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Evidence for a Precursor of the High-Affinity Metastasis-Associated Murine Laminin Receptor†

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ABSTRACT: The high-affinity cellular receptor for the basement membrane component laminin is differentially expressed during tumor invasion and metastasis. A cDNA clone encoding the murine laminin receptor was isolated and identified on the basis of sequence homology to the human laminin receptor [Wewer et al. (1986) *Proc. Natl. Acad. Sci. U.S.A. 83*, 7137-7141]. Primer extension experiments demonstrated that the clone contained the complete 5′ sequence of the murine laminin receptor mRNA. RNA blot data demonstrated a single-sized laminin receptor mRNA, approximately 1400 bases long, in human, mouse, and rat. The nascent laminin receptor predicted from the cDNA sequence is 295 amino acids long, with a molecular weight of 33 000, and contains one intradisulfide bridge, a short putative transmembrane domain, and an extracellular carboxy-terminal region which has abundant glutamic acid residues and multiple repeat sequences. The precursor of the laminin receptor is apparently smaller than the 67-kilodalton protein isolated from tissue. The apparent molecular weight on SDS-polyacrylamide gels of the rabbit reticulocyte cell-free translation product of selectively hybridized laminin receptor mRNA is 37 000. Antisera to three different domains of the cDNA-predicted receptor were used to study the relationship between the 37- and 67-kilodalton polypeptides. Antisera to cDNA-deduced synthetic peptides of the receptor immunoprecipitated a 37-kilodalton band both from cell-free translation products and from pulse-labeled cell extracts. On immunoblots of cell extracts, one antisynthetic peptide antiserum recognized only the 67-kilodalton receptor, while another antiserum identified both 37- and 67-kilodalton polypeptides, suggesting a precursor-product relationship between the two polypeptides.

Extracellular matrix molecules, notably laminin and fibronectin, play a significant role in important cellular events such as adhesion, morphology, spreading, migration, differentiation, tumor cell metastasis, and neurite outgrowth (Liotta et al., 1986, 1987; Ruoslahti, 1988). Studies on cell surface proteins which specifically interact with extracellular matrix molecules have identified specific receptors for these molecules and suggest that invasive and metastatic tumor cells have enhanced abilities to bind to the extracellular matrix (Liotta et al., 1986; Ruoslahti, 1988; Terranova et al., 1983; McCarthy et al., 1988; Humphries et al., 1986). Tumor cells must attach and interact with the basement membrane at several steps

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during the metastatic process (Liotta et al., 1983, 1986). Attachment to laminin, a major component of basement membranes, is thought to initiate a cascade of events involved in the invasive process (Liotta et al., 1983). A high-affinity laminin receptor with an apparent molecular weight of 67K has been isolated and characterized from human and rodent neoplastic cells, as well as from human placenta, bovine smooth muscle cells, and avian corneal epithelial cells (Lesot et al., 1983; Malinoff & Wicha, 1983; Rao et al., 1983; Wewer et al., 1986; Sugrue, 1988). By using a monoclonal anti-human laminin receptor antibody which blocks laminin binding to tumor cells (Liotta et al., 1985), we previously isolated and characterized a partial human cDNA clone for the laminin receptor (Wewer et al., 1986). Northern hybridization analysis indicated a strong, direct correlation between the levels of laminin receptor mRNA and the number of laminin receptors present on the cell surfaces of a variety of human carcinoma-derived cell lines. On the basis of these findings, we hypothesized that the laminin-mediated attachment of tumor cells to basement membranes may be regulated by the amount of translatable laminin receptor mRNA (Wewer et al., 1986). While this paper was in preparation, a more complete human laminin receptor cDNA clone was reported and increased mRNA expression of laminin receptor was demonstrated in human colon carcinoma (Yow et al., 1988).

Most experimental approaches to the study of tumor cell–basement membrane interaction utilize laminin isolated from a murine source (Liotta et al., 1986). We therefore set out to determine the sequence and structure of the murine counterpart of the high-affinity, metastasis-associated, human laminin receptor. We report here the isolation of a full-length murine laminin receptor cDNA clone which predicts a nascent laminin receptor polypeptide of 33 kilodaltons, and we demonstrate a relationship between the smaller precursor and the 67-kilodalton laminin receptor.

Experimental Procedures

Cell Lines. Human Panc-1 (Lieber et al., 1975) and HT-1080 (Rasheed et al., 1974) cells and murine NIH-3T3 cells were obtained from the American Type Culture Collection. Human A2058 cells (Todaro et al., 1980) were obtained from G. Todaro, National Cancer Institute. F9 teratocarcinoma cells (Strickland & Mahdavi, 1978) were obtained from W. Anderson, National Cancer Institute. Rat L2 cells were derived from a rat yolk sac carcinoma (Wewer, 1982) and have been described (Wewer et al., 1986). The cells were routinely grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum, penicillin (100 μg/mL), and streptomycin (100 μg/mL), except that NIH-3T3 cells were grown in 10% bovine serum instead of fetal bovine serum. The growth conditions for differentiation of F9 cells by retinoic acid and dibutyryl cyclic AMP have been described (Strickland et al., 1980).

Preparation of Antibodies to Synthetic Peptides. Peptides were synthesized according to predicted amino acid sequences based on the nucleotide sequence of the laminin receptor cDNA. Peptide 12 (AIVAIENPADVSVISSRNTG; see Figure 2, amino acids 64–83) and peptide 13 (SGALDVLQ; see Figure 2, amino acids 2–9) were synthesized on a Biosearch 9600 peptide synthesizer. Antiserum 4056 was raised against peptide 12 in the following manner. Two milligrams of peptide 12 was conjugated to 6 mg of bovine serum albumin in a volume of 10 mL of phosphate-buffered saline (PBS),[1] using 0.125% glutaraldehyde. Rabbits were immunized subcutaneously using complete Freund's adjuvant every 2 weeks. Antibodies were then affinity purified by heating serum to 56 °C for 30 min, followed by adsorbing the antibody to peptide 12–AffiGel 10 resin at 4 °C for 18 h with gentle agitation. After thorough washing with PBS, the adsorbed antibody was eluted from the resin with 1.0 M acetic acid. The eluate was neutralized to pH 7.0 with sodium hydroxide, and the resulting solution was concentrated and exchanged to PBS on Centricon 30 microconcentrators (Amicon, Danvers, MA). Antiserum 4099 was raised against peptide 13 in a similar manner. Peptide pro-20-ala has been described previously (Wewer et al., 1987) and corresponds to amino acids 263–282 of Figure 2. Antiserum 3801 was raised against peptide pro-20-ala as described (Wewer et al., 1987).

Preparation of Cell Extracts, Immunoprecipitations, and Immunoblots. Cells were grown on 100-mm tissue culture dishes as described above. For radiolabeling experiments, when the cells reached confluence, the medium was removed, and the cell layer was rinsed twice with 10 mL of serum-free Dulbecco–Vogt medium without methionine. The cells were then incubated at 37 °C for 30 min in 10 mL of the rinse medium containing 100 μCi of [^{35}S]methionine (1153 Ci/mmol, ICN). The medium was removed, and the cell layer was quickly rinsed twice with cold PBS.

For immunoprecipitations, radiolabeled cell layers were lysed in 2 mL of buffer D [50 mM Tris (pH 7.4)/0.5% Nonidet P40/0.5% aprotinin]. Equal aliquots of cell extract (800 μL) were incubated with 50 μL preimmune serum or anti-laminin receptor antiserum 4056. The antigen–antibody complexes were allowed to form during an overnight incubation at 4 °C and adsorbed to 100 μL of a 25% protein A–Sepharose suspension for 1 h at 4 °C. The Sepharose beads were pelleted by centrifugation and washed 3 times with 1 mL of buffer D and 2 times with buffer D containing 18% potassium chloride, followed by 2 times with buffer D again as described (Laurent et al., 1987). The pellets were resuspended in 40 μL of electrophoresis sample buffer [62.5 mM Tris (pH 6.8)/2.5% sodium dodecyl sulfate (SDS)/10% glycerol/0.65 M 2-mercaptoethanol/0.01% bromophenol blue] and boiled for 3 min, and the antibody-reactive proteins were analyzed on SDS–polyacrylamide gels as described (Laemmli, 1970; Laurent et al., 1987).

For immunoblots, unlabeled cells were lysed in 2.0 mL of 1% SDS and immediately boiled for 3 min. Aliquots (25 μL) were mixed with 2× electrophoresis sample buffer and electrophoresed on 10% SDS–polyacrylamide gels as described above. After electrophoresis, transfer of proteins to nitrocellulose filters was performed in a Trans-Blot apparatus (Bio-Rad, Richmond, CA), following the manufacturer's instructions, using a transfer buffer containing 20 mM Tris, 150 mM glycine, and 20% (v/v) methanol as described (Wewer et al., 1987). Residual binding sites on the filters were blocked by incubation with 5% nonfat dry milk, 1% ovalbumin, 5% fetal calf serum, and 7.5% glycine for 30 min at room temperature with gentle shaking. The filters were washed 3 times for 5 min each in washing solution (PBS containing 2% nonfat dry milk, 2% ovalbumin, and 10% fetal calf serum). Antisera (preimmune, and serum 4056 or 4099) were diluted 1:200 in 20 mM Tris (pH 7.5)/500 mM sodium chloride and incubated with the nitrocellulose filter at 4 °C overnight with gentle shaking. After three 10-min washes in the washing solution described above, the nitrocellulose filter was incubated for 30

[1] Abbreviations: PBS, phosphate-buffered saline; buffer D, 50 mM Tris (pH 7.4)/0.5% Nonidet P40/0.5% aprotinin; SDS, sodium dodecyl sulfate; SSC, 0.15 M sodium chloride and 0.015 M sodium citrate, pH 7.4; PIPES, piperazine-*N*,*N*′-bis(2-ethanesulfonic acid); bp, base pair(s); kbp, kilobase pair(s).

min at room temperature with secondary antibody (affinity-purified goat anti-rabbit conjugated to alkaline phosphatase; Boehringer-Mannheim) diluted 1:1000 in PBS without Ca or Mg. The filter was washed twice for 5 min each in washing solution and once for 5 min in 20 mM Tris (pH 7.5)/500 mM sodium chloride. Finally, the nitrocellulose filter was stained with HRP color development solution (Bio-Rad); the staining reaction was stopped by extensive washing in distilled water.

Isolation and Characterization of Murine Laminin Receptor cDNA Clones. An Okayama–Berg cDNA library of bovine papillomavirus type 1 transformed murine C127 fibroblasts (Yang et al., 1985) was kindly provided by Dr. C. Baker (National Cancer Institute). The plasmid library was transformed into *Escherichia coli* HB101 and screened by colony hybridization (Grunstein & Hogness, 1975) using as a probe the 650 base pair (bp) insert of the human laminin receptor cDNA clone pLR4-4 (Wewer et al., 1986) which had been labeled with ^{32}P by nick translation (Maniatis et al., 1975). Colony hybridizations were performed on Colony/PlaqueScreen hybridization transfer membranes (Du Pont) at 40 °C in a solution containing 50% formamide, 5× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), 10× Denhardt's solution without albumin, 0.5% SDS, 50 mM Tris, pH 7.5, 0.25 mg/mL boiled salmon sperm DNA, and 0.5 × 10^6 dpm/filter of human laminin receptor cDNA insert. Unbound probe was washed off filters by successive incubations at room temperature in 2× SSC, 1× SSC, and 0.4× SSC, all containing 0.5% SDS and 2.5 mM EDTA. After autoradiography, positive clones were selected and purified through at least three rounds of colony hybridization. Plasmid DNA was isolated and subjected to restriction endonuclease analysis and Southern hybridization as described (Wewer et al., 1986).

DNA and Protein Sequence Analysis. The nucleotide sequence of overlapping restriction fragments of clone pMLR21 was obtained by the chemical modification method (Gilbert & Maxam, 1973). Potential secondary structures in laminin receptor mRNA and protein were determined by using the Sequence Analysis Software Package of the Genetics Computer Group, University of Wisconsin, Version 5. Inverted repeats of laminin receptor mRNA were detected by using the Stemloop and Dotplot programs, using a window (minimum stem length) of 6, a maximum loop size of 20, and a stringency (minimum number of bonds/stem) of 12. G–T, A–T, and G–C bonds were scored as 1, 2, and 3 bonds, respectively. Secondary structures of mRNA were determined by using the Fold and Squiggles programs based on the method of Zuker and Stiegler (1981). The potential secondary structure of the laminin receptor protein was determined by the Peptide-Structure and PlotStructure programs, as described by Jameson and Wolf (Jameson & Wolf, 1988; Wolf et al., 1988).

RNA Extraction and Blotting. Total cellular RNA was isolated by either the guanidine thiocyanate or the guanidine hydrochloride methods as described (Glisin et al., 1974; Sobel et al., 1981; Ullrich et al., 1977). RNA was fractionated on 1.0% agarose gels containing methylmercuric hydroxide as the denaturing agent, transferred to activated (diazobenzyloxymethyl)cellulose paper (Schleicher & Schuell), and then prehybridized in hybridization buffer containing 50% formamide, 5× SSC, 1% glycine, 0.1% SDS, 1× Denhardt's solution without albumin, and 1.2 mg/mL yeast RNA at 42 °C for at least 3 h as described (Sobel et al., 1981). RNA blots were hybridized to nick-translated ^{32}P-labeled laminin receptor cDNA insert for 16–24 h at 42 °C. Blots were washed at room temperature successively in 2× SSC, 1× SSC, 0.5× SSC, and 0.25× SSC, all containing 0.2% SDS, and exposed to X-ray film using Cronex Hi-plus intensifying screens (Du Pont). Blots were stripped free of probe by incubating in 99% formamide and then water, both at 70 °C for 5 min, followed by exposure to X-ray film to ensure complete removal of probe before hybridization to another cDNA probe.

Primer Extension Analysis. A synthetic 17-base-long oligonucleotide AGGACATCCTCCTCCTT, complementary to murine laminin receptor mRNA (see Figure 2, bases 126–110), was purchased from Midland Certified Reagent Co. (Midland, TX). The primer was further purified from contaminating shorter oligomers by elution from 20% acrylamide–urea gels (Gilbert & Maxam, 1973) and chromatography on Sephadex G-50 (fine) molecular sieve columns, after which the oligomer was ethanol precipitated. Polyadenylated RNA was isolated on oligo(dT)–cellulose columns as described (Aviv & Leder, 1972). Annealing and reverse transcriptase reactions were based on modifications of previously described procedures (Williams & Mason, 1985). The primer was end-labeled with T4 polynucleotide kinase and [γ-^{32}P]ATP as described (Maniatis et al., 1975), and 250 000 dpm was annealed to 2 μg of poly(A) RNA in a 10-μL solution containing 0.4 M sodium chloride and 10 mM piperazine-*N*,*N*′-bis(2-ethanesulfonic acid) (PIPES), pH 6.4. The mixture was heated to 70 °C for 5 min and incubated at 45 °C for 3 h. The annealing reaction mixture was then diluted to 100 μL with 90 μL of reverse transcription buffer containing 50 mM Tris (pH 8.2)/10 mM dithiothreitol/6 mM magnesium chloride/2.5 μg of actinomycin D (Calbiochem)/0.5 mM each deoxynucleotide triphosphate/10 units of avian myeloblastosis virus reverse transcriptase enzyme (Promega Biotec). After a 60-min incubation at 42 °C, the nucleic acids were precipitated in the presence of carrier calf liver tRNA (Boehringer-Mannheim), rinsed with 95% ethanol, dissolved in 5 μL of 99% formamide plus tracking dyes, and fractionated on 5% denaturing polyacrylamide gels (Gilbert & Maxam, 1973). The primer extension products were visualized by autoradiography using Kodak XAR5 X-ray film at −70 °C. The size of the extended product was determined by comparison with the electrophoretic pattern of denatured ϕX174/*Hin*cII markers which had been 3′ end-labeled with terminal deoxytransferase and [α-^{32}P]cordycepin (New England Nuclear) as described (Wewer et al., 1986).

Laminin Receptor mRNA Hybrid Selections. Five micrograms of heat-denatured laminin receptor cDNA insert or linearized control pBR322 plasmid was immobilized to 5-mm-diameter (diazobenzyloxymethyl)cellulose paper circles as described (Goldberg et al., 1979). The filters were incubated 2–3 h at room temperature in 100 μL of hybridization buffer containing 50% deionized formamide, 0.9 M sodium chloride, 0.2% SDS, 1 mM EDTA, and 20 mM PIPES, pH 6.4. The filters were then hybridized to 200 μg of total cellular RNA suspended in hybridization buffer for 16–20 h at 37 °C. The unbound RNA was removed by repeated washings (6 times, 15 min each) at 37 °C in 1 mL of a wash buffer containing 50% formamide, 20 mM sodium chloride, 8 mM sodium citrate, 1 mM EDTA, and 0.5% SDS. The bound RNA was eluted by incubating the filters in 100 μL of buffer containing 80% formamide, 1 mM EDTA, 0.5% SDS, and 10 mM PIPES, pH 6.4, for 30 min at 37 °C. The RNA in the eluate was ethanol precipitated in the presence of 10 μg of calf liver tRNA (Boehringer Mannheim) and rinsed twice with 70% ethanol. The pellet was then suspended in 5 μL of water.

Cell-Free Translations. Either 1 μg of total cellular RNA or 2 μL of the hybrid-selected laminin receptor mRNA was used in a cell-free translation system using a micrococcal

nuclease treated rabbit reticulocyte cell lysate (Bethesda Research Laboratories) as previously described (Laurent et al., 1987). Each 9-μL reaction contained 20 μCi of [^{35}S]-methionine (1095 Ci/mol, Amersham), 83 mM potassium acetate, 48 mM potassium chloride, 1.16 mM magnesium chloride, and heat-denatured RNA. Protein synthesis was allowed to proceed for 1 h at 30 °C. Under these conditions, we have previously shown that translation is linear with time and RNA concentration (Laurent et al., 1987).

For immunoprecipitation experiments, 10 translation reactions were combined and diluted to 1 mL in buffer D. The preparation was centrifuged for 1 min at 13000*g* in a microfuge to remove high molecular weight RNA aggregates. The supernatant was then divided into two equal parts and incubated with 30 μL of rabbit antiserum 3801 against synthetic laminin receptor peptide pro-20-ala or preimmune serum (Wewer et al., 1987). The antigen–antibody complexes were allowed to form during an overnight incubation at 4 °C and adsorbed to 100 μL of a 25% protein A–Sepharose (Pharmacia) suspension for 2 h at 4 °C. The Sepharose beads were pelleted by centrifugation and washed as described above. The pellets were resuspended in 45 μL of electrophoresis sample buffer and boiled for 3 min, and the antibody-reactive proteins were analyzed on SDS–polyacrylamide gels.

Results

Identification and Analysis of a Murine Laminin Receptor cDNA. An Okayama–Berg cDNA library of transformed murine fibroblasts was screened with a cDNA insert of the human laminin receptor clone which had been previously characterized (Wewer et al., 1986). Selected clones were analyzed by *Bam*HI restriction endonuclease digestion and DNA blot hybridization to determine the size of each cDNA insert. Clone pMLR21 had the largest insert, of approximately 1.4 kb. Keeping in mind that the Okayama–Berg vector contributes approximately 130 bp of sequence, excluding the poly(A) tail, to the *Bam*HI fragment, we predicted that pMLR21 contained a laminin receptor cDNA insert of approximately 1.3 kb. Figure 1 presents a simplified restriction map of pMLR21, based on restriction endonuclease mapping and DNA sequence analysis of the cDNA insert. Included in Figure 1 are all the unique restriction sites (of commercially available enzymes) as well as the two *Pst*I sites which are referred to below. Among the unique restriction sites for pMLR21, the sites for *Bsp*MII, *Hin*cII, *Sac*I, *Sau*I, and *Sph*I are present in the same location in the human laminin receptor cDNA (Wewer et al., 1986; Yow et al., 1988). Although both murine and human laminin receptor cDNAs have two *Pst*I sites, neither site is conserved in the same location between the two species.

The nucleotide sequence of the positive (sense) strand of the pMLR21 cDNA insert is presented in Figure 2. Excluding the poly(A) tail, pMLR21 contains 1086 base pairs of sequence. As shown below in Figure 3, primer extension analysis confirms that pMLR21 contains the entire sequence of the murine laminin receptor mRNA. The murine laminin receptor mRNA has a 79-base-long 5′ untranslated region. Notable within this region is a pyrimidine-rich region at the extreme 5′ end, as well as three potential translation stop codons (underlined in Figure 2). The 5′ untranslated region is 87% homologous to the analogous human untranslated region, as determined from the primer-extended sequence from human laminin receptor mRNA (data not shown). The coding region of pMLR21 is 885 bases long, encoding a polypeptide of 295 amino acids. The derived protein sequence is shown below the nucleotides in Figure 2. The nucleic acid sequences of the coding region of the murine laminin receptor are 88.7%

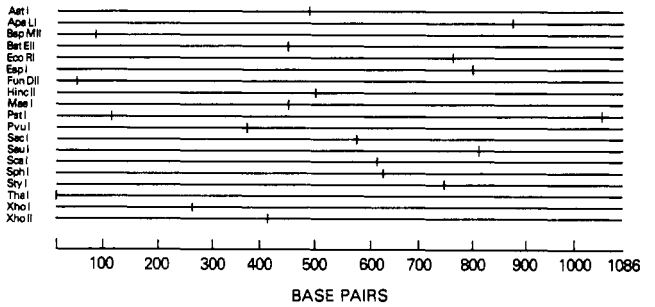

FIGURE 1: Restriction map of cDNA insert of pMLR21. Unique restriction sites as well as the location of the two *Pst*I sites are designated within the 1086 base pair full-length cDNA insert of pMLR21.

homologous to human laminin receptor. Since most of the base changes between the two species are in the wobble position, there are only four amino acids which differ between murine and human laminin receptors, resulting in a 98.6% identity at the protein level. The 3′ untranslated region of the murine laminin receptor mRNA, however, differs significantly from, and is 53 bases longer than, the human sequence (Wewer et al., 1986). A polyadenylation signal (heavy underline, Figure 2) is 68 bases upstream of the poly(A) tail.

Primer Extension of Laminin Receptor mRNA. To determine if the cDNA insert of pMLR21 encoded the full-length laminin receptor mRNA, a primer extension experiment was conducted (Figure 3). A 17-base-long oligonucleotide which was complementary to bases 126–110 of the pMLR21 insert (see Figure 2) was annealed to RNA from murine NIH-3T3 cells, and the complementary strand was synthesized. The length of the single extended product was determined to be 126 bases (Figure 3), consistent with the full-length coding capacity of pMLR21.

Predicted Secondary Structure of the Murine Laminin Receptor mRNA. Computer analysis of the nucleotide sequence of pMLR21 predicts an RNA molecule with significant secondary structure, including many short inverted repeats (Figure 4). Figure 5 presents two alternative secondary structures of the folded murine laminin receptor mRNA, each with the same free energy of −356.5 kcal/mol. The positions of the stem and loop structures shown in this two-dimensional representation are relatively constrained between the two structures, suggesting a rigid conformation of the mRNA. It is notable that the beginning of the 3′ untranslated region base pairs with the 5′ untranslated region. The most 3′ portion of the untranslated region forms its own stem and loop structure. The presumed initiating methionine codon is within a base-paired region of a stem and loop structure.

Predicted Structure of the Murine Laminin Receptor. The cDNA-derived protein sequence was analyzed for helical, β-sheet, coil, and turn configurations, as well as hydrophobicity (Figure 6). A putative, short transmembrane domain (Rao & Argos, 1986) was detected at amino acid residues 86–101 (Figures 2 and 6). Within the transmembrane domain is an alanine repeat structure AAA--A which is repeated at residues 216–221. No signal peptide is predicted by the cDNA-derived sequence, although the most amino-terminal region of the receptor contains two closely spaced hydrophobic areas. In general, the amino-terminal half of the receptor has more hydrophobic regions than the rest of the protein. There are two cysteine residues at positions 148 and 163, which most likely form an intradisulfide bond in the middle of the molecule, consistent with the faster mobility of the purified laminin receptor on native versus denatured polyacrylamide gels (Wewer et al., 1986).

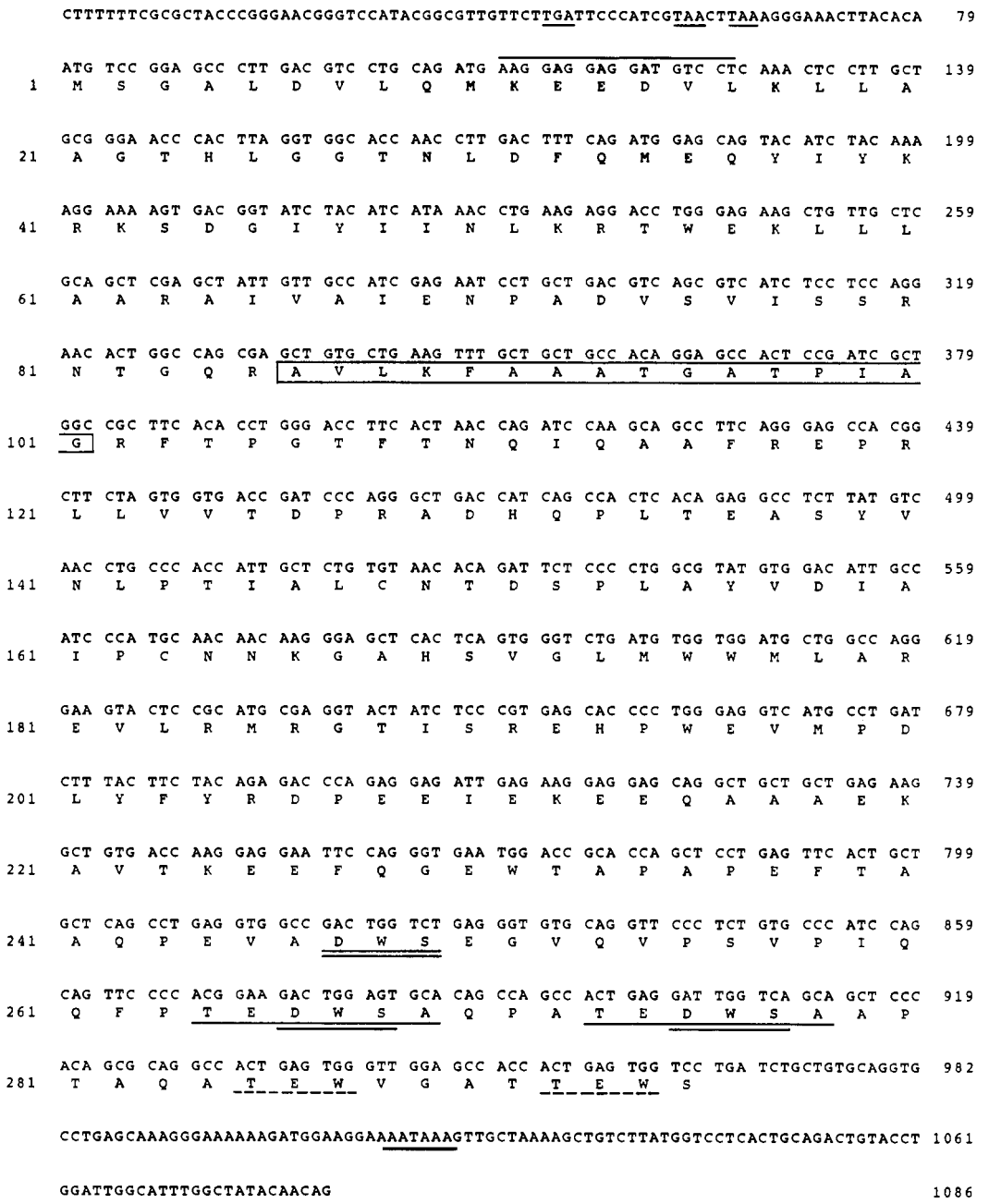

FIGURE 2: Nucleotide sequence of the cDNA insert of pMLR21 and derived protein sequence. Overlapping restriction fragments of clone pMLR21 were sequenced in both directions by the chemical modification method (Gilbert & Maxam, 1973). The derived amino acid is shown below the nucleotide sequence of the (+) strand of DNA. The number of nucleotides is shown in the right margin, and the amino acids are numbered in the left margin. The underlined nucleotide sequences (bases 45–47, 57–59, 62–64) designate stop codons in the 5′ untranslated region of the mRNA. The heavy bar underlining the nucleotide sequences 1013–1018 designates the polyadenylation signal. The line above nucleotides 110–126 shows the sequence from which the oligonucleotide used in primer extension experiments was derived. The amino acids within the box (86–101) are a putative transmembrane signal (Rao & Argos, 1986). The underlined amino acids (single, douuble, and dashed lines) designate a region of the carboxy terminus of the receptor which is rich in internal repeats.

We have previously defined the region of the receptor carboxy-terminal to residue 160 as containing the laminin binding site (Wewer et al., 1986), and immunofluorescence staining of nonpermeabilized cells, using antisera to carboxy-terminal synthetic peptides, showed that this region is extracellular (Wewer et al., 1987). Notable within the carboxy half of the receptor is a symmetrical sequence LMWWML from residues 173–178. The terminal third of the region is highly negatively charged, with 23% of the residues being aspartic acid and glutamic acid. Most of the glutamic acid residues are clustered together; 42% of the residues between positions 208 and 226 are glutamic acid. A trypsin-resistant fragment, defined by the absence of lysine and arginine residues, is carboxy-terminal to residue 224. This trypsin-resistant fragment is also highly negatively charged. We have previously described this region to be one of amino acid repeat structure in the human receptor (Wewer et al., 1986). The same region in the mouse contains a similar, but not identical, repeat pattern. A six amino acid repeat (underline, Figure 2), separated by three amino acids, contains within it a DWS tripeptide (double underline, Figure 2) which is repeated upstream. In the human sequence, this tripeptide is also present as the last three amino acid residues of the protein. In the mouse, however, the aspartate residue at position 293 is changed to a glutamate, providing another tripeptide repeat TEW (dashed lines, Figure 2) at the carboxy-terminal region of the receptor.

Species Specificity of Human and Murine Laminin Re-

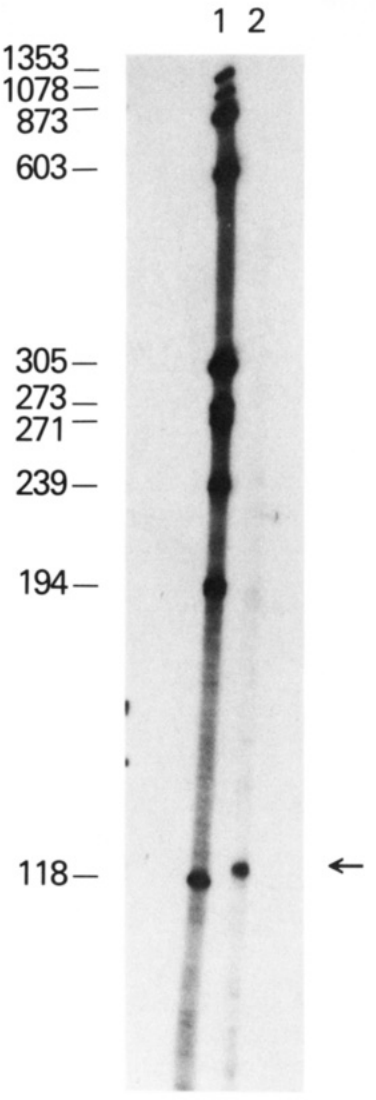

FIGURE 3: Primer extension of mouse laminin receptor mRNA. A synthetic oligonucleotide complementary to bases 126–110 of the murine laminin receptor mRNA was annealed to poly(A)-selected RNA from mouse NIH-3T3 cells at 45 °C, and a primer extension reaction was carried out as described under Experimental Procedures. The size of the extended product (lane 2) was determined on a 5% denaturing polyacrylamide gel by comparison to the electrophoretic pattern of denatured ϕX174/*Hin*cII markers (lane 1). The arrow to the right designates the single primer extension product of 126 bases.

ceptor cDNA Probes. Sequence analysis of the murine clone predicted a close identity between the human and mouse species. To confirm this, an RNA blot experiment was performed. RNA from human, rat, and mouse cells was hybridized to either human or mouse cDNA insert (Figure 7). In all cases, the cDNA inserts from both species hybridized to the same size mRNA. We had previously calculated the laminin receptor mRNA on RNA blots to be 1700 bases (Wewer et al., 1986); however, more accurate calculations, including comparison with RNA ladders, determined that the size was approximately 1400 bases, consistent with the data in Figures 2 and 3. Despite the significant amount of homology between human and mouse nucleic acid sequences, there was a differential ability to hybridize to the cross-species under stringent washing conditions. Thus, the human cDNA probe recognized the human mRNA of human Panc-1 cells more efficiently than did the murine cDNA probe, and vice versa. The rat mRNA of L2 cells was recognized relatively better by the murine cDNA probe.

We have previously reported that immunostaining of different tumors with anti-laminin receptor antibodies suggests a degree of correlation between the degree of differentiation of the tumor and the content of tumor cell laminin receptor. We wondered whether the level of laminin receptor mRNA varied with the state of cellular differentiation in an alternate

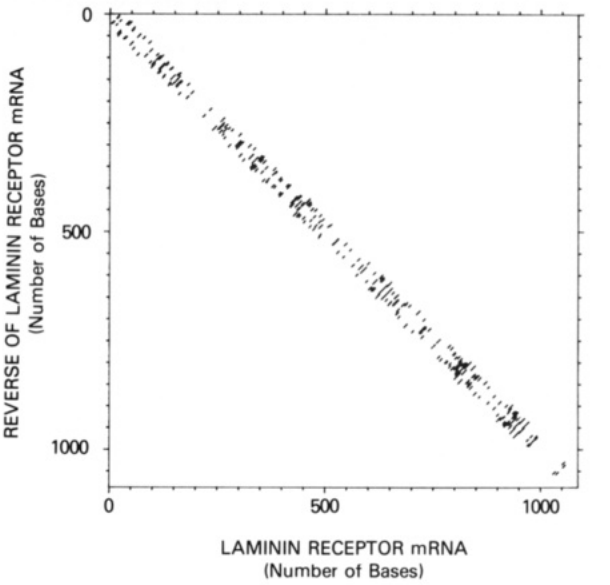

FIGURE 4: Inverted repeats within the murine laminin receptor mRNA. The nucleotide sequence of Figure 2 was analyzed by the StemLoop and DotPlot programs using the parameters described under Experimental Procedures. Inverted repeats within the laminin receptor mRNA are depicted along the diagonal.

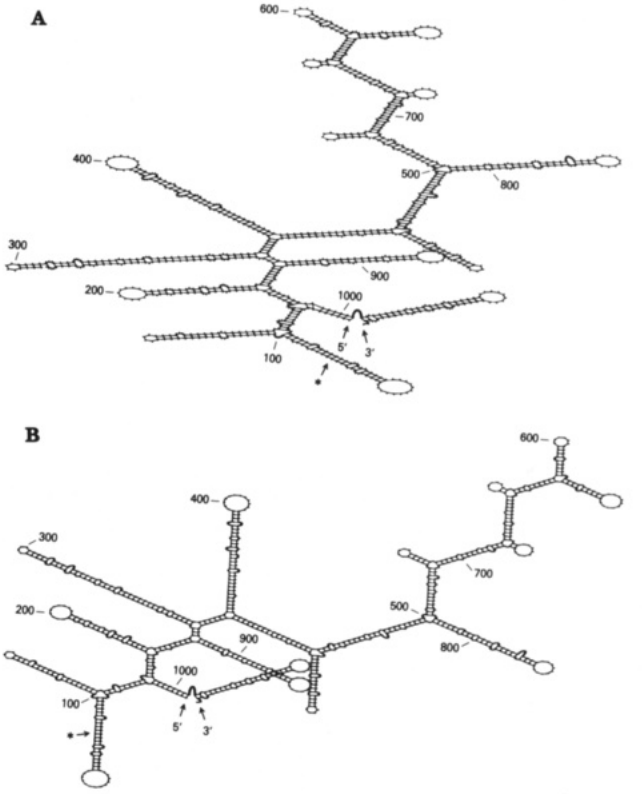

FIGURE 5: Potential secondary structure of murine laminin receptor mRNA. The nucleotide sequence of Figure 2 was analyzed by the Fold and Squiggles programs described under Experimental Procedures. Two potential configurations, each with a free energy of −356.5 kcal/mol, are shown. Bonds formed between bases are drawn as chords. The 5′ and 3′ ends of the mRNA are designated by arrows; the asterisk shows the initiating AUG codon for translation. The numbers refer to the nucleotide numbers of murine laminin receptor mRNA as described in Figure 2.

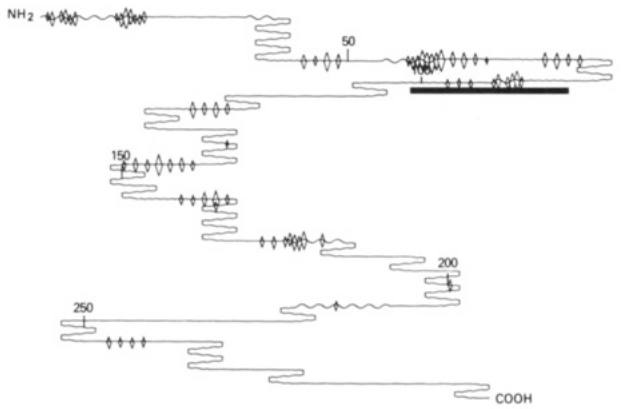

FIGURE 6: Potential secondary structure of the murine laminin receptor. The amino acid derived sequence shown in Figure 2 was analyzed by the PeptideStructure and PlotStructure programs as described under Experimental Procedures. A two-dimensional plot is presented, with predicted helices shown as a sine wave, β sheets as a sharp saw-tooth wave, and coils as a dull saw-tooth wave; turns are demonstrated with 180° turns. Superimposed on the plot are diamond-shaped symbols which designate relative hydrophobic regions. The size of each diamond is proportional to the amount of hydrophobicity of that domain. The bar designates a putative transmembrane domain as predicted by the method of Rao and Argos (1986). The numbers refer to the amino acid residue from the amino terminus, as shown in Figure 2.

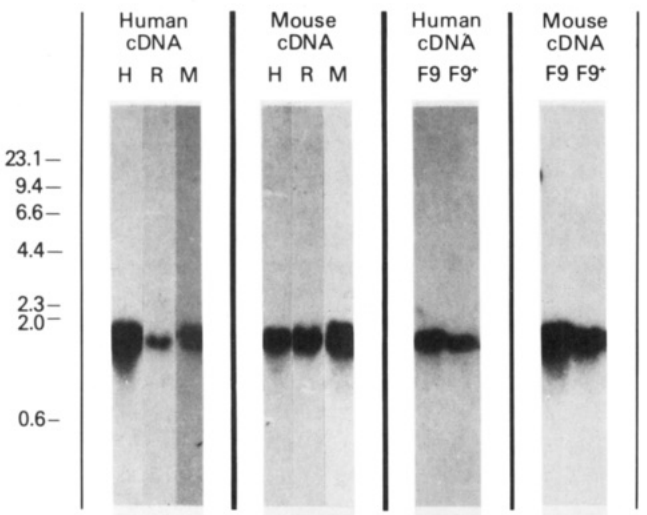

FIGURE 7: Cross-hybridization of human and murine laminin receptor cDNA probes with mRNA from three species. Total cellular RNA was extracted from human HT-1080 (H), rat L2 (R), and mouse NIH-3T3 (M) cells, separated on RNA gels, and transferred to filter paper as described under Experimental Procedures. Total cellular RNA was also extracted from mouse F9 teratocarcinoma cells grown under undifferentiating and differentiating conditions (Strickland et al., 1980) and analyzed. Duplicate blots, in which 5 μg of each RNA was analyzed, were hybridized either to the 650 bp insert of the human laminin receptor cDNA clone pLR4-4 (Wewer et al., 1986) or to the 650 bp *Pst*I–*Eco*RI insert of pMLR21 (see Figure 1). The length of the hybridized mRNA species was determined by comparison with the known sizes of rRNA, RNA ladder (Bethesda Research Laboratories), and with the sizes of λ DNA digested with *Hin*dIII. After radioautography, each blot was stripped free of probe and rehybridized with the other probe. In addition, blots were counterscreened with actin cDNA probe (Cleveland et al., 1980) to ensure that equal amounts of RNA were transferred.

system. We therefore isolated RNA from undifferentiated F9 teratocarcinoma cells, and from F9 cells which differentiated after treatment with retinoic acid and dibutyryl cyclic AMP. The level of laminin receptor RNA was greater in the undifferentiated F9 cells (Figure 7). In contrast, differentiated F9 cells contain greater levels of laminin RNA and type IV collagen RNA (Wang & Gudas, 1983), suggesting that in this

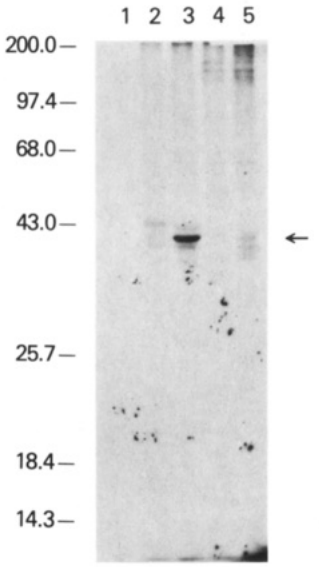

FIGURE 8: Immunoprecipitation of cell-free translation products. Human Panc-1 and murine NIH-3T3 total cellular RNAs were translated in a rabbit reticulocyte cell-free translation system and immunoprecipitated with antiserum 3801 directed against a carboxy-terminal synthetic peptide deduced from the cDNA clone, as described under Experimental Procedures. The translation products were analyzed on 12% SDS–polyacrylamide gels alongside ^{14}C protein markers (Bethesda Research Laboratories). Lane 1, endogenous translation products immunoprecipitated with antiserum 3801; lane 2, Panc-1 RNA translation products immunoprecipitated with preimmune serum; lane 3, Panc-1 RNA translation products immunoprecipitated with antiserum 3801; lane 4, NIH-3T3 RNA translation products immunoprecipitated with preimmune serum; lane 5, NIH-3T3 RNA translation products immunoprecipitated with antiserum 3801. The arrow designates the specific immunoprecipitated nascent laminin receptor.

system there is a lack of correlation between laminin and laminin receptor mRNA levels.

Cell-Free Translation Product of Laminin Receptor mRNA. The metastasis-associated high-affinity laminin receptor has been previously characterized as a 67-kilodalton polypeptide on SDS–polyacrylamide gels (Lesot et al., 1983; Malinoff & Wicha, 1983; Rao et al., 1983; Wewer et al., 1986). The coding capacity of the full-length murine laminin receptor cDNA described in this report, as well as of the complete human laminin receptor cDNA (Yow et al., 1988), is 295 amino acids, with a computer prediction of a nascent laminin receptor molecular weight of 33 000. We determined the apparent molecular weight of the nascent laminin receptor on SDS–polyacrylamide gels by translating total cellular RNA from human Panc-1 cells and murine NIH-3T3 cells in a rabbit reticulocyte cell-free translation system, followed by immunoprecipitation with antiserum 3801, which is directed against a cDNA-deduced synthetic peptide corresponding to the carboxy-terminal region of the laminin receptor. This antiserum has previously been shown to inhibit the attachment of cells to laminin (Wewer et al., 1987) and specifically recognizes purified 67-kilodalton human laminin receptor by ELISA (Wewer et al., 1987). On immunoblots of breast carcinoma cell extracts, this antiserum identifies a major 67-kilodalton band (Wewer et al., 1987). When compared to the background bands brought down by the preimmune serum, the only specific translation product recognized by the antisynthetic peptide antiserum 3801 has an apparent molecular weight of 37 000 (Figure 8, lanes 3 and 5). As expected from RNA blot data (data not shown), there was more in vitro synthesized laminin receptor from the Panc-1 RNA than from the NIH-3T3 RNA.

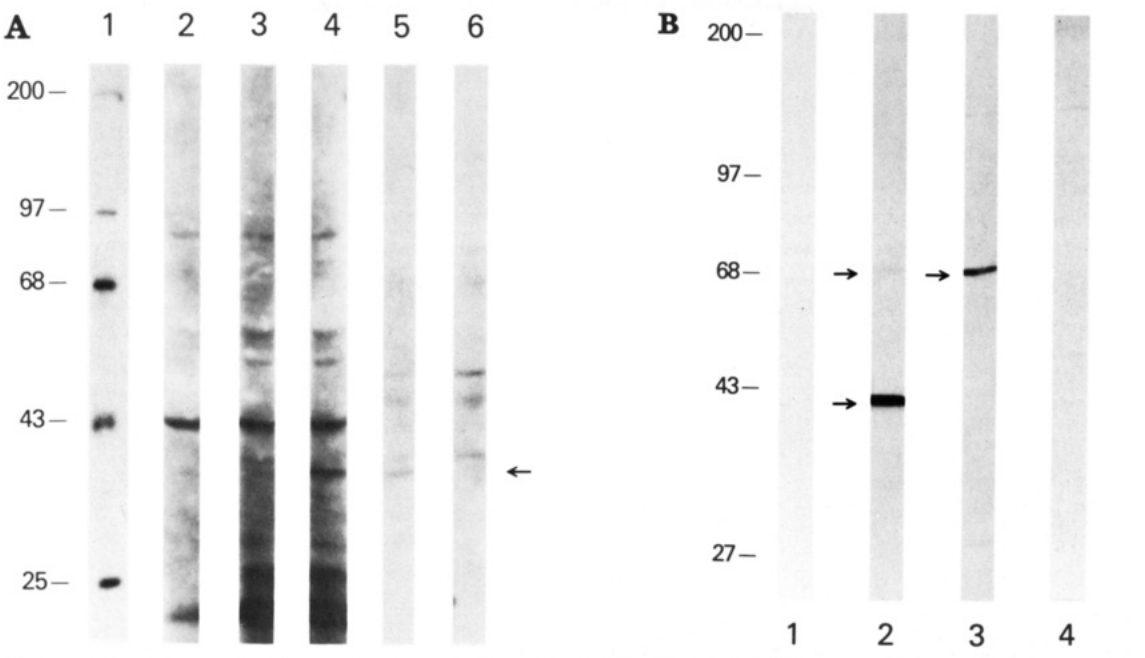

FIGURE 9: (A) Nascent laminin receptor from cell-free translation and metabolically labeled cell extracts. Human laminin receptor mRNA was purified from Panc-1 cells by hybrid-selection and translated in a cell-free rabbit reticulocyte lysate as described under Experimental Procedures. Human A2058 melanoma cells were metabolically labeled with [^{35}S]methionine, and cell extracts were immunoprecipitated with antiserum 4056 directed against a relatively amino-terminal domain of the laminin receptor deduced from the cDNA clone, as described under Experimental Procedures. The translation and cell products were analyzed on 10% SDS–polyacrylamide gels. Lane 1, ^{14}C protein markers (Bethesda Research Laboratories); lane 2, endogenous translation reaction; lane 3, translation of RNA selected with *Pvu*II-digested (linearized) control pBR322 plasmid; lane 4, translation of RNA selected with the 650 bp human laminin receptor cDNA insert of pLR4-4 (Wewer et al., 1986); lane 5, metabolically labeled A2058 cell extract immunoprecipitated with antiserum 4056; lane 6, metabolically labeled A2058 cell extract immunoprecipitated with preimmune serum. The arrow designates the specific nascent laminin receptor polypeptide. (B) Immunoblot of laminin receptor. Total cellular-extracted proteins from HT 1080 cells were separated on 10% SDS–polyacrylamide gels, transferred to nitrocellulose paper, and immunoblotted as described under Experimental Procedures. The extracts were run alongside prestained protein markers (Bethesda Research Laboratories). Lane 1, preimmune serum to rabbot 4056; lane 2, antiserum 4056; lane 3, antiserum 4099; lane 4, preimmune serum to rabbit 4099. The arrows designate the 37- and 67-kilodalton polypeptides specifically recognized by the antisera.

We also determined the relative mobility of the nascent laminin receptor on gels in hybrid-selected translation experiments. Laminin receptor cDNA insert was immobilized on filters and hybridized to Panc-1 RNA. The specifically bound RNA was eluted and translated in vitro, and the translation products were analyzed on SDS–polyacrylamide gels. When compared to the endogenous translation reaction and the translation of RNA selected from control filters, the only specific polypeptide synthesized had a mobility on SDS–polyacrylamide gels equivalent to an apparent molecular weight of 37 000 (Figure 9A, lane 4).

Identification of the Nascent Laminin Receptor in Cell Extracts. To determine if the 37-kilodalton form of the laminin receptor could be identified in cell extracts, we metabolically labeled human melanoma A2058 cells and immunoprecipitated the cell extracts with affinity-purified antibodies directed against a cDNA-deduced synthetic peptide corresponding to a relatively amino-terminal region of the laminin receptor. We compared the size of the immunoprecipitated cell product with that of the hybrid-selected laminin receptor RNA translation product on SDS–polyacrylamide gels (Figure 9A). The antisynthetic peptide antiserum 4056 immunoprecipitated a metabolically labeled polypeptide (Figure 9A, lane 5) identical in size with that of the hybrid-selected translation product (Figure 9A, lane 4). Thus, a laminin receptor precursor of 37 kilodaltons predicted by the in vitro translation experiments is also present in extracts of cells expressing laminin receptor.

In immunoblot experiments, the antisynthetic laminin receptor peptide antibody 4056 specifically recognized the 37-kilodalton polypeptide from extracts of human fibrosarcoma HT1080 cells (Figure 9B, lane 2). The antibody also recognized a minor band at 67 kilodaltons. The antisynthetic laminin receptor peptide antibody 4099, which was raised against the most amino-terminal domain of the receptor, specifically recognized the 67-kilodalton polypeptide, and not the precursor (Figure 9B, lane 3). Preimmune sera failed to detect either the 37- or the 67-kilodalton bands (Figure 9B, lanes 1 and 4). Given the fact that we detect a single-size mRNA on blots in all samples analyzed, and the original cDNA clone was identified by immunoreactivity toward a monoclonal antibody directed against the 67-kilodalton laminin binding polypeptide, the data suggest that the 37-kilodalton nascent laminin receptor may be a precursor of the 67-kilodalton polypeptide.

Discussion

In the present study, we report on the identification and analysis of a full-length cDNA clone for the high-affinity metastasis-associated mouse laminin receptor. The identification of the clone was based on sequence homology to a partial cDNA clone for the human laminin receptor. The original human cDNA clone (Wewer et al., 1986) was isolated from an expression cDNA library using a monoclonal antibody to human laminin receptor which inhibited the binding of laminin to cells, to amnion basement membrane, and to plasma membranes (Liotta et al., 1985; Toga et al., 1985; Wewer et al., 1986). The identity of the human cDNA was further confirmed by comparison of the cDNA-derived protein sequence with authentic, purified, cyanogen bromide generated fragments of human placental laminin receptor (Wewer et al., 1986). We previously demonstrated that antibodies to synthetic peptides predicted from the human cDNA clone inhibit cell attachment and haptotaxis to laminin (Wewer et al., 1987).

The nucleic acid and amino acid sequences of the murine and human laminin receptors are remarkably homologous, as are the sequences of the ligand laminin in these two species (Pikkarainen et al., 1987), suggesting that these important biological molecules have been conserved through evolution. The cDNA-predicted sequence of the high-affinity laminin receptor which we describe in this report predicts an intradisulfide bond between two cysteine residues at positions 148 and 163, close to the middle of the polypeptide. This is consistent with our finding that the mobility of the receptor on nonreduced SDS–polyacrylamide gels is faster than on reduced gels (Wewer et al., 1986).

The region of the receptor which is carboxy-terminal to residue 160 was previously shown to contain the laminin binding domain (Wewer et al., 1986). We have also previously demonstrated that this region of the receptor is extracellular (Wewer et al., 1987). The putative transmembrane domain of the laminin receptor predicted by the cDNA sequence is relatively amino-terminal, suggesting that approximately two-thirds of the receptor may be extracellular. The computer-predicted (Rao & Argos, 1986) transmembrane domain is unusual is that it contains only 16 amino acid residues. Examples of other proteins which contain shorter than 20-residue-long transmembrane domains include the β subunit of the T cell receptor (Hedrick et al., 1984; Yanagi et al., 1984) and mutated forms of the vesicular stomatitis virus glycoprotein (Adams & Rose, 1985). In such situations, it has been suggested that the transmembrane domain is not helical, but assumes a more extended conformation (Adams & Rose, 1985). This is, indeed, the case for the laminin receptor (see Figure 6). No traditional signal peptide is found in the predicted sequence, although the amino terminus is relatively hydrophobic. Although somewhat unusual, some of these features are also found in other receptors such as the transferrin receptor (McClelland et al., 1984; Stearne et al., 1985) and the asialoglycoprotein receptor (Drickamer et al., 1984). The carboxy-terminal third of the receptor, in addition to being extracellular, is highly negatively charged and contains multiple short amino acid repeats and a trypsin-resistant fragment. We have previously reported that laminin binding to tumor cells is trypsin-sensitive (Rao et al., 1983). We are currently further defining the ligand binding region of the receptor to determine if it is located in the carboxy-terminal 71-residue-long trypsin-resistant fragment.

Polyclonal antiserum as well as monoclonal antibodies to natural laminin receptor purified from liver metastases of human breast carcinomas recognizes a polypeptide with an approximate molecular weight of 67 000 (Liotta et al., 1985; Wewer et al., 1986). Several lines of evidence point to the fact that the nascent laminin receptor before posttranslational modification has an apparent molecular weight on SDS–polyacrylamide gels of 37 000 and is a precursor of the 67-kilodalton polypeptide. The original human laminin receptor cDNA clone was initially isolated from an expression library using a monoclonal antibody directed against the 67-kilodalton laminin binding polypeptide (Wewer et al., 1986). That monoclonal antibody has been demonstrated to inhibit the binding of laminin to cells, amnion basement membranes, and plasma membranes of tumor tissue (Liotta et al., 1985; Togo et al., 1985). The coding capacity of the murine laminin receptor cDNA described in this report, as well as that of the human laminin receptor cDNA (Wewer et al., 1986; Yow et al., 1988), is that of a 295 amino acid residue protein, with a predicted molecular weight of 33 000. Primer extension experiments (Figure 3) unequivocally demonstrate that pMLR21 encodes a full-length laminin receptor mRNA, and the hybrid-selected translation experiments (Figure 9A) show that a specific polypeptide with a mobility on SDS–polyacrylamide gels of approximately 37 kilodaltons is the nascent protein product of laminin receptor mRNA. We have used three different antisera directed against distinct synthetic peptides deduced from the laminin receptor cDNA clone to demonstrate that the 37- and 67-kilodalton polypeptides are related to each other. Antiserum 3801 is directed against a carboxy-terminal, extracellular, domain of the laminin receptor. It recognizes a 67-kilodalton polypeptide on immunoblots of human breast carcinoma cell extracts, as well as a minor species with an approximate molecular weight of 37 000 (Wewer et al., 1987). By ELISA, this antiserum specifically recognizes purified 67-kilodalton human laminin receptor and inhibits the attachment of melanoma cells to laminin (Wewer et al., 1987). In this report, we demonstrate that antiserum 3801 immunoprecipitates a polypeptide with an apparent molecular weight of 37 000 from the translation products of total cellular RNA (Figure 8). We also used an antiserum directed against a different laminin receptor synthetic peptide. Antiserum 4056 was raised against a hypothetical intracellular, and relatively amino-terminal, domain of the laminin receptor. This antiserum specifically immunoprecipitated a 37-kilodalton polypeptide from extracts of melanoma cells and recognized a major 37-kilodalton polypeptide on immunoblots of several human tumor cell extracts (Figure 9). In addition, this antiserum recognized a minor polypeptide band with an apparent molecular weight of 67 000 on immunoblots. Serum albumin was not recognized by the antiserum. Apparently, the 67-kilodalton form is not efficiently recognized by this antiserum. In contrast, antiserum 4099, which was raised against the most amino-terminal domain of the receptor, specifically recognized only the 67-kilodalton polypeptide and not the precursor form. These results demonstrate that antisynthetic peptide antibodies must be used cautiously, since not all domains of a protein may be available for recognition during different stages of the biosynthetic process. We have therefore demonstrated that the 37- and 67-kilodalton polypeptides are immunologically related using three different antisera which are directed against distinctly different domains of the laminin receptor predicted by the cDNA clone. Significantly, RNA gel blots show that the laminin receptor mRNA in all species tested is approximately 1400 bases long, which is consistent with the size of the full-length cDNA insert of pMLR21 with a 250-base-long poly(A) tail. Furthermore, a single-size mRNA species is detected on RNA gel blots, even after overexposure of radioautographs. It is therefore unlikely that the 37- and 67-kilodalton polypeptides are encoded by two different mRNA species.

Our current hypothesis is that the cell surface laminin receptor is a highly substituted molecule derived from the nascent 37-kilodalton translation product. Although there are no N-linked carbohydrate attachment sites in the predicted sequence of the receptor, there are many serine residues, and O-linked oligosaccharides may significantly alter the molecular weight of the receptor. We are currently testing this hypothesis. We have also not ruled out the possibility that the 37-kilodalton precursor may be covalently attached to another peptide or to lipid components when it is incorporated in the membrane. In any case, the mobility of receptors on polyacrylamide gels is often misleading (Tanford & Reynolds, 1976). It should be noted that we have used globular proteins as standards to estimate apparent molecular weights on

acrylamide gels. Since the predicted carboxy-terminal domain of the laminin receptor is highly negatively charged, acidic protein standards may give a different size estimate for the nascent and mature receptor species. It is thus difficult at this time to precisely define the biochemical mechanism by which a polypeptide with an approximate molecular weight of 67 000 on SDS–polyacrylamide gels is synthesized from a nascent translation product of 37 kilodaltons. Pulse–chase studies in our laboratory have recently established a precursor–product relationship between the 37- and 67-kilodalton proteins (unpublished data). Such studies will assist in the determination of the biosynthetic pathway for the laminin receptor.

Several other proteins have been identified which bind to laminin (Clegg et al., 1988; Hall et al., 1988; Horwitz et al., 1985; Kleinman et al., 1988; Krotoski et al., 1986; Ruoslahti, 1988; Gehlsen et al., 1988). Some of these appear to be specific for neurite formation (Kleinman et al., 1988), while others may be more ubiquitous (Krotoski et al., 1986). The integrin family of extracellular matrix receptors also appears to play an important role in laminin binding (Horwitz et al., 1985; Krotoski et al., 1986; Gehlsen et al., 1988). The high-affinity laminin receptor which we have cloned and reported on in this paper is unique from these other proteins. Its sequence is different, and antibodies between the different types of receptors do not cross-react. Different receptors appear to be involved in laminin versus fibronectin haptotaxis of human melanoma cells (Wewer et al., 1987), suggesting that both the integrin-type receptor and the high-affinity laminin receptor can exist on the same cell. It is not yet clear if these receptors interact with each other, and what role each plays in cell migration, invasion, and metastasis. However, there is no doubt that the high-affinity receptor which we have cloned in this report is closely associated with the metastatic process. While this paper was in preparation, the isolation of a cDNA clone by differential expression in colon carcinoma was reported (Yow et al., 1988). When sequenced, that cDNA clone was shown to be identical with the partial human laminin receptor cDNA which we previously identified (Wewer et al., 1986).

Previous studies have suggested that the steady-state level of laminin receptor mRNA in the cell may be a major regulatory factor in controlling laminin receptor expression (Wewer et al., 1986; Yow et al., 1988). The current study indicates that other factors may be important as well. There may be considerable posttranslational modification of the receptor, as suggested by the different mobilities of translation product and natural receptor on SDS–polyacrylamide gels. Furthermore, there may be more than one translation initiation site. For example, analysis of the predicted amino acid sequence of the murine laminin receptor reveals that neither the first methionine nor the second methionine at amino acid residue 10 is surrounded by a typical translation consensus sequence (Kozak, 1986). Evidence that the first possible methionine is, in fact, the initiating codon can be inferred from human sequence data (Yow et al., 1988; and unpublished observations). In the human sequence, an out-of-reading frame methionine codon (which also has no consensus sequence) is present between residues 1 and 10, due to a base change at position 97 from a C in the mouse to a T in the human. The correct reading frame was previously established by comparing the human cDNA-derived amino acid sequence with authentic laminin receptor peptide sequence. Furthermore, antiserum 4099, raised against residues 2–9 of the cDNA-predicted receptor, specifically recognizes the 67-kilodalton receptor on immunoblots. It therefore seems that the laminin receptor mRNA, like that of several other messengers, does not have a consensus sequence for efficient translation. The laminin receptor mRNA appears to have a great deal of secondary structure, with multiple short inverted repeat sequences. The 5′ and 3′ untranslated regions of the mRNA can form a stem structure, perhaps affecting the translatability or stability of the mRNA. All the potential methionine initiation residues are in predicted stem structures. Thus, expression of the high-affinity laminin receptor may be regulated at transcriptional, RNA processing, translational, and also post translational levels in a variety of cell types.

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The Yeast *GAL1-10* UAS Region Readily Accepts Nucleosomes in Vitro†

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ABSTRACT: To test if the absence of nucleosomes on the UAS region of the yeast *GAL1-10* genes in vivo could be due to a low inherent affinity of this DNA for histones, DNA fragments containing the UAS and various amounts of flanking DNA were reconstituted into chromatin. Restriction enzyme and DNase I digestion analyses show that DNA in the UAS becomes protected against digestion in the reconstitutes. Thus, nucleosomes can assemble on the UAS region in vitro. The level of protection of the UAS and of the flanking DNA regions is comparable and remains so at various levels of nucleosome loading, suggesting that the UAS DNA has no tendency to exclude nucleosomes. In fact, DNase I results suggest that the UAS elements themselves preferentially bind histones.

Hypersensitive regions are a common feature of eukaryotic chromatin [cf. Gross and Garrard (1988)]. In many cases, these regions correspond to nucleosome-free gaps in the chromatin structure. Gene-regulating DNA elements often lie in these gaps.

It has been shown that histones and transcriptional regulatory proteins can bind to the same piece of regulatory DNA [cf. Gottesfeld (1987) and Workman et al. (1988)]. Generally, for gene expression to occur, regulatory factor assembly must prevail over nucleosome assembly. Thus, maintaining nucleosome-free gaps around DNA regulatory elements could be one aspect of eukaryotic transcriptional regulation.

The UAS,[1] or upstream activation sequences, are the DNA elements which mediate induction of gene expression of the yeast *GAL1-10* genes (Guarente et al., 1982). During growth in galactose or glycerol/ethanol, but not in glucose, the regulatory factor GAL4 binds to these small (~17 bp) elements (Giniger et al., 1985; Lohr & Hopper, 1985). In galactose, the bound GAL4 promotes expression of the genes. There is a large (~170 bp) nucleosome-free gap around the UAS elements under all growth conditions (Lohr, 1984; Lohr & Hopper, 1985). It is of interest to know how the cell maintains

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[1] Abbreviations: bp, base pair(s); DNase I, deoxyribonuclease I (EC 3.1.4.5); UAS, upstream activation sequence(s).

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