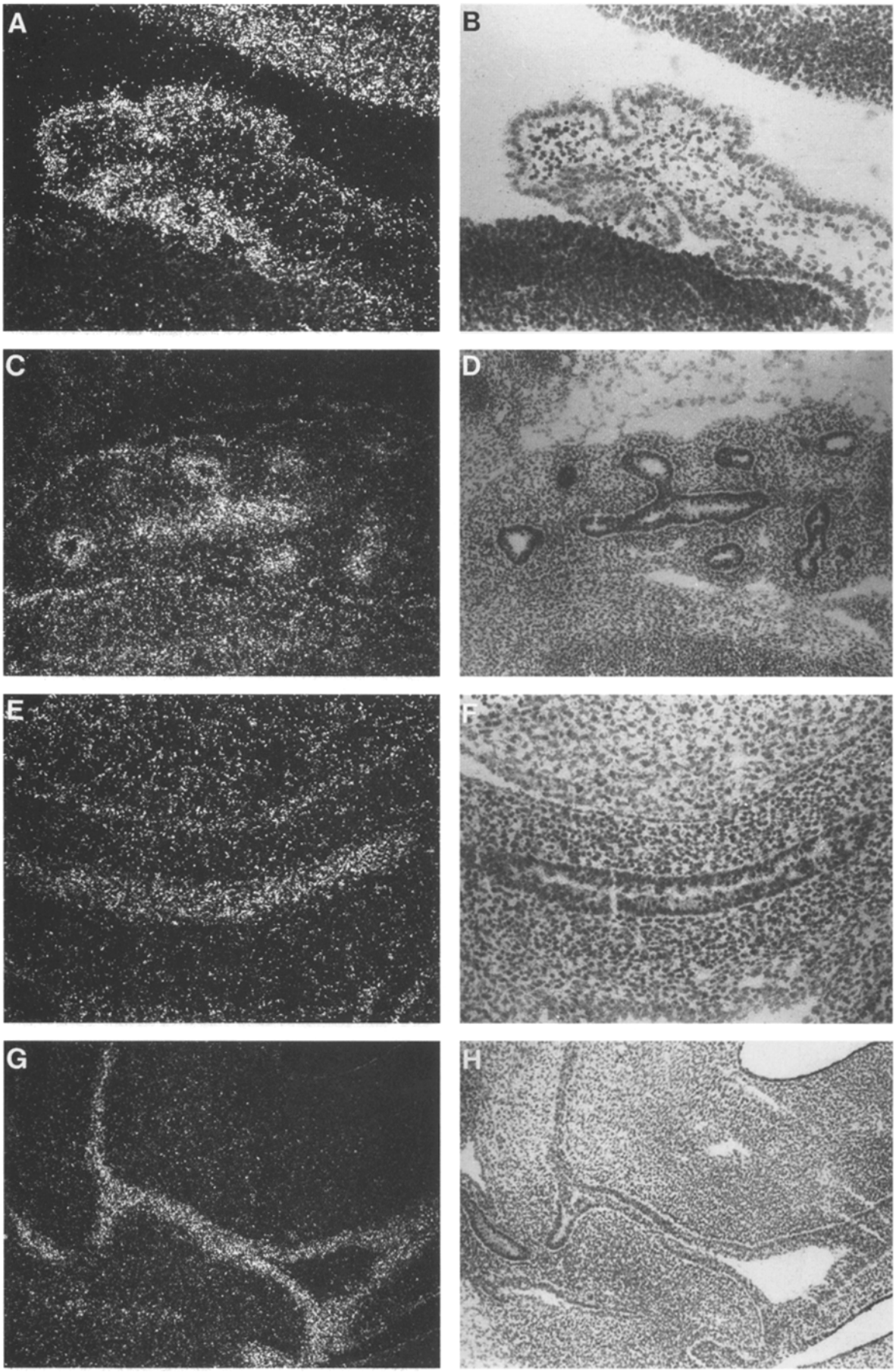
For a long time, integrins have been considered to be the main receptors linking epithelial cells to basement membranes (Sorokin et al., 1990; Korhonen et al., 1990; Schoenenberger et al., 1994). Our current study suggests that the dystroglycan complex acts as another important receptor between cells and the basement membrane during epithelial morphogenesis. Dystroglycan mRNA could be detected in developing epithelial cells. Development of epithelial cells in the embryonic kidney could be perturbed in vitro by antibodies known to block the interaction between α-dystroglycan and laminins. α-Dystroglycan binds to E3 fragment of laminins (Ervasti and Campbell, 1993; Gee et al., 1993) and one possibility is that α-dystroglycan acts as a receptor for E3 fragment of laminin-1 during epithelial morphogenesis.

A role for the E3 fragment of laminin-1 in kidney epithelial cell development was suggested several years ago (Klein et al., 1988), but no receptors for it were identified. Since the 156 kD dystrophin-associated glycoprotein, α-dystroglycan, recently was shown to bind to both laminin-1 and -2 (Ervasti and Campbell, 1993), we reasoned that this complex could be a receptor for laminin-1 during embryonic development. Binding of α-dystroglycan to laminin was abolished by heparin (Ibraghimov-Beskrovnaya et al., 1992), and heparin perturbs formation of kidney epithelium in vitro (Ekblom et al., 1978). However, it remained unclear whether the binding of α-dystroglycan to laminin-1 observed in the overaly assays with purified molecules (Ervasti and Campbell, 1993) was physiologically relevant. The present in situ hybridization studies were therefore highly significant. As expected, dystroglycan mRNA was highly expressed in embryonic muscle. In muscle the dystroglycan complex acts as a receptor for laminin α2 chain. Laminin α2 chain is expressed in non-muscle tissues also, and an obvious possibility was that α-dystroglycan acts as a binding protein for laminin-2 in non-muscle tissues. In most parts of the body, expression of laminin-1 and laminin-2 are mutually exclusive. Laminin-1 as defined by its α1 chain is expressed by epithelial cells, whereas laminin-2 as defined by the α2 chain is confined to mesenchymal cells in non-muscle tissues such as the kidney (Ekblom et al., 1990; Vuolteenaho et al., 1994).

It was striking to note that dystroglycan mRNA was strictly expressed in epithelial cells in the developing kidney. Northern blots revealed that dystroglycan mRNA was transiently expressed at high levels during embryonic kidney development and downregulated in adult kidney. In situ hybridization showed that expression was confined to the epithelial sheets and dystroglycan mRNA became strongly expressed with onset of epithelial cell polarization. The focal expression in developing epithelial cells was verified in the organ culture model system for epithelial cell development. When kidney mesenchyme is induced to differentiate in vitro epithelial cells begin to develop on day two of culture. The level of dystroglycan mRNA was very low in the uninduced mesenchyme but on day two of in vitro development, strong signals for α-dystroglycan were seen. Moreover, the strict focal expression of dystroglycan mRNA in epithelial cells was noted in all other non-muscle embryonic tissues studied. A codistribution of the dystroglycan polypeptides and the E3 fragment of laminin-1 in the basement membrane region was shown by immunofluorescence of embryonic kidney. Integrin α6 subunit is also found on the basal side of epithelial cells (Sorokin et al., 1990) but it is not as strictly confined to the basal side as dystroglycan.

Taken together, both the expression of the mRNA and the subcellular localization of the protein strongly suggest that dystroglycan acts as an epithelial basement membrane receptor during morphogenesis. This is supported by the antibody perturbation experiments. Antibody IIH6 has previously been shown to block binding of α-dystroglycan to laminin (Ervasti and Campbell, 1993) and agrin (Campanelli et al., 1994; Gee et al., 1994). Application of IIH6 to organ cultures drastically altered the development of kidney epithelium, whereas a control antibody against dystrophin in no apparent way altered development. The histology of the explants treated with the IIH6 blocking antibody was interesting and revealed an apparent increase in the stromal compartment.

The number of basement membrane components that bind α-dystroglycan is still unknown. It apparently does not bind nidogen, fibronectin, collagens or heparan sulphate proteoglycans, but binding to both laminin-1 and -2



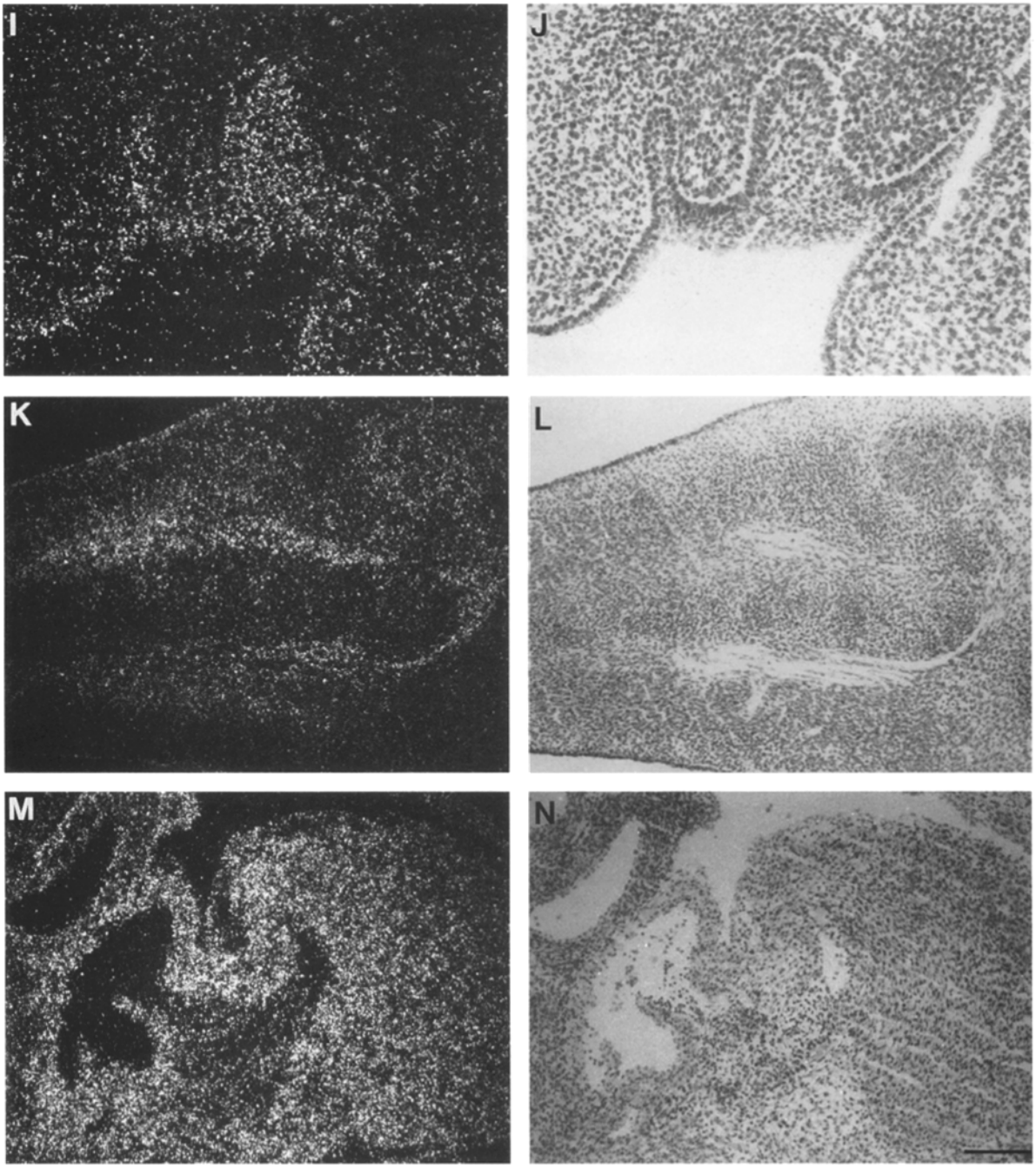
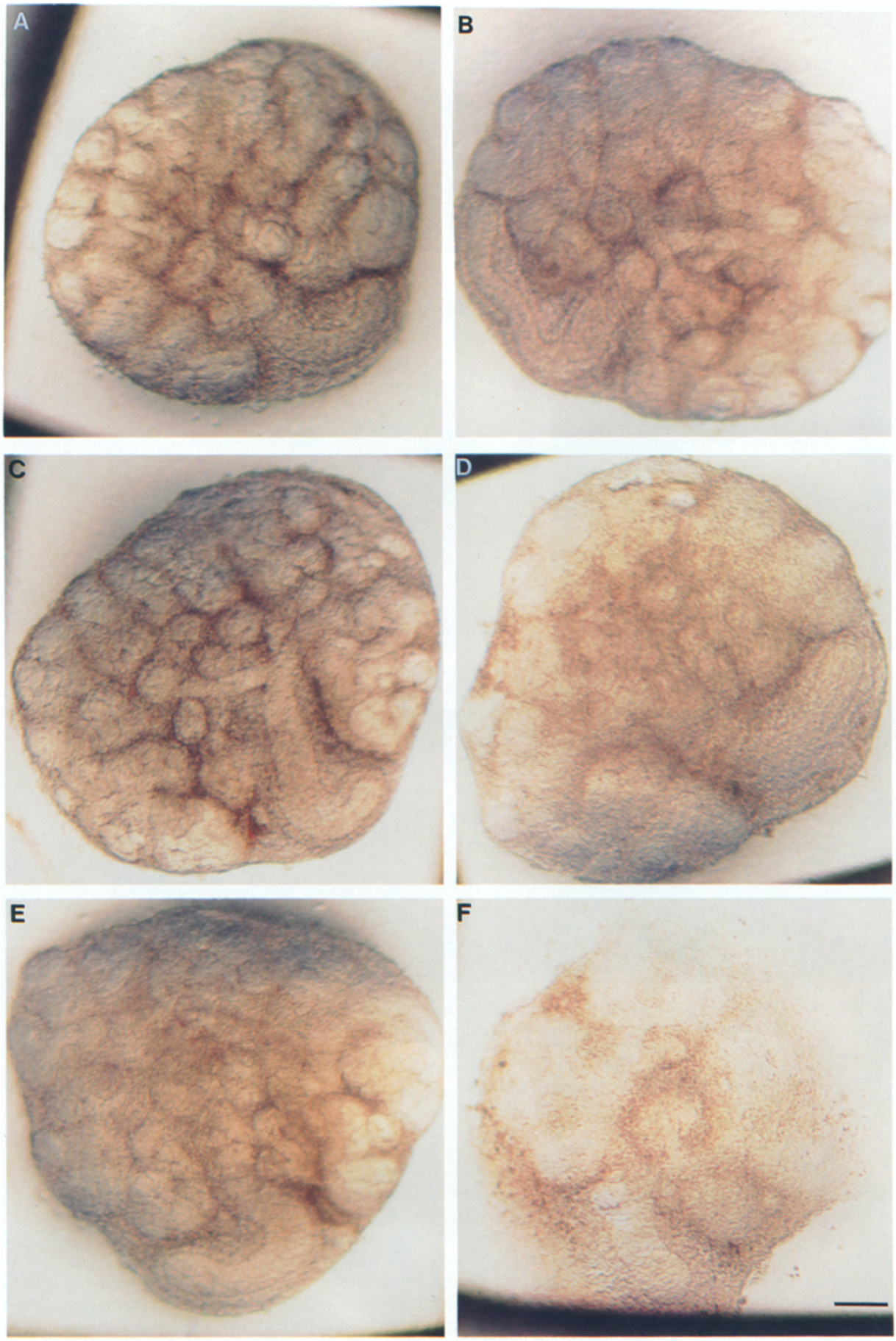


Figure 6. Expression of dystroglycan mRNA is restricted to epithelium and muscle in 13-d-old mouse embryo. Parasagittal sections of 13-d-old embryos were hybridized with a synthetic oligonucleotide probe against dystroglycan (*DG*) mRNA. Darkfield (*A, C, E, G, I, K,* and *M*) and brightfield (*B, D, F, H, J, L,* and *N*) micrographs are shown. DG mRNA is expressed in epithelium of several non-muscle organs such as in the epithelium of choroid plexus, (*A* and *B*); lung epithelium, (*C* and *D*); gut epithelium (*E* and *F*); urogenital epithelium, (*G* and *H*); and tooth epithelium (*I* and *J*). Skeletal muscle in the tail (*K* and *L*) expresses DG mRNA. The strongest signal of DG mRNA is detected in cardiomyocytes (*M* and *N*). Bar, 92 μm.

has been demonstrated (Ervasti and Campbell, 1993). Our expression studies suggest that laminin-1 rather than laminin-2 can act as a ligand in epithelial cells. We emphasize that it was not directly shown in the current study that the E3 domain of laminin-1 is the ligand for α-dystroglycan in epithelial cells, and it cannot be excluded that other basement membrane components also bind α-dystroglycan. Agrin in the neuromuscular junctions binds α-dystroglycan (Campanelli et al., 1994; Gee et al., 1994).

Although the ligand specificity of epithelial α-dystroglycan has not been fully clarified, it seems clear that the dystroglycan complex forms an important receptor system which links epithelial cells to basement membranes. Integrins (Sorokin et al., 1990) are another well characterized



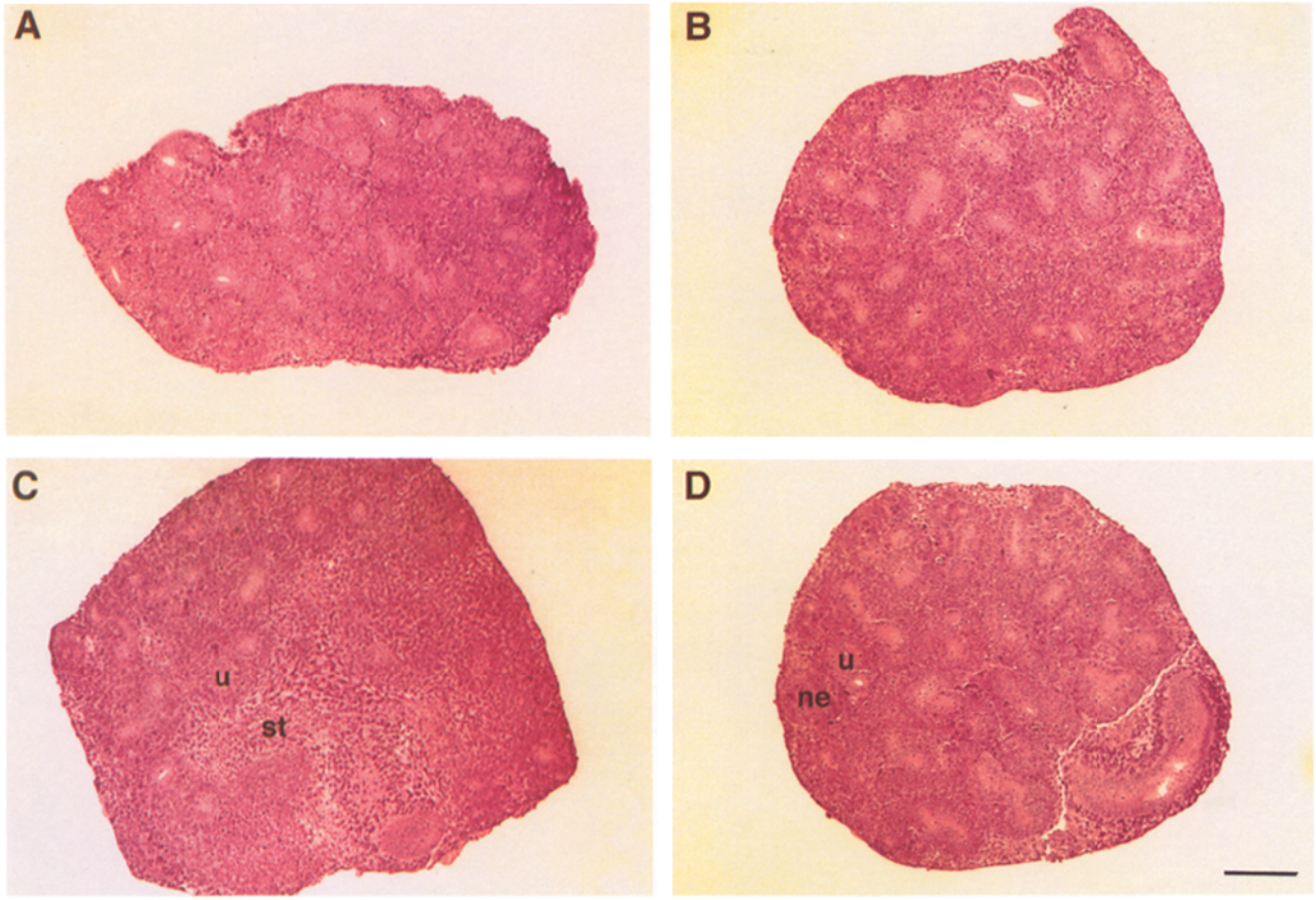


Figure 8. Histological sections of kidneys cultured in the presence of IIH6 and XIXC2 antibodies. 12-d-old embryonic kidneys were cultured in the presence of 50 μg/ml of IIH6 (*A*), 50 μg/ml of XIXC2 (*B*), 100 μg/ml of IIH6 (*C*), and 100 μg/ml of XIXC2 (*D*), and were then processed for histology. Note that few new tubules have formed in kidneys treated with 100 μg/ml of IIH6 and instead the central part of the explant contains a well-organized stromal compartment. *u*, ureter; *ne*, new epithelium; *st*, stroma. Bar, 96 μm.

adhesion receptor system for kidney epithelial cells. The observed additive effects of the antibodies suggest that the two receptor systems perform distinct events. However, it is still unclear how these receptors affect the epithelial cells at the molecular level. Some recent studies suggest that E3 fragment of laminin-1 could be involved in basement membrane assembly. Antibodies against integrin α6 subunit and antibodies against laminin E3 fragment both perturbed epithelial cell development of submandibular gland. Antibodies against E3 perturb formation of new basement membranes, whereas antibodies against integrin α6 subunit leave the basement membrane intact (Kadoya et al., 1995). One possibility is therefore that the dystroglycan complex acts as a nucleation site for basement membrane assembly, whereas integrin α6 subunit gives differentiation signals to epithelial cells. Integrin-mediated signal transduction leading to altered gene expression has been documented (Hynes, 1992). The availability of different blocking antibodies should now make it possible to experimentally analyze the different roles of integrins and α-dystroglycan in the kidney model system. In the current study, long term effects of antibodies in a two day culture were monitored but it should be possible to use a variety of other methods to identify the earlier molecular events affected by the blocking antibodies.

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Figure 7. Branching morphogenesis in the presence of antibodies against α-dystroglycan and dystrophin. Kidneys from 12-d-old embryonic mice were cultured without added antibodies (*A*) and in the presence of 50 μg/ml antibody IIH6 (*B*); 100 μg/ml XIXC2 (*C*); 100 μg/ml IIH6 (*D*); 200 μg/ml XIXC2 (*E*); and 200 μg/ml IIH6 (*F*). Cultures were examined after 2 d by stereomicroscopy. Bar, 38 μm.

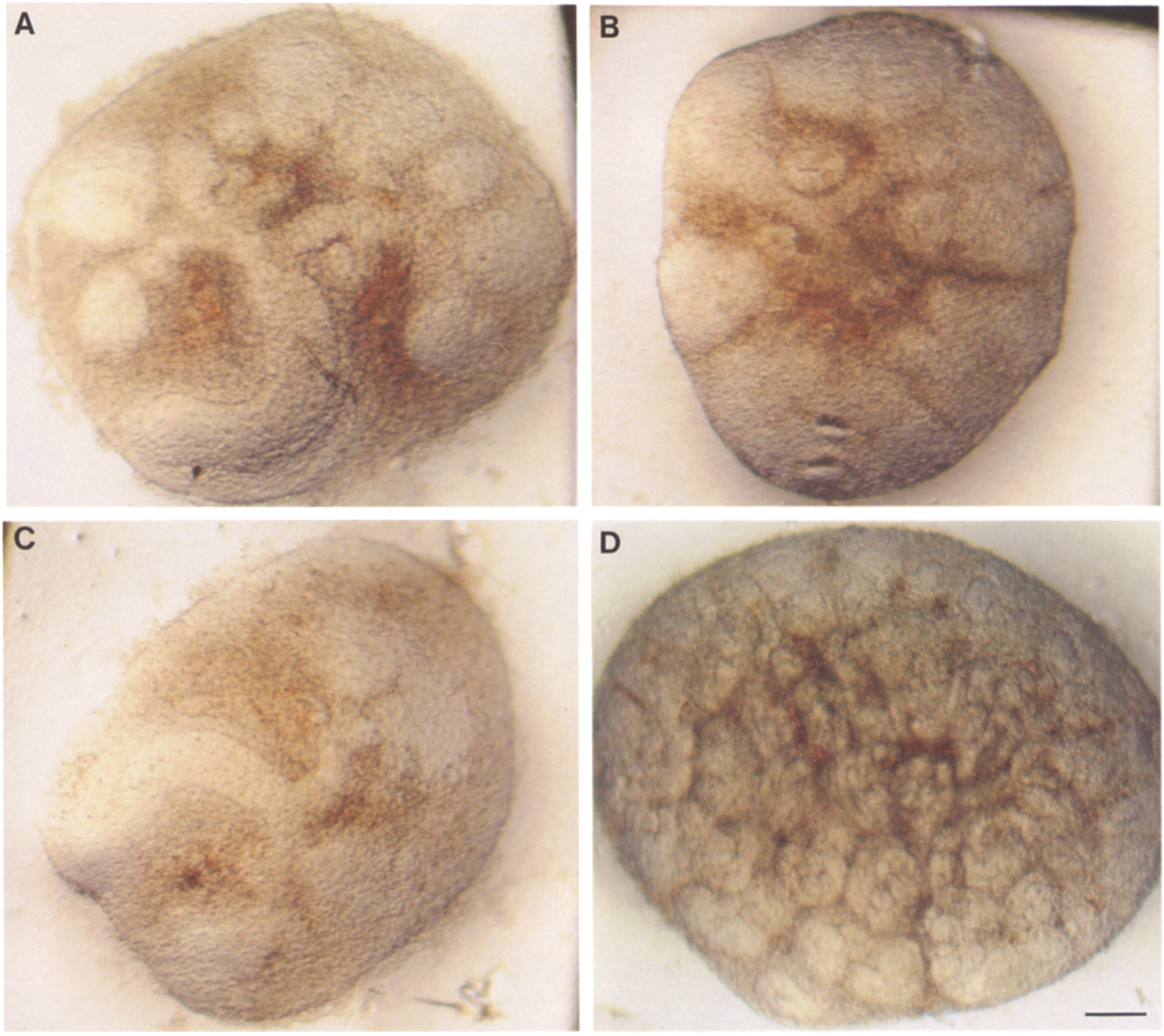

Figure 9. Branching morphogenesis in the presence of antibodies against α-dystroglycan and α-6 integrin subunit. Kidneys from 12-d-old embryonic mice were cultured in the presence of 200 μg/ml of IIH6 (*A*), 100 μg/ml of GoH3 (*B*), 200 μg/ml of IIH6, and 100 μg/ml of GoH3 (*C*) and Rat IgG at 300 μg/ml. Cultures were examined after 2 d by stereomicroscopy. Bar, 38 μm.

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