

In Situ Hybridization Reveals Temporal and Spatial Changes in Cellular Expression of mRNA for a Laminin Receptor, Laminin, and Basement Membrane (Type IV) Collagen in the Developing Kidney

G. W. Laurie, S. Horikoshi, P. D. Killen, B. Segui-Real, and Y. Yamada

Laboratory of Developmental Biology and Anomalies, National Institute of Dental Research, Bethesda, Maryland 20892

Abstract. The appearance of extracellular matrix molecules and their receptors represent key events in the differentiation of cells of the kidney. Steady-state mRNA levels for a laminin receptor, the laminin B1, B2, and A chains, and the $\alpha 1$ -chain of collagen IV ($\alpha 1$ [IV]), were examined in mouse kidneys at 16 d gestation and birth, when cell differentiation is active, and 1–3 wk after birth when this activity has subsided. Northern analysis revealed that mRNA expression of laminin receptor precedes the $\alpha 1$ (IV) and laminin B chains whereas laminin A chain mRNA expression was very low. In situ hybridization reflected this pattern and revealed the cells responsible for expression. At 16 d gestation, laminin receptor mRNA

was elevated in cells of newly forming glomeruli and proximal and distal tubules of the nephrogenic zone located in the kidney cortex. These cells also expressed mRNA for $\alpha 1$ (IV) and laminin chains. At birth, mRNA expression of receptor and all chains remained high in glomeruli but was reduced in proximal and distal tubules. At 1 wk after birth, expression was located in the medulla over collecting ducts and loops of Henle. Little expression was detectable by 3 wk. These results suggest that cellular expression of steady-state mRNA for laminin receptor, laminin, and collagen IV is temporally linked, with laminin receptor expression proceeding first and thereafter subsiding.

BASEMENT membrane is a thin extracellular matrix that forms soon after cytodifferentiation on the basal surface of epithelial and endothelial cells, and on all surfaces of muscle cells (20). Current evidence using components derived from Englebreth-Holm-Swarm basement membrane suggests that this sheet is formed by the coassembly of its main components (8, 28) particularly collagen IV, a triple helical filament of two $\alpha 1$ -chains and one $\alpha 2$ -chain (48); laminin, a cruciform-shaped glycoprotein (13) composed of a B1, B2, and A chain; heparan sulfate proteoglycan, consisting of one chain with two to three glycosaminoglycans attached to one end (29, 38); and entactin (nidogen), a dumbbell-shaped molecule formed of one chain (6, 48).

Various functions have been attributed to parts of these molecules (1, 48), for example, certain laminin regions are involved in interactions with collagen IV, heparan sulfate proteoglycan, and entactin (nidogen). Also, cells bind to laminin, collagen IV (48), and heparan sulfate proteoglycan (8a) through specific cell surface receptors. Basement

membrane molecules through cell receptors thereby influence cell adhesion, migration, differentiation, and growth (26).

The appearance of basement membrane molecules appear to represent key events in the differentiation of cells of the kidney (12). During nephrogenesis, undifferentiated cortical mesenchymal cells are locally induced by branching ureter bud epithelium to aggregate and transform into epithelial comma- and S-shaped bodies through the acquisition of indentations. S-shaped bodies give rise to nephrons consisting of glomeruli, proximal and distal tubules, and loops of Henle (23, 54). Examination of this process by immunofluorescence (12) has revealed that laminin, collagen IV, and heparan sulfate proteoglycan first appear as spots on aggregating cells, and then form a semicontinuous sheet on the surface of comma- and S-shaped bodies, suggesting that these molecules may be involved initially in cell aggregation and later in epithelial differentiation.

Since laminin is one of the most potent regulators of cell attachment and differentiation, laminin receptors may play an important role in this process. Several putative laminin receptors have been characterized (15), including a high affinity 67-kD receptor (32, 33, 39, 46) whose antibodies bind to endothelial (52) and epithelial cells (18), and inhibit both the attachment of cells to laminin (51) and the invasion of tumor cells through basement membrane. Recently, a 32-

G. W. Laurie's present address is Department of Anatomy and Cell Biology, University of Virginia, Charlottesville, Virginia 22908. P. D. Killen's present address is Department of Pathology, University of Michigan, Ann Arbor, Michigan. B. Segui-Real's present address is Department of Cell Biology and Histology, University of the Basque Country, Leioa, Vizcaya, Spain.

kD laminin receptor was identified and found to show functional and immunological similarities with the 67-kD receptor, suggesting that the two proteins may be products of the same gene with size differences due to posttranslational modifications (Segui-Real, B., P. Savagner, R. Reich, R. C. Ogle, G. R. Martin, and Y. Yamada, manuscript submitted for publication). Initial cloning studies resulted in a partial human cDNA clone identified by Wewer et al. (50) as coding for the 67-kD receptor by comparison to a limited protein sequence. However, continued cloning to full length has indicated that both the human and mouse cDNA code for a protein with a molecular mass of 32 kD (53; Segui-Real, B., P. Savagner, R. Reich, R. C. Ogle, G. R. Martin, and Y. Yamada, manuscript submitted for publication). Considering the uncertain relationship between the 67-kD and 32-kD proteins, we therefore refer to them together as the "67/32-kD laminin receptor."

In the present study, we have used Northern analysis and in situ hybridization to study the temporal expression of mRNA for the 67/32-kD laminin receptor, the three laminin chains, and the $\alpha 1$ -chain of type IV collagen ($\alpha 1$ [IV])¹ in cells of developing kidneys. Expression of laminin receptor mRNA was found to precede laminin and collagen IV, suggesting a role for the 67/32-kD laminin receptor in kidney development.

Materials and Methods

Preparation of Probes

Mouse cDNA for the 67/32-kD laminin receptor was isolated and sequenced. Complete conservation with the published partial human amino acid sequence (50) was observed. Mouse cDNA (Fig. 1) for the 67/32-kD laminin receptor (nucleotides 737-1,030), and for the laminin B1 (nucleotides 566-1,698 and 4,028-5,126; reference 43), laminin A (nucleotides 5,232-6,640; reference 44), and $\alpha 1$ (IV) (nucleotides 145-981; references 25 and 37) chains was subcloned into pGEM vectors (Promega Biotec, Madison, WI) containing SP6 and a T7 RNA polymerase promoters. Mouse cDNA for the laminin B2 chain (nucleotides 4,409-6,120, 666 of which are translated; reference 42) was subcloned into Bluescript vector (Stratagene, La Jolla, CA) containing T3 RNA polymerase and T7 RNA polymerase promoters (Fig. 1). Rat cDNA in pGEM for cartilage proteoglycan core (nucleotides 5,844-6,554; reference 10) and link protein (nucleotides 570-1,344; reference 40) was provided by Dr. Kurt Doege (Shriners Hospital, Portland, OR). Before transcription, plasmids were linearized such that RNA probes complementary (antisense) or noncomplementary (sense) to mRNA could be produced. Transcription was performed as described (35, 36) using [³⁵S]UTP (New England Nuclear, Boston, MA) and the appropriate RNA polymerase. DNA templates were digested with RNase-free DNase I and then the [³⁵S]RNA probes were purified. Probe size was determined by electrophoresis on a 5% polyacrylamide/7 M urea gel. Probes were stored up to 3 wk at -70°C in 10 mM DTT containing 1 U of RNasin. In addition, the same $\alpha 1$ (IV) and laminin B2 cDNA, as well as cDNA for 28-S rRNA (7) were nick translated (34) using [³²P]deoxynucleotides.

Preparation of Tissues

Kidneys were removed from C57BL mice either as embryos at 16 d gestation, at birth, and at 1, 2, and 3 wk after birth. For Northern analysis, kidneys were immediately frozen in liquid nitrogen, and RNA was extracted with 6 M guanidine-HCl as described by Han et al. (17). RNA from differentiated F9 teratocarcinoma cells (45) was provided by Dr. P. Burbelo (National Institute of Dental Research, Bethesda, MD). For in situ hybridization, kidneys were fixed with 5% formaldehyde (30) in 0.08 M NaPO₄, pH 6.8, for 3 h (at 4°C), washed in PBS for 2 h (4°C), then dehydrated and infiltrated with paraffin (2). Parietal yolk sac (9) from mice at 12 d gestation were similarly fixed and embedded in paraffin.

1. *Abbreviation used in this paper:* $\alpha 1$ (IV), the $\alpha 1$ -chain of type IV collagen.

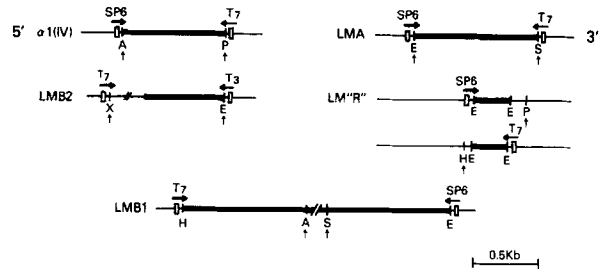


Figure 1. Orientation and length of DNA templates used for in vitro transcription of [³⁵S]RNA probes to $\alpha 1$ (IV), the laminin B2 (LMB2), laminin B1 (LMB1), and laminin A (LMA) chains, as well as to the 67/32-kD laminin receptor (LM'R'). Boxes indicate promoter sites for T7, SP6, or T3 RNA polymerases which allow transcription either from the noncoding strand (thick horizontal arrow) for antisense RNA, or from the coding strand (thin horizontal arrow) for sense RNA. Before transcription, plasmids were linearized at the restriction site (vertical arrows; A, Ava I; P, Pst I; E, Eco RI; H, Hind III; S, SST I; and X, Xba I) distal to the chosen promoter. The position of DNA templates in the full length sequence is given in Materials and Methods.

Northern Analysis

Equal amounts (7 μ g) of total RNA from each age of kidney and from differentiated F9 cells were electrophoresed under denaturing conditions through a 1% agarose/formaldehyde gel, transferred to Nytran (Schleicher & Schuell, Inc., Keene, NH), baked, and hybridized (34) overnight with each of the [³⁵S]RNA probes (4×10^6 cpm/ml), using the same conditions as for in situ hybridization (see below). The washing procedure was similar (see below) except that higher wash temperatures (65°C) were used to compensate for the omission of the RNase step. Filters were exposed to x-ray film for 2 d to 2 wk (-70°C), and were hybridized (37°C) to a [³²P]28-S rRNA probe (0.5×10^6 cpm/ml), washed (0.1 \times SSC; 60°C), and exposed to x-ray film overnight to check for uniform RNA transfer. In addition, filters were hybridized (37°C) to [³²P]cDNA probes (0.5×10^6 cpm/ml) for the laminin B2 and $\alpha 1$ (IV) chains, washed in a solution of 50% formamide, 5 \times SSC at 37°C for 1 h and exposed for 1 wk to x-ray film. Radioautograms in the linear range were scanned with a Helena Laboratories (Beaumont, TX) or LKB Instruments, Inc. (Bromma, Sweden) densitometer.

Immunoprecipitation

Primary cultures of newborn kidney epithelia were prepared by the method of Taub and Sato (47), grown to confluency, and incubated for 60 min with [³⁵S]methionine in the presence of β -aminopropionitrile (15 μ g/ml) and ascorbate (50 μ g/ml). Cells were scraped, extracted in a solution of 0.1 M Tris, 0.15 M NaCl, 1% Triton X-100, 0.1% SDS, 1% deoxycholate, and 1% aprotinin, then immunoprecipitated by the method of Ledbetter et al. (31) using anti-67/32-kD laminin receptor antiserum, antilaminin antiserum (provided by Dr. J. R. Hassell, University of Pittsburgh, Pittsburgh, PA), and anticollagen IV antibodies (provided by Dr. C. D. Little, University of Virginia, Charlottesville, VA). The anti-67/32-kD laminin receptor antiserum was prepared against fusion protein containing receptor plus β -galactosidase synthesized in bacteria from cDNA in a pEX vector. Immunoprecipitates were examined after SDS-PAGE (5%) by the method of Laemmli (27) and subjected to fluorography using Autofluor (National Diagnostics, Inc., Manville, NJ).

Western Blotting

Primary cultures of 1-wk kidney epithelia were prepared and extracted as for immunoprecipitation. Extracts were separated by SDS-PAGE (10%), transferred to Nytran (Schleicher & Schuell, Inc.; 200 mA for 1 h), blocked with 3% BSA, 3% nonfat dry milk in 50 mM Tris, 10 mM EDTA, 1% Triton X-100 for 2 h at 37°C, and then incubated with anti-67/32-kD laminin receptor antiserum (1/100) in 100 mM Tris, 0.9% NaCl, 0.1% Tween-20 for 30 min at room temperature. Blots were washed with Tween 20-TBS for 15 min and bound antibody was detected using Vectastain ABC (Vector Laboratories, Inc., Burlingame, CA).

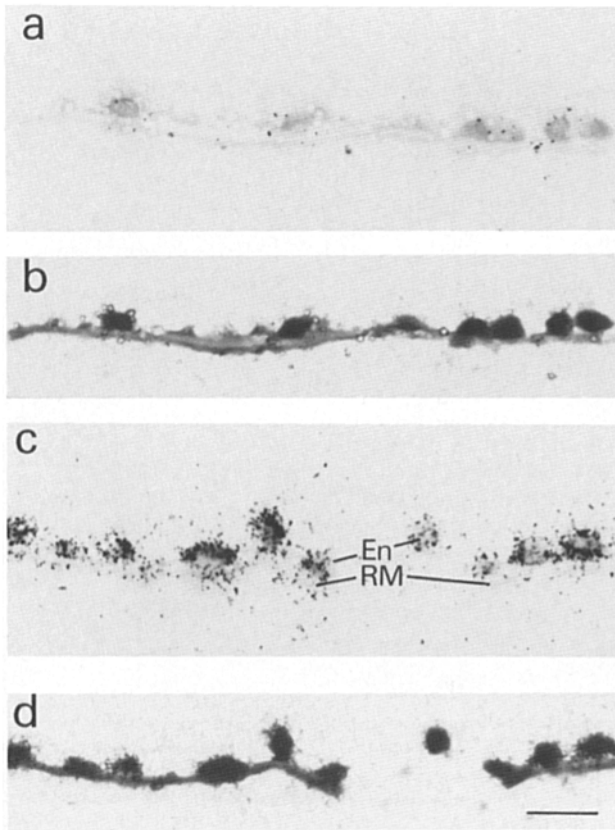


Figure 2. Light micrographs of parietal yolk sac after in situ hybridization with ^{35}S -control RNA (*a*), or with ^{35}S antisense RNA for the laminin B1 chain (*c*). *b* and *d* are methylene blue-stained versions of *a* and *c*, respectively. Parietal yolk sac sections were included as a positive control on slides containing kidney sections. RNA probes were hybridized with sections, washed, and then coated with nuclear emulsion and exposed for 1 wk. The presence of silver grains indicates the location of hybridized ^{35}S RNA. Few silver grains are seen in the control. The antisense probe detects a high level of laminin B1 chain mRNA in the cytoplasm of endoderm cells (*En*). *RM*, Reichert's membrane. Bar, 15 μm .

In Situ Hybridization

Paraffin sections were cut 1- μm thick on an ultramicrotome and allowed to adhere overnight at 42°C on slides freshly coated with polylysine (2). Sections of 16 d gestation, newborn, and 1-wk-old kidneys were grouped together on the same slides; and sections of 1-, 2-, and 3-wk-old kidneys were similarly grouped on other slides. As an internal positive control, sections of parietal yolk sac from mouse concepti at 12 d gestation were included (Fig. 2), since parietal yolk sac endoderm cells, like differentiated F9 cells, are known to synthesize large amounts of basement membrane molecules (9). The sections were deparaffinized, pronase treated (0.25 mg/ml for 10 min; see references 4, 16), acetylated (22), and then prehybridized in a solution of 50% formamide, 2 \times SSC, 10 mM DTT at 52°C for 10 min. Hybridization was performed overnight at 49°C in a humidified chamber (14) using 10 μl per slide of a hybridization solution (19) of 50% formamide, 2 \times SSC, 100 mM DTT, 1 mg/ml tRNA, 1 mg/ml sonicated salmon sperm DNA, 2 mg/ml BSA, and ^{35}S RNA probe (3 \times 10⁷ cpm/ml). The sections soaked in hybridization solution were covered with a plastic square (12 mm²) cut from a Kapak Pouch (Kapak Corp., St. Louis Park, MN). Sections were washed in several changes of 50% formamide, 5 \times SSC, 10 mM DTT (52°C) for 3 h, then for 20 min in a solution of 50% formamide, 2 \times SSC, 10 mM DTT (52°C). Sections were treated with 30 μl per slide of RNase A (100 $\mu\text{g/ml}$)/RNase T1 (1 $\mu\text{g/ml}$; references 14, 19) for 30 min at 37°C, and were washed for 1 h in three changes of a solution of 0.1 \times SSC, 10 mM DTT (52°C). Slides were then placed in a cassette with x-ray film (Amersham Corp., Arlington Heights, IL) for 4 wk at 4°C, or coated with nuclear

emulsion (14; NTB-2, Eastman Kodak Co., Rochester, NY; diluted 1:2) and exposed for 1 wk at 4°C. Radioautograms from x-ray film were photographically enlarged (final magnification, 17.8 \times). Quantitation was performed from photographic enlargements (final magnification, 6,250 \times) of emulsion-coated sections in which the area per field (36 fields per time-point per probe examined) of glomeruli, proximal and distal tubules, collecting ducts, and

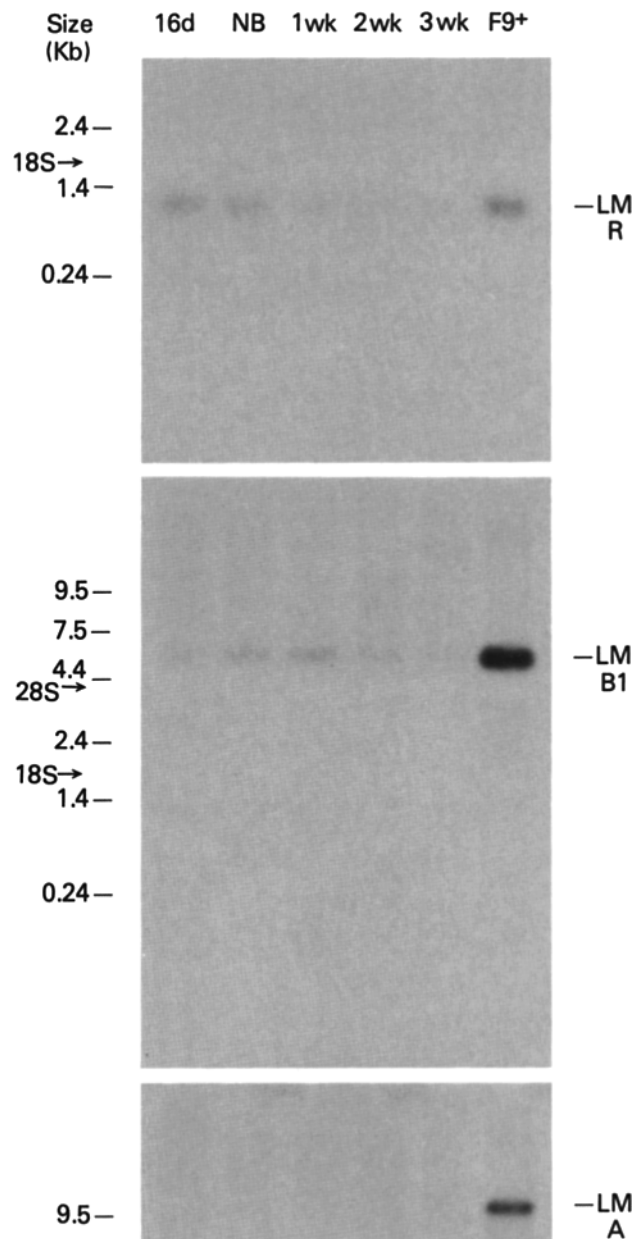


Figure 3. Northern analysis of mRNA for the 67/32-kD laminin receptor (*LM R*), the B1 (*LM B1*), and the A (*LM A*) chains of laminin in RNA prepared from kidneys at 16 d gestation (16 d), birth (NB), and 1–3 wk after birth, and from differentiated F9 teratocarcinoma cells (F9+). Total cellular RNA was electrophoresed and transferred to a Nytran filter. The same filter was sequentially hybridized with ^{35}S -labeled antisense RNA for the laminin B1 chain, the laminin receptor, and the laminin A chain. Laminin receptor mRNA expression peaks at 16 d gestation then declines, whereas laminin B1 expression is maximal at 1 wk. Expression of laminin A chain is detected in differentiated F9 cells but not in kidney. Exposure time was 1 wk. The lanes contained similar amounts of RNA as confirmed by hybridization with a ^{32}P 28-S rRNA probe.

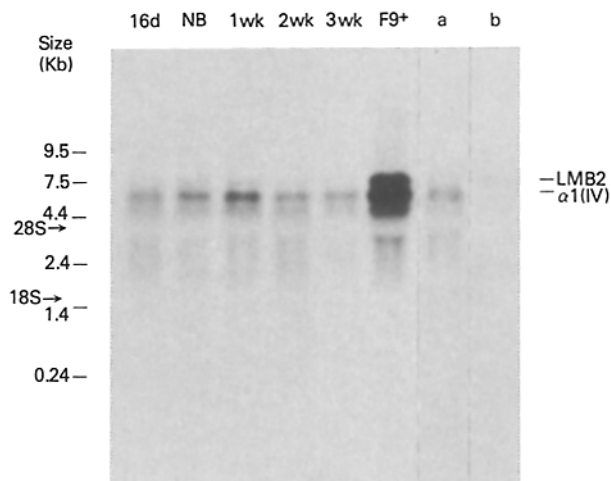


Figure 4. Northern analysis of mRNA for $\alpha 1(IV)$, and for the laminin B2 chain (LMB2) in RNA extracts of different aged kidneys and differentiated F9 teratocarcinoma cells (F9+). At left (16 d to F9+), hybridization was performed simultaneously with [^{35}S]antisense RNA for the $\alpha 1(IV)$ and laminin B2 chains. $\alpha 1(IV)$ and laminin B2 chain expression is maximal at 1 wk. Laminin B2 expression is quite weak. Hybridization with a [^{32}P]28-S rRNA probe confirmed that the lanes contained similar amounts of RNA. At right (lanes a and b), hybridization was performed separately with [^{32}P]cDNA for the $\alpha 1(IV)$ (a) and laminin B2 (b) chains on blots containing RNA from 1-wk-old kidneys. Exposure time was 4 d.

loops of Henle was measured by tracing with a ZIDAS digitizer (Carl Zeiss, Oberkochen, West Germany). The corresponding number of grains per measured structure was then counted and expressed as the mean \pm 2 SEM.

Results

Laminin Receptor Expression Precedes Laminin and Collagen IV

Steady-state mRNA levels of the laminin receptor, laminin B1, B2, and A chains, and $\alpha 1(IV)$ were examined in kidneys at 16 d gestation, birth, and 1–3 wk after birth (Figs. 3–5).

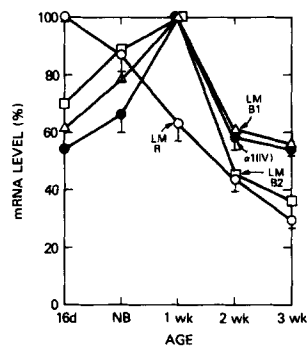


Figure 5. Quantitation of Northern blot hybridization by densitometric scanning. Laminin receptor mRNA is maximal at 16 d gestation whereas laminin B1, laminin B2, and $\alpha 1(IV)$ chain mRNA is maximal at 1 wk. Data is expressed as a percentage of the maximal level of expression and has been standardized using the [^{32}P]28-S rRNA hybridization results. For laminin receptor and $\alpha 1(IV)$, for which three to four North-

ern analyses were performed on separate blots, the data are presented as the mean \pm 2 SEM. Two separate laminin B1 and four separate laminin B2 analyses were performed on different blots; two of the B2 blots were quantifiable. These data are presented as the mean; the ranges for laminin B1 and B2 at each time were as follows: 58–65% and 60–70% (16 d); 78–79% and 80–90% (newborn, NB); 100–100% and 100–100% (1 wk); 51.3–71% and 38–53% (2 wk); and 49–63% and 26–46% (3 wk), respectively.

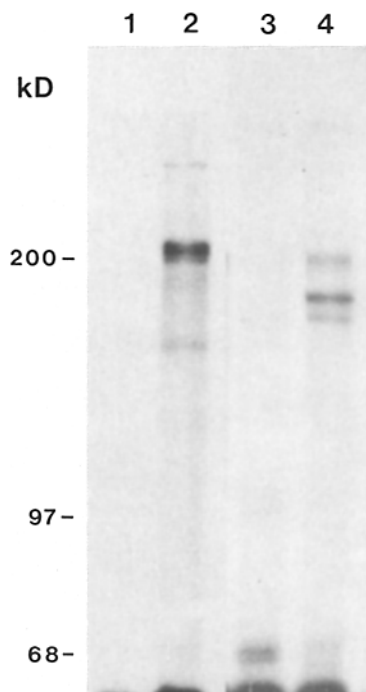


Figure 6. Immunoprecipitation of laminin receptor, laminin, and collagen IV from primary cultures of newborn kidney epithelial cells. Lane 1 is the preimmune serum control, no protein is precipitated. Lane 2 shows laminin A (400 kD), laminin B1 (210 kD), and laminin B2 (200 kD) chains and some contaminating entactin (nidogen; 150 kD) precipitated with antilaminin antiserum. Lane 3 shows the 67/32-kD laminin receptor precipitated with antireceptor antiserum; this antiserum poorly precipitates the 32-kD portion which would not have been included in the 5% gel. The 67-kD portion commonly (reference 33) runs as two bands, the reason for which is not known. Lane 4 shows the $\alpha 1$ (185 kD) and $\alpha 2$ (165 kD) chains of collagen IV and some contaminating laminin (200 kD) after precipitation with anticollagen IV antibodies. All samples were run on a single 5% SDS-polyacrylamide gel. The figure represents a composite of two fluorographs exposed for 1 d (lanes 1 and 2) or 21 d (lanes 3 and 4). No bands were present in lane 1 at 21 d.

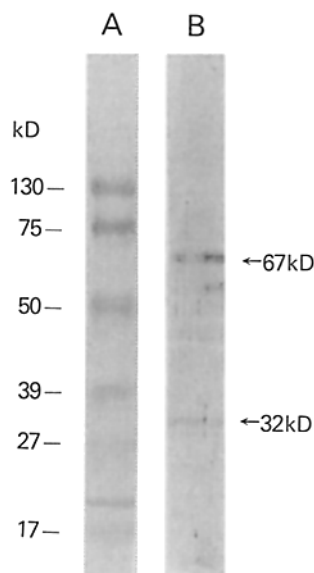


Figure 7. Western blot analysis of laminin receptor from primary cultures of 1-wk-old kidney epithelial cells. Lane A contains molecular mass standards stained with Amido black. Lane B shows the 67-kD and 32-kD portions of the laminin receptor detected with the same antireceptor antiserum used in Fig. 6. The doublet nature of the 67-kD portion is not as apparent in this preparation. Samples were run on a 10% SDS-polyacrylamide gel before transfer to Nytran.

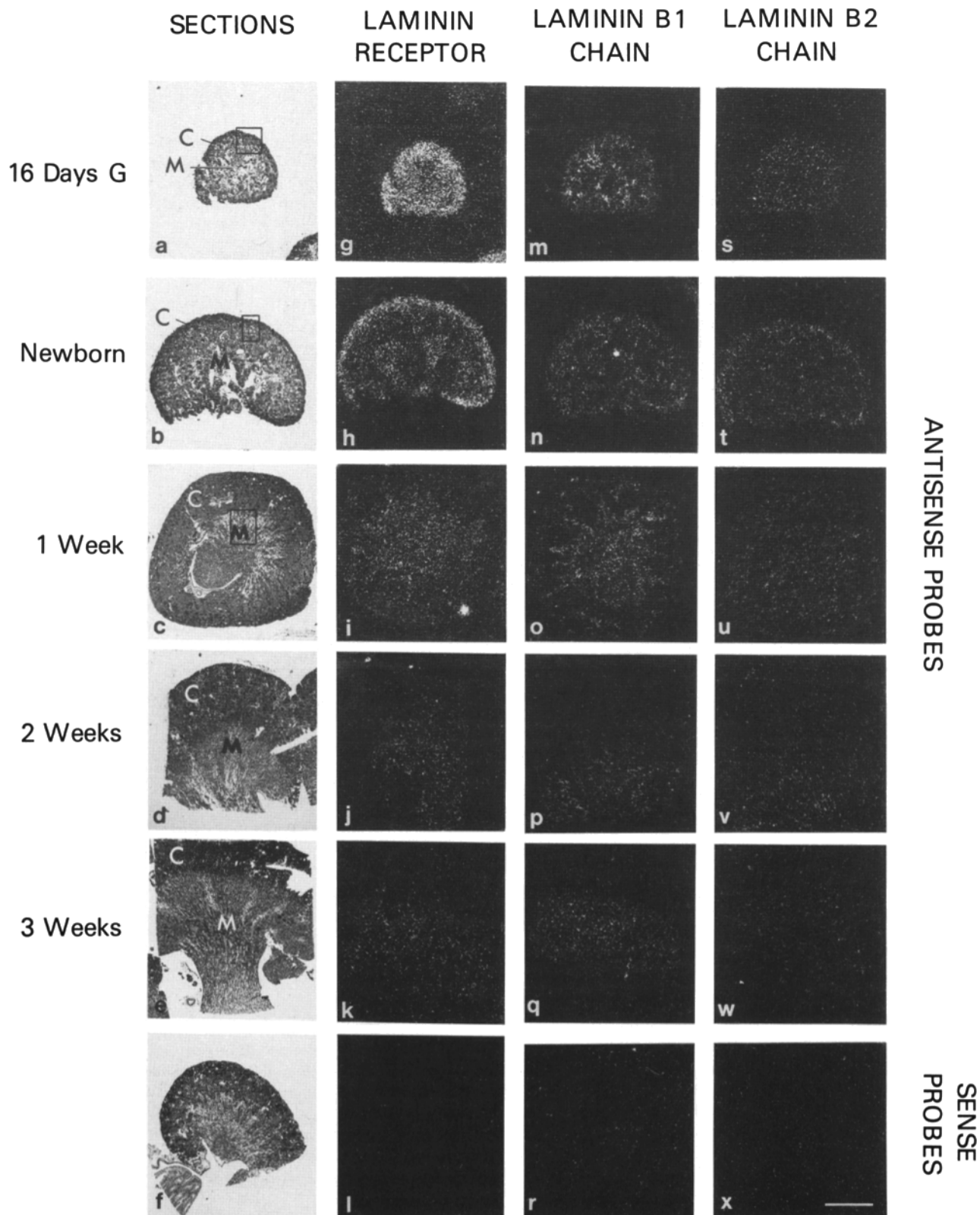


Figure 8. Micrographs of kidney at 16 d gestation (*G*), birth (*Newborn*), and 1–3 wk after birth, after in situ hybridization with [³⁵S]antisense RNA for the 67/32-kD laminin receptor (*g–k*), laminin B1 chain (*m–q*), and laminin B2 chain (*s–w*), or in controls with [³⁵S]sense RNA for the receptor (*l*), and B1 (*r*) and B2 (*x*) chains. The tissue sections at left (*a–f*) correspond to the adjacent radioautograms (*g–l*). Boxes in tissue sections (*a–c*) indicate approximate regions enlarged in Fig. 9. Sections were hybridized, washed, and exposed to x-ray film for 4 wk, then the x-ray film was photographically enlarged in negative image so that the regions of white indicate the location of hybridized probe. No labeling is seen with the control probes (*l*, *r*, and *x*). In contrast, at 16 d gestation laminin receptor (*g*), laminin B1 (*m*), and laminin B2 (*s*) expression occurs throughout the kidney, but is particularly strong for the laminin receptor in the nephrogenic zone of the cortex (*C*). At birth the nephrogenic zone expression is more distinct, especially for the laminin receptor (*h*). The expression shifts to the medulla (*M*) at one week (*i* and *o*), but is very weak for laminin B2 (*u*). At 2 and 3 wk, labeling for all three mRNAs is weak or not detectable. Bar, 500 μm.

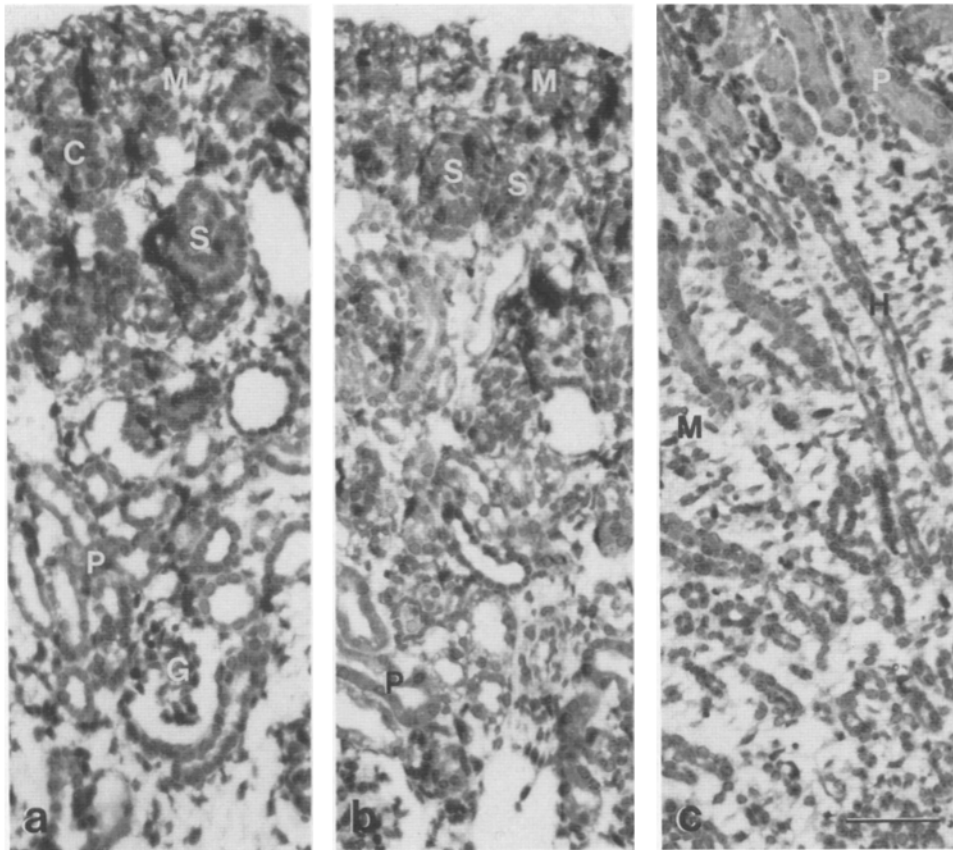


Figure 9. Light micrographs corresponding approximately to boxed regions in Fig. 8 from kidney sections at (a) 16 d gestation, (b) birth, and (c) 1 wk. (a) Top half of micrograph is nephrogenic zone containing mesenchyme (M) and several structures including a comma-shaped (C) and an S-shaped (S) body. Bottom half is junction between nephrogenic zone and medulla, in which a glomerulus (G) and proximal tubules (P) are present. (b) Corresponds to cortex with mesenchyme (M) and S-shaped bodies (S) in the nephrogenic zone and other structures including proximal tubules (P) below. (c) Cortico-medullary junction with cortical proximal tubules (P) above and medullary mesenchyme (M) and loops of Henle (H) below. Micrographs were taken from sections cut serially to those boxed in Fig. 8. Bar, 50 μ m.

RNA from differentiated F9 teratocarcinoma cells was included as a positive control. Single mRNA species of 1.1 kb for the laminin receptor, 6 kb for laminin B1 (Fig. 3), 7.5 kb for laminin B2, and 6 kb for α 1(IV) (Fig. 4) were detected. Previously the laminin receptor message was thought to be 1.7 kb (50), but repeated Northern analyses have pointed to a 1.1-kb (Segui-Real, B., P. Savagner, R. Reich, R. C. Ogle, G. R. Martin, and Y. Yamada, manuscript submitted for publication) or 1.2-kb (53) size; the laminin and collagen IV message sizes are in keeping with previous observations (5, 11, 25). Densitometric scans (Fig. 5) illustrated that laminin receptor mRNA was maximal at 16 d gestation and then decreased, whereas laminin B1, laminin B2, and α 1(IV) chain mRNAs peaked 1 wk after birth. Laminin A chain mRNA of 10 kb was detected in differentiated F9 but not in 7 μ g of total kidney RNA (Fig. 3), reflecting the low level of laminin A chain expression in kidney (25). All lanes exhibited similar levels of hybridization with the probe for 28-S rRNA (not shown).

Immunoprecipitation of Laminin Receptor, Laminin, and Collagen IV

To confirm the synthesis of laminin receptor, laminin, and collagen IV in kidney, primary cultures of newborn kidney epithelial cells (47) were incubated in [35 S]methionine for 60 min. Cell extracts were immunoprecipitated with antibodies specific for laminin receptor (Fig. 6, lane 3), the three chains of laminin (Fig. 6, lane 2), and the chains of collagen IV (Fig. 6, lane 4); all three proteins were synthesized. As observed by others (33), the 67-kD portion of the receptor appeared as a doublet, the reason for which is not known.

In agreement with the Northern analysis, the synthesis of the laminin A chain was significantly less than the B chains. Some difficulty was encountered precipitating the 32-kD portion of the receptor with the anti-67/32-kD antiserum, but by Western blotting both bands were readily detected (Fig. 7).

Visualization of mRNAs by In Situ Hybridization

Exposure of hybridized sections of whole kidney to x-ray film revealed the location of mRNAs detected by Northern analysis (Figs. 8 and 10). Histological enlargements of expressing regions are presented in Figs. 9 and 11. At 16 d gestation, laminin receptor mRNA was concentrated in the nephrogenic zone of the kidney cortex (Fig. 8 g) where glomeruli and tubules are forming (Fig. 9 a). Expression of laminin B1 (Fig. 8 m), laminin B2 (Fig. 8 s), and α 1(IV) (Figs. 10 g and 11 a) chain mRNAs was lighter with α 1(IV) greater than laminin B1 which was greater than laminin B2 at this time and later, but these differences may be due to other factors such as probe size and hybridization efficiency. Expression of laminin A chain mRNA was less than laminin B2 and barely detectable (Fig. 10 m). At birth, expression of laminin receptor mRNA was less intense, but still was located in the nephrogenic zone (Figs. 8 h and 9 b). Laminin B1 (Fig. 8 n), laminin B2 (Fig. 8 t), and α 1(IV) (Figs. 10 h and 11 b) mRNAs were slightly elevated in the nephrogenic zone. Laminin A mRNA (Fig. 10 n) remained less than laminin B2 and barely detectable although some nephrogenic zone labeling was suggested in other exposures and in subsequent quantitative analysis (Fig. 14). By 1 wk, expression had shifted to the medulla for the laminin receptor (Figs. 8 i and 9 c), and laminin B1 (Fig. 8 o), laminin B2 (Fig. 8 u)

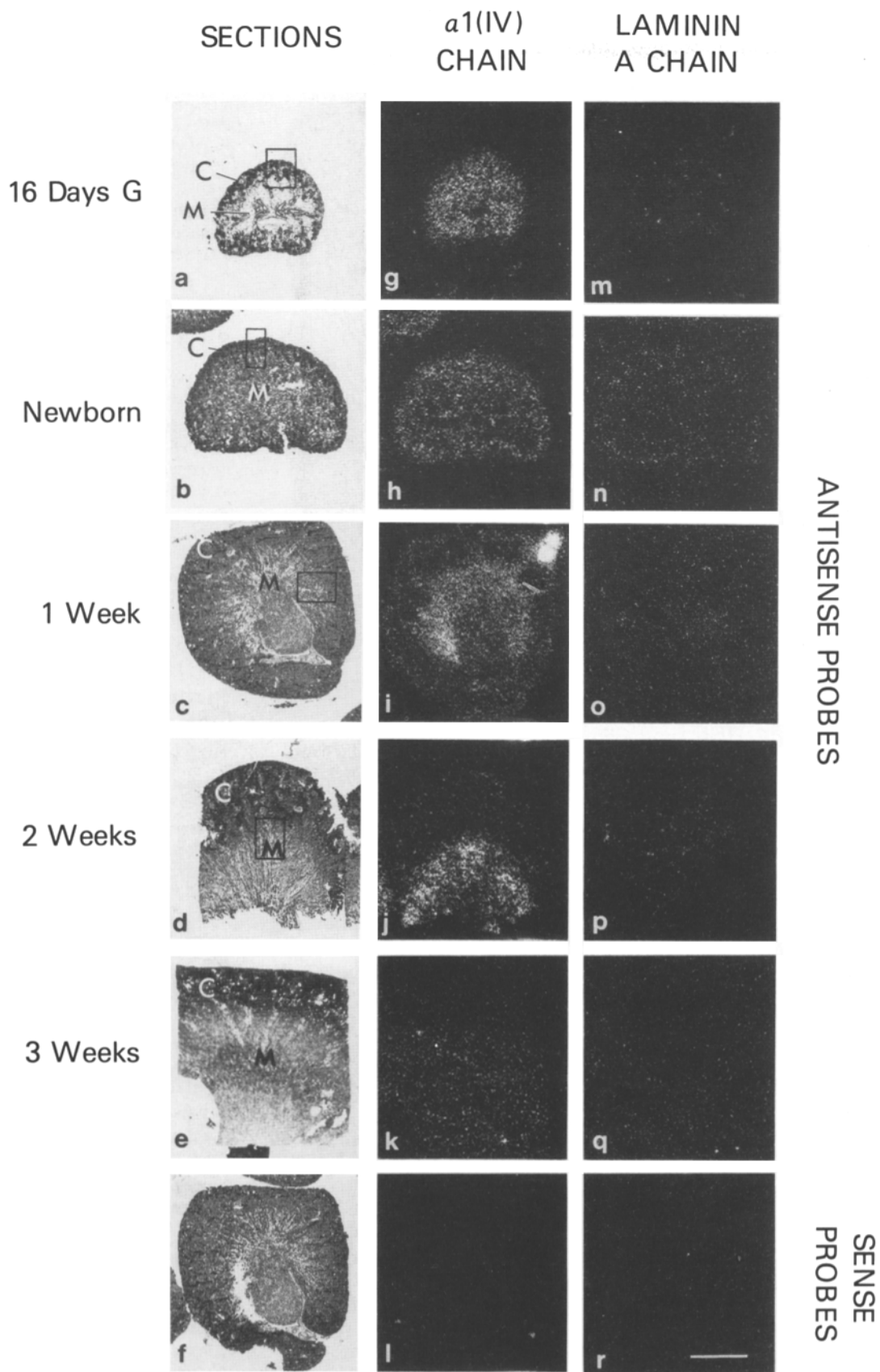


Figure 10. Micrographs of various ages of kidney after in situ hybridization with [³⁵S]antisense RNA for the $\alpha 1(\text{IV})$ chain (g–k) and laminin A chain (m–q), or in controls with [³⁵S]sense RNA for the $\alpha 1(\text{IV})$ (l) and laminin A (r) chains. The sections at left (a–f) correspond to the adjacent radioautograms (g–l) and were processed as in Fig. 8. Expression of mRNA for the $\alpha 1(\text{IV})$ chain is present throughout the 16 d gestation kidney (g), then becomes more prominent in the nephrogenic zone at birth (h), and shifts to the medulla at 1 wk (i). Expression remains strong in the medulla at 2 wk (j), then decreases (k). Laminin A chain mRNA is lighter than laminin B2 and is faintly detectable at 16 d gestation (m) and birth (n). No labeling is observed with the control probes (l and r). Bar, 500 μm .

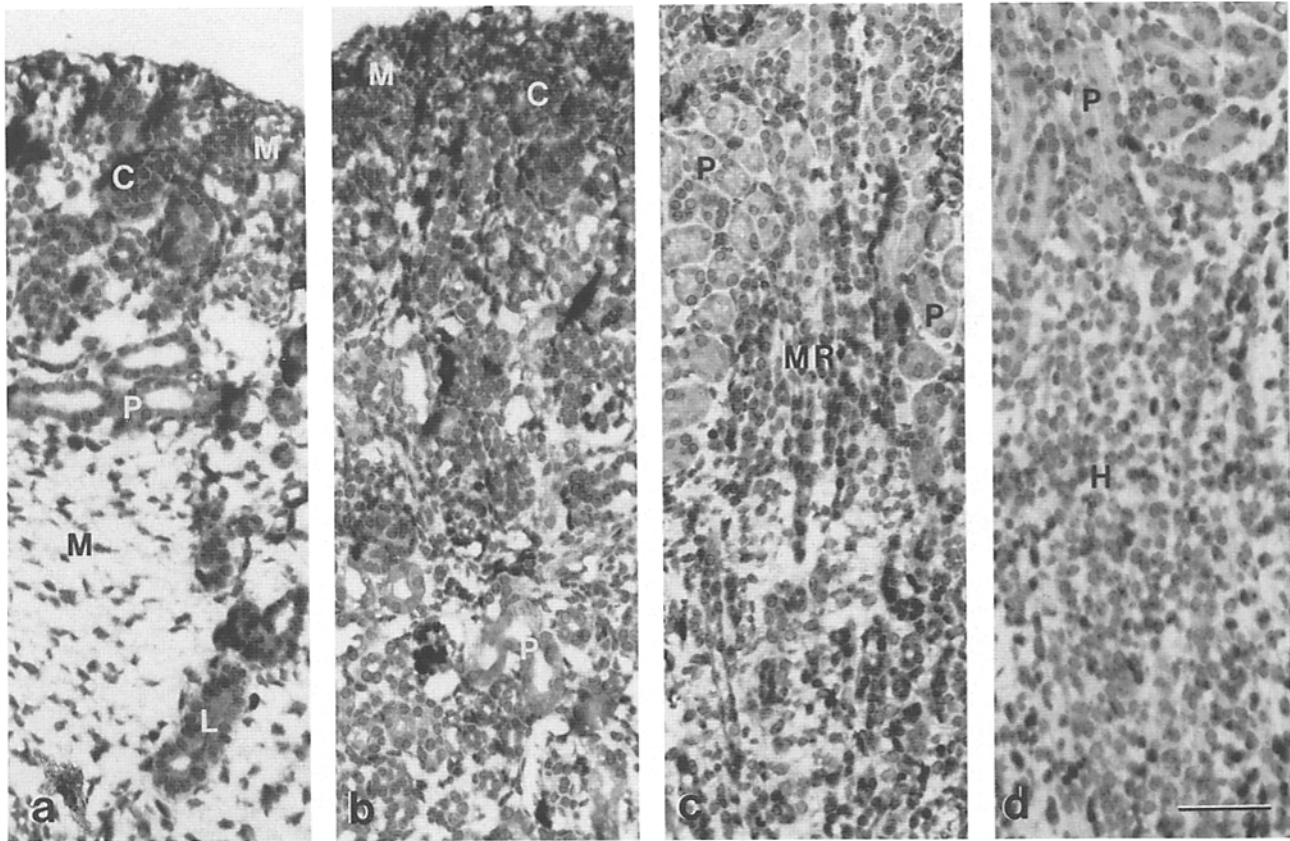


Figure 11. Light micrographs corresponding approximately to boxed regions in Fig. 10 from kidney sections at (a) 16 d gestation, (b) birth, (c) 1 wk, and (d) 2 wk. (a) Top half of micrograph is nephrogenic zone with mesenchyme (M), and several structures including a comma-shaped body (C) and proximal tubules (P). Bottom half is medulla containing mesenchyme (M) and collecting ducts (L). (b) Cortex with nephrogenic zone in top third of micrograph containing a comma-shaped body (C) and mesenchyme (M). Elsewhere, other structures including proximal tubules (P) are seen. (c) Cortico-medullary junction featuring a medullary ray (MR) extending up from the medulla into the cortex. On either side of the ray are proximal tubules (P). (d) Cortico-medullary junction with proximal tubules (P) in top third and several structures including loops of Henle (H) below. Micrographs were taken from sections cut serially to those boxed in Fig. 10. Bar, 50 μm .

and $\alpha 1(\text{IV})$ (Figs. 10 *i* and 11 *c*) chain mRNAs. Laminin B2 mRNA was very light and laminin A mRNA (Fig. 10 *o*) was not detected. At 2 wk, mRNA for the $\alpha 1(\text{IV})$ chain was still expressed in the medulla (Figs. 10 *j* and 11 *d*), but was weaker for the laminin receptor (Fig. 8 *j*), and laminin B1 (Fig. 8 *p*) and B2 (Fig. 8 *v*) chains. By 3 wk, expression of all mRNAs was barely or not detectable (Figs. 8, *k*, *q*, and *w*, and 10, *k* and *q*).

No labeling was present in controls using sense probes noncomplementary to mRNA for the laminin receptor (Fig. 8 *l*), or for the laminin B1 (Fig. 8 *r*), laminin B2 (Fig. 8 *x*), $\alpha 1(\text{IV})$ (Fig. 10 *l*), or laminin A (Fig. 10 *r*) chains. Labeling was also absent using antisense probes for rat cartilage proteoglycan core and link protein (not shown) which cross-hybridize with mouse RNA (Laurie, G. W., unpublished observations).

Comparison of Cellular Expression at Different Ages

To identify cell types expressing laminin receptor, laminin, and collagen IV mRNAs in kidney (Figs. 12–15), hybridized sections were exposed to a thin layer of nuclear emulsion and developed. The cellular location of hybridized probe was revealed by the presence of silver grains (Figs. 2 and 12).

At 16 d gestation and birth, labeled cells were apparent in comma-shaped bodies (not shown), S-shaped bodies (Fig. 12 *a*), and forming glomeruli (Fig. 12 *b*) of the nephrogenic zone, although the labeling intensity was much less than previously observed (Fig. 2) in parietal endoderm cells. Labeled cells were less frequent in glomeruli from 2-wk-old kidneys (Fig. 12 *c*).

Cellular expression was quantitated by determining the number of silver grains per 100 μm^2 of tissue (Figs. 13–15). A representative sense probe was used as control (Fig. 13 *a*). Proximal and distal tubules, glomeruli, collecting ducts, and loops of Henle accounted for most of the hybridization signal. Several similarities in the temporal appearance of these mRNAs were observed. Most striking was the transient expression of mRNA for the laminin receptor, and laminin B1, B2, and A, and $\alpha 1(\text{IV})$ chains in cells of proximal and distal tubules at 16 d gestation (Figs. 13, *d* and *g*, 14, *c* and *f*, and 15 *c*). Glomerular expression was maximal at 16 d gestation and birth for all mRNAs (Figs. 13, *b* and *e*, 14, *a* and *d*) with the exception of $\alpha 1(\text{IV})$ chain mRNA which remained high at 1 wk (Fig. 15 *a*).

The expression of these mRNAs differed significantly in collecting duct and loop of Henle cells. At 16 d gestation, only collecting ducts were apparent and these expressed peak

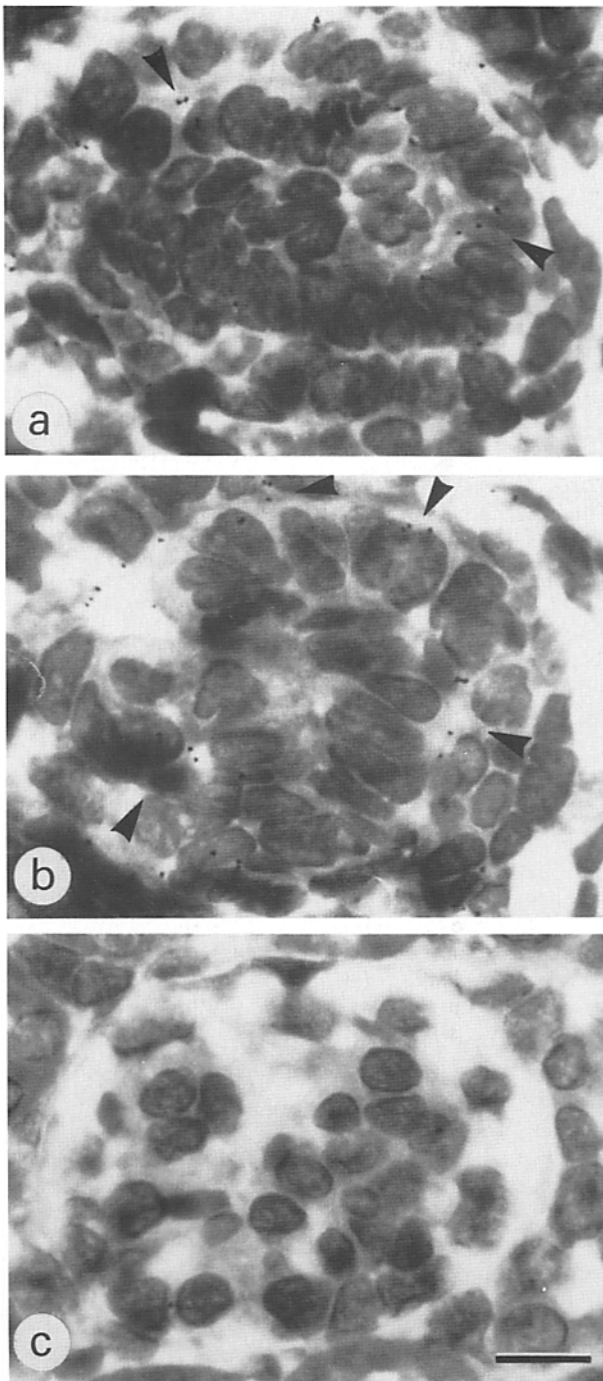


Figure 12. Light micrographs of an S-shaped body (a) and a forming glomerulus (b) from a 16-d-gestation kidney, as compared to a glomerulus (c) from a 2-wk-old kidney after in situ hybridization with [³⁵S]antisense RNA for the 67/32-kD laminin receptor. Prepared as in Fig. 2. Labeling (arrowhead) density is much less than for endodermal cells of parietal yolk sac. In the S-shaped body (a), the region of future developing tubules appears to be labeled. The visceral epithelium at the periphery of the forming glomerulus (b), and other glomerular, perhaps endothelial, cells are labeled. Labeling is absent in a 2-wk-old glomerulus (c). Bar, 3 μ m.

levels of laminin receptor mRNA (Fig. 13 c). At birth, both collecting ducts and loops of Henle were highly labeled. In contrast, laminin B1 (Fig. 13 f) and α 1(IV) (Fig. 15 b) chain mRNA expression peaked at 1 or 2 wk. Expression of mRNA

for laminin B2 (Fig. 14 b) and laminin A (Fig. 14 e) chains in collecting ducts and loops of Henle did not appear to significantly differ with time.

Discussion

Formation of the kidney occurs in several distinct steps (12, 23, 54). Initially, nephrogenesis predominates in the kidney cortex with the aggregation of mesenchymal cells induced by adjacent ureter bud epithelium. Aggregates transform into epithelial comma- and S-shaped bodies. Comma- and S-shaped bodies develop into nephrons consisting of glomeruli, proximal and distal tubules, and loops of Henle. After birth, nephrogenesis subsides and the kidney medulla expands as collecting ducts and loops of Henle lengthen, with adult size reached by three months in the rat.

Since nephrogenesis involves epithelial-mesenchymal contacts, particular attention has been paid to the role of extracellular matrix molecules in vesicle formation and epi-

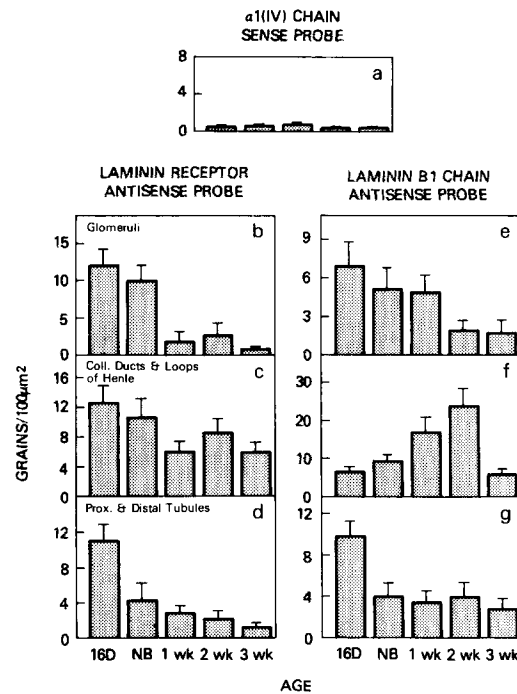


Figure 13. Quantitation of in situ hybridization for the 67/32-kD laminin receptor (b-d) and the laminin B1 (e-g) chain in cells of glomeruli, proximal and distal tubules, collecting ducts, and loops of Henle. Expression levels in collecting ducts and loops of Henle, and in proximal and distal tubules were similar; data from these paired structures were combined. Comma- and S-shaped bodies were considered as glomeruli for this analysis. Background labeling was \sim 0.6 grains/100 μ m² (a). At 16 d gestation and birth, laminin receptor mRNA expression is relatively high in glomeruli (b), and collecting ducts and loops of Henle (c). However, no loops of Henle were observed at 16 d gestation, thus labeling in the latter category could all be assigned to collecting ducts. Laminin receptor expression is maximal in proximal and distal tubules at 16 d gestation (d). The glomerular expression pattern for the laminin B1 chain is similar (e) to the laminin receptor, but remains elevated at 1 wk. Laminin B1 chain mRNA peaks at 2 wk in collecting ducts and loops of Henle (f), and is maximal at 16 d gestation in proximal and distal tubules (g).

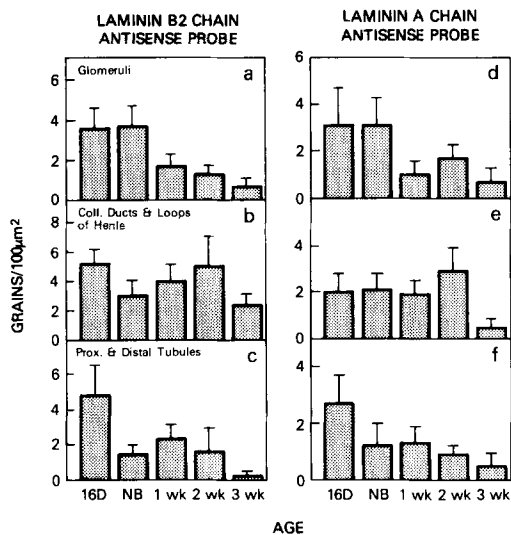


Figure 14. Quantitation of in situ hybridization for the laminin B2 (a-c) or laminin A (d-f) chains in glomeruli, proximal and distal tubules, and collecting ducts and loops of Henle. Quantitation was performed as in Fig. 13. Expression of laminin B2 chain mRNA is maximal at 16 d gestation and birth over glomeruli (a), but shows no definite pattern over collecting ducts and loops of Henle, and is elevated at 16 d gestation over proximal and distal tubules (c). Laminin A chain mRNA is detectable over glomeruli at 16 d gestation and at birth (d), and is elevated over proximal and distal tubules at 16 d gestation (f). No clear pattern is apparent for collecting ducts and loops of Henle (e).

thelial differentiation. The use of immunofluorescence has demonstrated spots of laminin, collagen IV, and heparan sulfate proteoglycan on aggregating cells, later to form sheets on comma- and S-shaped bodies (12). Similarly, uvomorulin (L-CAM) has been observed on ureter bud and embryonic tubule cells (49); whereas tenascin (3) and the disialoganglioside G_{D3} (41) localize to the adjacent mesenchyme.

The goal of the present study was to localize cellular mRNAs for a laminin receptor, the laminin B1, B2, and A chains, and $\alpha 1(IV)$ during kidney development. For this purpose, [^{35}S]RNA probes complementary to mRNA for these proteins were prepared by in vitro transcription and purified. Probe specificity was demonstrated in several ways. They were derived from well-characterized cDNA clones (25, 37, 42-44; Segui-Real, B., P. Savagner, R. Reich, R. C. Ogle, G. R. Martin, and Y. Yamada, manuscript submitted for publication) and hybridized to single mRNA species of the appropriate size. The $\alpha 1(IV)$ probe mainly spanned the noncollagenous (NC1) domain sequence (37), thus minimizing the possibility of cross-hybridization with other collagen mRNAs, as had been observed for RNA probes to the triple helical sequence of collagen I and II (21). Finally, in vitro transcription off the opposite DNA strand formed [^{35}S]RNA probes identical to mRNA which, as expected, showed no hybridization.

Northern analysis of steady-state mRNA showed that laminin receptor expression was maximal at 16 d gestation and expression of laminin B and $\alpha 1(IV)$ chains peaked at 1 wk after birth. Similarly, in our initial dot blot examination of $\alpha 1(IV)$ and laminin B chain expression in developing kidney (11), $\alpha 1(IV)$ chain expression was maximal at 1 wk but high levels of laminin B chain were observed both at birth and 1 wk with peak expression at birth. The laminin B2 result does

not differ significantly. The laminin B1 difference may be due to difficulties with the dot blot method in which possible nonspecific hybridizations would not be readily apparent.

In situ hybridization revealed that the cellular mRNA expression of laminin receptor, laminin chains, and $\alpha 1(IV)$ chain followed specific spatial and temporal patterns with early activity in glomerular and tubular cells of the nephrogenic zone, followed by activity in the medulla as collecting ducts and loops of Henle lengthened. By 3 wk after birth, expression had diminished, and presumably remained at low levels thereafter since residual laminin B and $\alpha 1(IV)$ chain mRNA expression have been detected in kidneys from animals 8 wk and older using dot blots (5, 25). These results reflected the Northern data and indicated that early high laminin receptor mRNA expression in total kidney RNA was due to early elevated expression in all cell types examined, whereas the delayed laminin B1 mRNA expression could be attributed to high 1-2 wk levels in collecting ducts and loops of Henle. Thus expression of these mRNAs followed three phases: the nephrogenic phase, corresponding to the differentiation and growth of cells giving rise to nephrons at 16 d gestation and birth; the medullary phase, when collecting ducts and loops of Henle lengthen at 1-2 wk after birth; and finally the phase of diminished steady-state mRNA after 2 wk.

Considered in light of epithelial-mesenchymal interactions during nephrogenesis, it was apparent that the laminin receptor, laminin, and $\alpha 1(IV)$ chain mRNAs were concentrated over epithelial structures including comma- and S-shaped bodies; and were minimal or absent from uninduced mesenchymal cells, in keeping with immunofluorescence data for laminin and collagen IV (12). Particularly striking was the early high level of laminin receptor mRNA in collecting ducts which, as derivatives of the ureter bud, remain active in mesenchymal induction. This observation raises the possibility that aggregation of induced mesenchymal cells on the collecting duct terminus may arise from the interaction of collecting duct laminin receptor with newly synthesized mesenchymal cell laminin.

Laminin A chain mRNA was low despite the age of kidney examined, in agreement with previous Northern analyses of 8 wk or older mouse kidney, heart, spleen, liver, and lung

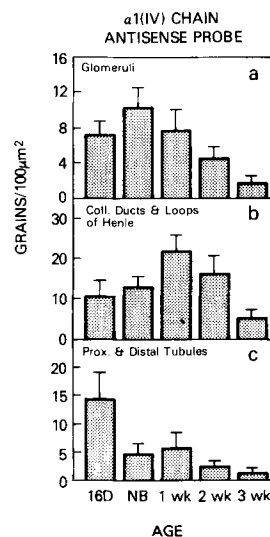


Figure 15. Quantitation of in situ hybridization for $\alpha 1(IV)$ in glomeruli, proximal and distal tubules, and collecting ducts and loops of Henle. Performed as in Fig. 13. Expression is maximal in glomeruli at birth (a), in collecting ducts and loops of Henle at 1 wk (b), and in proximal and distal tubules at 16 d gestation (c).

(25). This observation has raised the possibility that the apparent B1/B2/A chain composition of laminin in thick basement membranes such as the Englebreth-Holm-Swarm basement membrane and Reichert's membrane may differ from that in more common thin basement membranes, with B1/B2 dimers prevalent and perhaps A chains independently secreted according to local requirements. Indeed, A chain synthesis was low in our tubular epithelial cell cultures, and in 12–16 d kidney organ cultures (24) even though the A chain terminus was recently found to influence tubule cell polarization (24). On the other hand, A chain synthesis may be rate limiting for laminin B1/B2/A secretion; or A chain mRNA may be more rapidly degraded.

In summary, mRNA expression of laminin receptor, laminin chains, and $\alpha 1(IV)$ chain follows three phases during kidney development. Assuming a correlation of steady-state mRNA to protein synthesis, it is apparent that the laminin receptor, as well as laminin and collagen IV are mainly synthesized in the first two phases, with synthesis thereafter diminishing in the third phase after 2 wk. The lack of receptor expression in the third phase suggests that the laminin receptor–basement membrane complex may be a more stable arrangement than previously suspected.

The authors gratefully acknowledge the help of Dr. S. M. Laurie in the preparation of the manuscript.

Received for publication 25 September 1988 and in revised form 25 May 1989.

References

- Abrahamson, D. R. 1986. Recent studies on the structure and pathology of basement membranes. *J. Pathol.* 149:257–278.
- Angerer, L. M., and R. C. Angerer. 1981. Detection of poly(A)⁺ mRNA in sea urchin eggs and embryos by quantitative in situ hybridization. *Nucleic Acids Res.* 9:2819–2840.
- Aufderheide, E., R. Chiquet-Ehrismann, and P. Ekblom. 1987. Epithelial–mesenchymal interactions in the developing kidney lead to expression of tenascin in the mesenchyme. *J. Cell Biol.* 105:599–608.
- Berger, C. N. 1986. In situ hybridization of immunoglobulin-specific RNA in single cells of the B lymphocyte lineage with radiolabelled DNA probes. *EMBO (Eur. Mol. Biol. Organ.) J.* 5:85–93.
- Boot-Handford, R. P., M. Kurkinen, and D. J. Prockop. 1987. Steady-state levels of mRNAs coding for the type IV collagen and laminin polypeptide chains of basement membranes exhibit marked tissue-specific stoichiometric variations in the rat. *J. Biol. Chem.* 262:12475–12478.
- Carlin, B. E., R. Jaffe, B. Bender, and A. E. Chung. 1981. Entactin, a novel basal-lamina associated sulfated glycoprotein. *J. Biol. Chem.* 256:5209–5214.
- Chan, Y.-L., and I. G. Wool. 1983. The structure of rat 28S ribosomal ribonucleic acid inferred from the sequence of nucleotides in a gene. *Nucleic Acids Res.* 11:7819–7831.
- Charonis, A. S., E. C. Tsilibary, P. D. Yurchenco, and H. Furthmayr. 1985. Binding of laminin to type IV collagen: a morphological study. *J. Cell Biol.* 100:1848–1853.
- Clément, B., B. Segui-Real, J. R. Hassell, Y. Yamada, and G. R. Martin. 1989. Identification of a cell surface binding protein for the core protein of the basement membrane proteoglycan. *J. Biol. Chem.* In press.
- Cooper, A. R., M. Kurkinen, A. Taylor, and B. L. M. Hogan. 1981. Studies on the biosynthesis of laminin by murine parietal endoderm cells. *Eur. J. Biochem.* 119:189–197.
- Doege, K., M. Sasaki, E. Horigan, J. R. Hassell, and Y. Yamada. 1987. Complete primary structure of the rat cartilage proteoglycan core protein deduced from cDNA clones. *J. Biol. Chem.* 262:17757–17767.
- Ebihara, I., P. D. Killen, G. W. Laurie, T. Huang, Y. Yamada, G. R. Martin, and K. S. Brown. 1988. Altered mRNA expression of basement membrane components in a murine model of polycystic kidney disease. *Lab. Invest.* 58:262–269.
- Ekblom, P. 1981. Formation of basement membrane in the embryonic kidney: an immunohistological study. *J. Cell Biol.* 91:1–10.
- Engel, J., E. Odermatt, A. Engel, J. Madri, H. Furthmayr, H. Rohde, and R. Timpl. 1981. Shapes, domain organizations and flexibility of laminin and fibronectin, two multifunctional proteins of the extracellular matrix. *J. Mol. Biol.* 150:97–120.
- Gall, J. G., and M. L. Pardue. 1971. Nucleic acid hybridization in cytological preparations. *Methods Enzymol.* 21:470–480.
- Gehlsen, K. R., L. Dillner, E. Engvall, and E. Ruoslahti. 1988. The human laminin receptor is a member of the integrin family of cell adhesion receptors. *Science (Wash. DC).* 241:1228–1229.
- Hafen, E., M. Levine, R. L. Garber, and W. J. Gehring. 1983. An improved in situ hybridization method for the detection of cellular RNAs in *Drosophila* tissue sections and its application for localizing transcripts of the homeotic *Antennapedia* gene complex. *EMBO (Eur. Mol. Biol. Organ.) J.* 2:617–623.
- Han, J. H., C. Stratowa, and W. J. Rutter. 1987. Isolation of full-length putative rat lysophospholipase cDNA using improved methods for mRNA isolation and cDNA cloning. *Biochemistry.* 26:1617–1625.
- Hand, P. H., A. Thor, J. Schlom, C. N. Rao, and L. A. Liotta. 1985. Expression of laminin receptor in normal and carcinomatous human tissues as defined by a monoclonal antibody. *Cancer Res.* 45:2713–2719.
- Harper, M. E., L. M. Marselle, R. C. Gallo, and F. Wong-Staal. 1986. Detection of lymphocytes expressing human T-lymphotropic virus type III in lymph nodes and peripheral blood from infected individuals by in situ hybridization. *Proc. Natl. Acad. Sci. USA.* 83:772–776.
- Hay, E. D. 1981. Cell Biology of Extracellular Matrix. Plenum Publishing Corp., New York. 417 pp.
- Hayashi, M., Y. Ninomiya, J. Parsons, K. Hayashi, B. R. Olsen, and R. L. Trelstad. 1986. Differential localization of mRNA's of collagen types I and II in chick fibroblasts, chondrocytes, and corneal cells by in situ hybridization using cDNA probes. *J. Cell Biol.* 102:2302–2309.
- Hayashi, S., I. C. Gillian, A. D. Delaney, and G. M. Tener. 1978. Acetylation of chromosome squashes of *Drosophila melanogaster* decreases the background in autoradiographs from hybridization with [¹²⁵I] labeled RNA. *J. Histochem. Cytochem.* 26:677–679.
- Kazimierzczak, J. 1971. Development of the renal corpuscle and the juxtaglomerular apparatus. *Acta Pathol. Microbiol. Scand. Sect. A Pathol.* 218(Suppl.):1–64.
- Klein, G., M. Langegger, R. Timpl, and P. Ekblom. 1988. Role of laminin A chain in the development of epithelial cell polarity. *Cell.* 55:331–341.
- Kleinman, H. K., I. Ebihara, P. D. Killen, M. Sasaki, F. B. Cannon, Y. Yamada, and G. R. Martin. 1987. Genes for basement membrane proteins are coordinately expressed in differentiating F9 cells but not in normal adult murine tissues. *Dev. Biol.* 122:373–378.
- Kleinman, H. K., J. Graf, Y. Iwamoto, G. T. Kitten, R. C. Ogle, M. Sasaki, Y. Yamada, G. R. Martin, and L. Luckenbill-Edds. 1987. Role of basement membranes in cell differentiation. *Ann. NY Acad. Sci.* 513:134–145.
- Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (Lond.)* 227:680–685.
- Laurie, G. W., J. T. Bing, H. K. Kleinman, J. R. Hassell, M. Aumailley, G. R. Martin, and R. J. Feldmann. 1986. Localization of binding sites for laminin, heparan sulfate proteoglycan and fibronectin on basement membrane (type IV) collagen. *J. Mol. Biol.* 189:205–216.
- Laurie, G. W., S. Inoue, J. T. Bing, and J. R. Hassell. 1988. Visualization of the large heparan sulfate proteoglycan from basement membrane. *Am. J. Anat.* 181:320–326.
- Lawrence, J. B., and R. H. Singer. 1985. Quantitative analysis of in situ hybridization methods for the detection of actin gene expression. *Nucleic Acids Res.* 13:1777–1799.
- Ledbetter, S. R., B. Tyree, J. R. Hassell, and E. A. Horigan. 1985. Identification of the precursor protein to basement membrane heparan sulfate proteoglycans. *J. Biol. Chem.* 260:8106–8113.
- Lesot, H., U. Kuhl, and K. von der Mark. 1983. Isolation of a laminin-binding protein from muscle cell membranes. *EMBO (Eur. Mol. Biol. Organ.) J.* 2:861–865.
- Malinoff, A. L., and M. S. Wicha. 1983. Isolation of a cell surface receptor protein for laminin from murine fibrosarcoma cells. *J. Cell Biol.* 96:1475–1479.
- Maniatis, T., E. F. Fritsch, and J. Sambrook. 1982. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY. 545 pp.
- Melton, D. A., P. A. Krieg, M. R. Rebagliati, T. Maniatis, K. Zinn, and M. R. Green. 1984. Efficient in vitro synthesis of biologically active RNA and RNA hybridization probes from plasmids containing a bacteriophage SP6 promoter. *Nucleic Acids Res.* 12:7035–7056.
- Nomura, S., A. J. Wills, D. R. Edwards, J. K. Heath, and B. L. Hogan. 1988. Developmental expression of 2ar (osteopontin) and SPARC (osteonectin) RNA as revealed by in situ hybridization. *J. Cell Biol.* 106:441–450.
- Oberbaumer, I., M. Laurent, U. Schwartz, Y. Sakurai, Y. Yamada, G. Vogeli, T. Voss, B. Siebold, R. Glanville, and K. Kuhn. 1985. Amino acid sequence of the non-collagenous globular domain (NCI) of the $\alpha 1(IV)$ chain of basement membrane collagen as derived from complementary DNA. *Eur. J. Biochem.* 149:217–224.
- Paulsson, M., P. D. Yurchenco, G. C. Ruben, J. Engel, and R. Timpl. 1987. Structure of low density heparan sulfate proteoglycan isolated from a mouse tumor basement membrane. *J. Mol. Biol.* 197:297–313.
- Rao, C. N., S. H. Barsky, V. P. Terranova, and L. A. Liotta. 1983. Isolation of a tumor cell laminin receptor. *Biochem. Biophys. Res. Commun.*

- 111:804-808.
40. Rhodes, C., K. Doege, M. Sasaki, and Y. Yamada. 1988. Alternative splicing generates two different mRNA species for rat link protein. *J. Biol. Chem.* 263:6063-6067.
 41. Sariola, H., J. E. Aufderheide, H. Bernhard, S. Henke-Fahle, W. Dippold, and P. Ekblom. 1988. Antibodies to cell surface ganglioside G_{D3} perturbs inductive epithelial-mesenchymal interactions. *Cell.* 54:235-245.
 42. Sasaki, M., and Y. Yamada. 1987. The laminin B2 chain has a multidomain structure homologous to the B1 chain. *J. Biol. Chem.* 262:17111-17117.
 43. Sasaki, M., S. Kato, K. Kohno, G. R. Martin, and Y. Yamada. 1987. Sequence of the cDNA encoding the laminin B1 chain reveals a multidomain protein containing cysteine-rich repeats. *Proc. Natl. Acad. Sci. USA.* 84:935-939.
 44. Sasaki, M., H. K. Kleinman, H. Huber, R. Deutzmann, and Y. Yamada. 1988. Laminin, a multidomain protein. The A chain has a unique globular domain and homology with the basement membrane proteoglycan and the laminin B chains. *J. Biol. Chem.* 263:16536-16544.
 45. Strickland, S., K. K. Smith, and K. R. Marotti. 1980. Hormonal induction of differentiation in teratocarcinoma stem cells: generation of parietal endoderm by retinoic acid and dibutyryl cAMP. *Cell.* 21:341-347.
 46. Sugrue, S. P. 1984. The interaction of extracellular matrix with the basal surface of embryonic corneal epithelia. *Prog. Clin. Biol. Res.* 151:77-87.
 47. Taub, M., and G. Sato. 1980. Growth and functional primary cultures of kidney epithelial cells in defined medium. *J. Cell. Physiol.* 105:369-378.
 48. Timpl, R., and M. Dziadek. 1986. Structure, development, and molecular pathology of basement membranes. *Int. Rev. Exp. Pathol.* 29:1-112.
 49. Vestweber, D., R. Kemler, and P. Ekblom. 1985. Cell-adhesion molecule uvomorulin during kidney development. *Dev. Biol.* 112:213-221.
 50. Wewer, U. M., L. A. Liotta, M. Jaye, G. A. Ricca, W. N. Drohan, A. P. Claysmith, C. N. Rao, P. Wirth, J. E. Coligan, R. Albrechtsen, M. Mudryj, and M. E. Sobel. 1986. Altered levels of laminin receptor mRNA in various human carcinoma cells that have different abilities to bind laminin. *Proc. Natl. Acad. Sci. USA.* 83:7137-7141.
 51. Wewer, U. M., G. Taraboletti, M. E. Sobel, R. Albrechtsen, and L. Liotta. 1987. Role of laminin receptor in tumor cell migration. *Cancer Res.* 47:5691-5698.
 52. Yannariello-Brown, J., U. Wewer, L. Liotta, and J. A. Madri. 1988. Distribution of a 69-kD laminin-binding protein in aortic and microvascular endothelial cells: modulation during cell attachment, spreading, and migration. *J. Cell Biol.* 106:1773-1786.
 53. Yow, H., J. M. Wong, H. S. Chen, C. Lee, G. D. Steele, Jr., and L. B. Chen. 1988. Increased mRNA expression of a laminin-binding protein in human colon carcinoma: complete sequence of a full-length cDNA encoding the protein. *Proc. Natl. Acad. Sci. USA.* 85:6394-6398.
 54. Zamboni, L., and C. DeMartino. 1968. Embryogenesis of the human renal glomerulus. *Arch. Pathol.* 86:279-291.