

The 67-kDa Laminin Receptor Originated from a Ribosomal Protein that Acquired a Dual Function During Evolution

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The 67-kDa laminin receptor (67LR) is a nonintegrin cell surface receptor that mediates high-affinity interactions between cells and laminin. Overexpression of this protein in tumor cells has been related to tumor invasion and metastasis. Thus far, only a full-length gene encoding a 37-kDa precursor protein (37LRP) has been isolated. The finding that the cDNA for the 37LRP is virtually identical to a cDNA encoding the ribosomal protein p40 has suggested that 37LRP is actually a component of the translational machinery, with no laminin-binding activity. On the other hand, a peptide of 20 amino acids deduced from the sequence of 37LRP/p40 was shown to exhibit high laminin-binding activity. The evolutionary relationship between 23 sequences of 37LRP/p40 proteins was analyzed. This phylogenetic analysis indicated that all of the protein sequences derive from orthologous genes and that the 37LRP is indeed a ribosomal protein that acquired the novel function of laminin receptor during evolution. The evolutionary analysis of the sequence identified as the laminin-binding site in the human protein suggested that the acquisition of the laminin-binding capability is linked to the palindromic sequence LMWWML, which appeared during evolution concomitantly with laminin.

Introduction

In 1983, three independent laboratories reported the purification of a 67-kDa molecule, designated the 67-kDa laminin receptor (67LR), by affinity chromatography on laminin-Sepharose columns (Lesot, Kuhl, and Von der Mark 1983; Malinoff and Wicha 1983; Rao et al. 1983). This protein, which binds laminin with high affinity, was found to be expressed in a wide variety of mammalian cells and to be particularly abundant on the cancer cell surface (Sobel 1993). The strong correlation between 67LR overexpression and the metastatic potential of tumor cells (Castronovo 1993; Martignone et al. 1993) suggests that the receptor plays a significant role in the acquisition of the metastatic phenotype. We recently suggested that this 67LR is an auxiliary molecule involved in regulating and stabilizing the interaction of laminin with the bona fide laminin receptor of the integrin family (Ardini et al. 1997). Interest in the study of the 67LR has recently grown since, using yeast two-hybrid technology, this molecule has been identified as receptor for the pathogenic prion protein PrP^{Sc} on mammalian cells (Rieger et al. 1997).

Screening of a human cDNA expression library using monoclonal antibodies directed against the purified protein (Rao et al. 1989) led to isolation of the full-length cDNA, but the protein encoded had a calculated molecular mass of 32 kDa. Likewise, *in vitro* translation of the selectively hybridized mRNA led to a protein with an apparent molecular mass of 37 kDa as estimated by SDS-polyacrylamide gel electrophoresis (Rao et al.

1989). Although pulse-chase experiments have clearly demonstrated a precursor-product relationship (Castronovo et al. 1991), the mechanism by which this 37-kDa polypeptide gives rise to its mature form is still unknown. Recent data suggest that a posttranslational modification of the 37LRP, involving acylation by fatty acid palmitate, oleate, and stearate, leads to dimerization of the 37LRP (Landowski, Dratz, and Starkey 1995).

The most intriguing observation is that this 37LRP cDNA is virtually identical to a cDNA encoding a mouse protein, p40, initially identified in a screening for mRNAs under translational control in a variety of mouse tumor cells (Makrides et al. 1988). Evidence suggests that the 37LRP/p40 protein is a component of the translational machinery, being specifically associated with the 40S ribosomal subunit (Auth and Brawerman 1992).

37LRP/p40 genes have recently been identified in many different organisms, such as *Saccharomyces cerevisiae* (Davis, Tzagoloff, and Ellis 1992), *Arabidopsis thaliana* (Garcia-Hernandez, Davies, and Staswick 1994), *Drosophila melanogaster* (Melnick, Noll, and Perrimon 1993), *Urechis caupo* (Rosenthal and Wordeman 1995), and *Chlorohydra viridissima* (Keppel and Schaller 1991). The recent finding that one open reading frame in the genome of the archaeobacterium *Haloarcula marismortui* encodes a protein 40% identical to the 37LRP/p40 led to the identification of a parental relationship with the S2 family of procaryotic ribosomal proteins (Ouzonis, Kyrpides, and Sander 1995).

In oocytes and embryos of the sea urchin *Urechis caupo*, the 37LRP/p40 appears to be associated exclusively with polysomes with no localization on the cell surface, suggesting a role for this molecule in protein synthesis during early embryogenesis (Rosenthal and Wordeman 1995). In *S. cerevisiae*, two genes closely related to 37LRP, named YST1 and YST2, were recently isolated; disruption of either YST1 or YST2 leads to a reduction in cell proliferation, and disruption of both

Abbreviations: 67LR, 67-kDa laminin receptor (67LR); 37LRP, 37-kDa laminin receptor precursor.

Key words: evolution, ribosomal proteins, monomeric laminin receptor, adhesion molecules, tumor aggressiveness, metastasis.

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genes is lethal (Demianova, Formosa, and Ellis 1996). Since yeasts do not express laminin and presumably do not interact with laminin-containing extracellular matrices, it seems unlikely that the 37LRP/p40 functions as laminin receptor in these organisms.

These observations have led to the hypothesis that the 37LRP is actually a ribosome-associated molecule involved in protein synthesis and not in laminin binding. However, a convincing demonstration of the relationship between the 37LRP/p40 and laminin-binding function came from the use of a 20-amino-acid peptide (IPCNNKGAHSGVGLMWWMLAR) derived from the 37LRP/p40 sequence which was shown to bind laminin, to elute 67LR from a laminin affinity chromatography column, and to dramatically increase laminin binding on tumor cells (Castronovo, Taraboletti, and Sobel 1992; Magnifico et al. 1996). The same activity was observed using the inversely synthesized peptide (Castronovo, Taraboletti, and Sobel 1992), suggesting that the palindromic sequence LMWWML is the active site, consistent with the observation that two consecutive tryptophan residues are often associated with protein-protein interaction (Macias et al. 1997).

In an attempt to clarify the evolutionary relationships between the 37LRP proteins found in vertebrates and other homologous ribosomal proteins present in all living organisms, as well as to obtain information on the appearance of laminin-binding capability during evolution, we carried out an evolutionary analysis of 23 homologous protein sequences, including the eukaryotic 37LRP/p40 and the prokaryotic S2 ribosomal proteins.

Materials and Methods

Evolutionary Analysis

The 37LRP/p40 homologous protein sequences were retrieved using both a keyword-based search with the ACNUC retrieval system (Gouy et al. 1985) and a sequence-similarity-based search with the BLAST program (Altschul et al. 1990).

Sequences were aligned using the PILEUP program from the GCG package (GCG 1994, version 8, Genetic Computer Group, Madison, Wis.), followed by manual editing to obtain maximum similarity alignments using the SEAVIEW package (Galtier, Gouy, and Gautier 1996). Protein data were analyzed using the PHYLO-WIN program (Galtier, Gouy, and Gautier 1996) with a Poisson correction to calculate genetic distances, and the neighbor-joining method (Saitou and Nei 1987) for tree reconstruction. Statistical significance of the tree nodes was assessed by bootstrap simulation.

The pairwise divergence between all members of 37LRP/p40 homologous protein sequences was calculated using the DISTANCE program (GCG 1994, version 8). The pattern of amino acid conservation among the proteins was calculated using the PLOTSIMILARITY program of the GCG package (GCG 1994, version 8) with a sliding window of 10 residues.

Reagents

Peptide G (IPCNNKGAHSGVGLMWWMLAR), corresponding to the 161–180 region of the human

37LRP/p40; scrambled peptide Gx (PMLRWGCHI-AMVKNKLSWGNA); peptide Gc (VPCNNKGERSI-GLMWWMLAR), corresponding to the 161–180 region of the 37LRP/p40 from *Caenorhabditis elegans*; and peptide Gs (IPCNNRGKHSIGLIWYLLAR), corresponding to the 160–179 region of the 37LRP/p40 from *S. cerevisiae*, were obtained from Neosystem (Strasbourg, France) in N-terminal biotinylated form. High-pressure liquid chromatography analysis showed 95% purity for each peptide. Peptides were dissolved in distilled water at 500 µg/ml and stored at –20°C.

Cells and Culture Conditions

Human vulvar epidermoid carcinoma cell line A431 was provided by ATCC (Rockville, Md.). Cells were maintained in RPMI-1640 medium (Sigma Chemical Co., St. Louis, Mo.) supplemented with 10% fetal calf serum (FCS), penicillin (100 mg/ml), and streptomycin (100 mg/ml). Experiments involving treatment with laminin were performed using human laminin purified from placenta (Sigma).

Solid-Phase Binding Assay

Microwells of a 96-well polyvinyl chloride plate (Becton Dickinson, Mountain View, Calif.) were coated with 100 µl of laminin (10 µg/ml) in PBS for 2 h at 37°C. After three washes with PBS, nonspecific binding sites were blocked with 1% BSA in PBS for 30 min at 37°C. Biotinylated peptide G, Gx, Gc, or Gs was added at a concentration of 1 µg/ml in 100 µl of PBS supplemented with 0.05% Tween 20 and 1% BSA, and wells were incubated for 2 h at 4°C. After four washes, wells were incubated with ¹²⁵I-avidin (1 × 10⁵ cpm/well; Amersham Corp., Little Chalfont, U.K.) for 1 h at room temperature. After washing, wells were treated with 100 µl of 2 N NaOH for 20 min at room temperature, and radioactivity was measured in a gamma counter. All experiments were performed in triplicate. Specific bound was calculated as the difference between cpm bound to laminin and cpm bound to BSA-coated wells.

Flow Cytometric Analysis

Indirect immunofluorescence was used to analyze the binding of biotinylated peptides and the presence of exogenous laminin. Live cells (3 × 10⁵) treated with human laminin (50 µg/ml) for 30 min at 37°C were incubated with biotinylated peptides at 37°C for 30 min. After three washings, cells were assayed for bound peptide G, Gx, Gc, or Gs with fluorescein-labeled streptavidin (5 µg/ml; Amersham Corp).

Human laminin treated with peptides (50 µg/ml) or untreated was incubated with cells at 37°C for 30 min and detected using a rabbit polyclonal serum directed against human laminin (Sigma) and fluorescein-isothiocyanate-conjugated goat anti-rabbit Ig. Labeled cells were analyzed using a FACScan flow cytometer with LYSYS II software (Becton Dickinson).

Each experiment was repeated at least three times, with highly reproducible results. Staining of cells with fluorescein-isothiocyanate-conjugated goat anti-rabbit Ig or fluorescein-labeled streptavidin was performed to determine background values.

Table 1
LRP/p40 Protein Sequences Considered in this Study

Organism	Taxon	Accession Number	Length (aa)
Eukaryota			
<i>Drosophila melanogaster</i>	Animalia, Arthropoda	M90422	270
<i>Gallus gallus</i>	Animalia, Aves	X94368	296
<i>Chlorohydra viridissima</i>	Animalia, Coelenterata	X63849	293
<i>Tripneustes gratilla</i>	Animalia, Echinodermata	U02371	316
<i>Urechis caupo</i>	Animalia, Echiura	U02370	317
<i>Bos taurus</i>	Animalia, Mammalia	M64923	295
<i>Cricetulus griseus</i>	Animalia, Mammalia	L16589	295
<i>Homo sapiens</i>	Animalia, Mammalia	J03799	295
<i>Mus musculus</i>	Animalia, Mammalia	J02870	295
<i>Rattus rattus</i>	Animalia, Mammalia	D25224	295
<i>Caenorhabditis elegans</i>	Animalia, Nematoda	Z37983	276
<i>Echinococcus granulosus</i>	Animalia, Platyhelminthes	L33460	268
<i>Acanthamoeba castellanii</i> (mt)	Protozoa	U12386	312
<i>Pneumocystis carinii</i>	Fungi, Ascomycotina	U09451	295
<i>Saccharomyces cerevisiae</i> (mt)	Fungi, Hemiascomycetes	M82841	224
<i>Saccharomyces cerevisiae</i>	Fungi, Hemiascomycetes	M88277	252
<i>Neurospora crassa</i>	Fungi, Ascomycotina	U36470	293
<i>Nicotiana tabacum</i> (cp)	Planta, Angiospermae	Z00044	236
<i>Pinus thunbergiana</i> (cp)	Planta, Coniferophyceae	D17510	234
<i>Arabidopsis thaliana</i>	Planta, Magnoliophyta	X69056	298
Prokaryota			
<i>Haloarcula marismortui</i>	Archaeobacteria	M76567	266
<i>Escherichia coli</i>	Bacteria	V00343	240
<i>Pediococcus acidilactici</i>	Bacteria	X70925	261

NOTE.—For each entry, the GenBank/EMBL accession number and the length in amino acids are given. Mitochondrial (mt) and chloroplast (cp) proteins are also indicated.

Results

The 37LRP/p40 sequences used in the evolutionary analysis are listed in table 1. All available eukaryotic sequences and some representative prokaryotic sequences, including two mitochondrial (*Acanthamoeba castellanii* and *S. cerevisiae*) and two chloroplast (*Nicotiana tabacum* and *Pinus thunbergiana*) homologous protein sequences, were analyzed. Figure 1 shows the multiple alignment of the sequences. Multiple alignment was not obvious due to the high divergence between some protein sequences and their length heterogeneity. By manual editing of the multiple alignment, similar nonidentical residues were aligned to optimize the overall similarity among all protein sequences considered. Indeed, only eight residues were conserved in all the proteins analyzed. Four of these were found in the two signature patterns for ribosomal protein S2 described in the PROSITE entries (PS00962 and PS00963) corresponding to residues 15–26 and 119–143 of the human sequence (Bairoch, Bucher, and Hofmann 1997). It is striking to note that despite the high divergence observed, particularly at the C-terminal region, vertebrate proteins are almost completely identical. Protein folding is unknown, as no clear homologs can be found in the PDB database with the exception of a significant similarity between the N-terminal part of the protein and various members of the HSP70 family (data not shown; see also Chen, Fornace, and Laszlo 1996). Figure 2 shows the phylogenetic tree calculated using the neighbor-joining method on a pairwise distance matrix calculated with a Poisson correction (Galtier, Gouy, and

Gautier 1996). The phylogenetic relationships between mammals were not resolved, resulting in a polytomy of all mammalian species, with chicken as the sister taxon. The observed polytomy derives from the extremely high degree of sequence conservation of mammalian proteins, all of which are almost identical. As expected, the closest relative of vertebrates was *Tripneustes gratilla* (Echinodermata), since Vertebrata and Echinodermata both belong to the Deuterostoma group, with all other animal species considered in this study belonging to the Protostoma group.

All Metazoa, animals, plants, and fungi, form a clear-cut monophyletic cluster with plants (*A. thaliana*) more closely related to animals than to fungi (*Neurospora crassa*, *Pneumocystis carinii*, *S. cerevisiae*), which in turn form a statistically significant clade. The Archaeobacteria (*H. marismortui*) are more closely related to Eukaryota than to Prokaryota, consistent with the observation that most of the archaeobacterial genes involved in transcription, translation, and replication are more similar to eukaryotic than to prokaryotic homologous genes (Bult et al. 1996).

In accord with the theory that the endosymbiosis of ancestral prokaryotic cells led first to the mitochondrion in the ancestral eukaryotic cell and then to the chloroplast in the ancestral plant cell (Margulis 1981; Gray 1993), we found that both the mitochondrial and chloroplast proteins are more closely related to the prokaryotic than to the eukaryotic proteins. The most recent endosymbiotic event which originated chloroplasts results in a closer relationship of chloroplast proteins with prokaryotic proteins than with mitochondrial proteins.

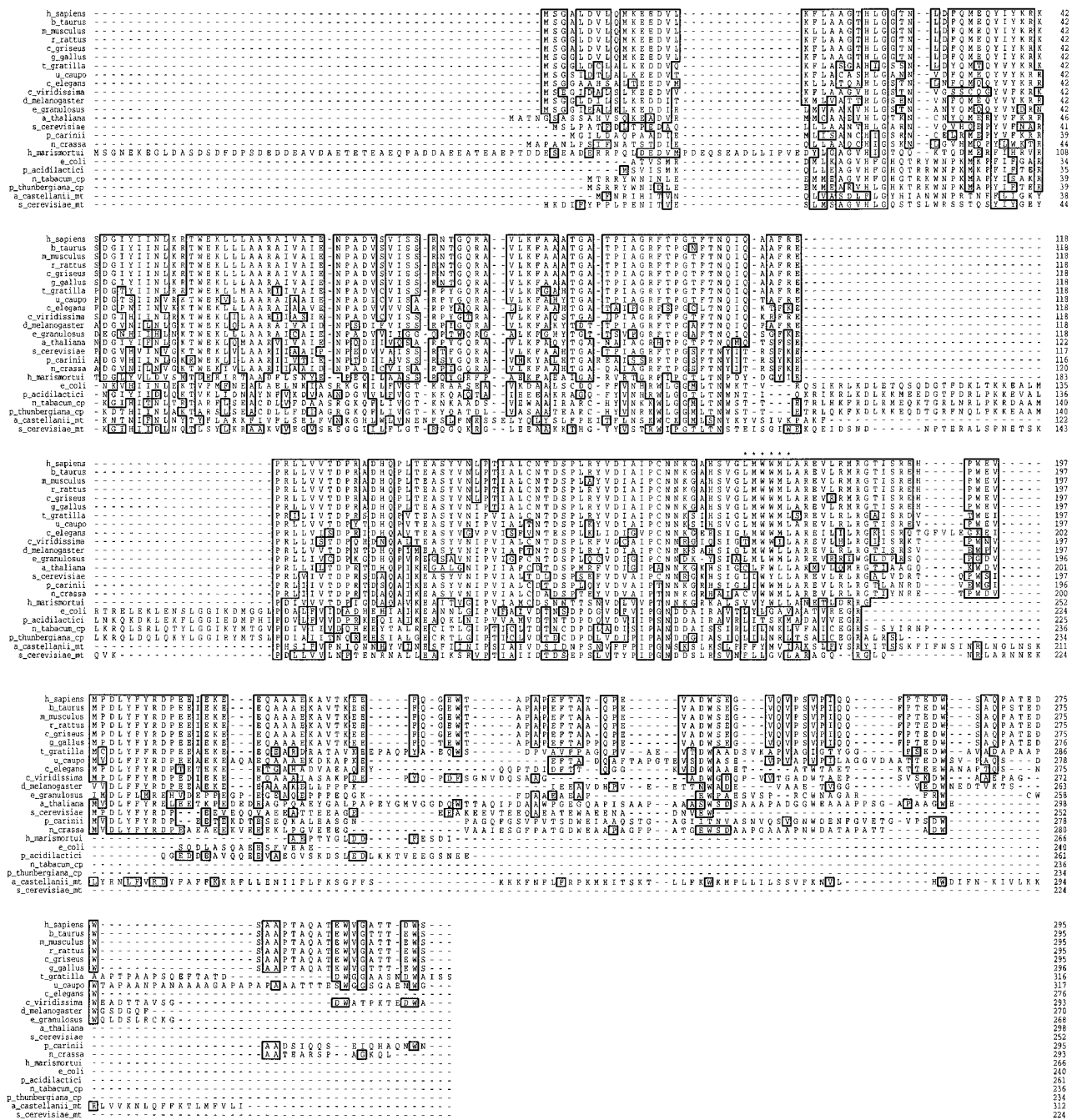


FIG. 1.—Multiple alignment of the 23 37LRP/p40 protein sequences. Sequence codes (on the left) derive from the organism names listed in table 1 (first letter of genus and species). The laminin-binding palindromic sequence LMWWML is marked by an asterisk.

The strong congruence between the phylogenetic relationships obtained using the 37LRP/p40 protein sequences and the widely accepted phylogeny of living organisms suggests that all protein sequences considered here derive from orthologous and not paralogous genes, i.e., they originated from speciation rather than from gene duplication events. Thus, it is likely that the function of the 37LRP/p40 as a ribosomal protein is maintained during evolution from prokaryotes to eukaryotes and that the laminin-binding capability is actually an additional function gained during evolution. Since the sequence of 20 amino acids, designated peptide G, has

been identified as the laminin-binding site, we analyzed the evolution of this sequence in association with the appearance of laminin and laminin-related molecules. As shown in table 2, the appearance of the sequence bearing the palindromic sequence LMWWML (marked by an asterisk in the multiple alignment in fig. 1) corresponds to the appearance of laminin or laminin-related proteins.

We recently demonstrated that peptide G binds to laminin and induces conformational changes in the adhesion molecule which greatly increase its affinity for integrins. Based on our conclusions concerning the evolution

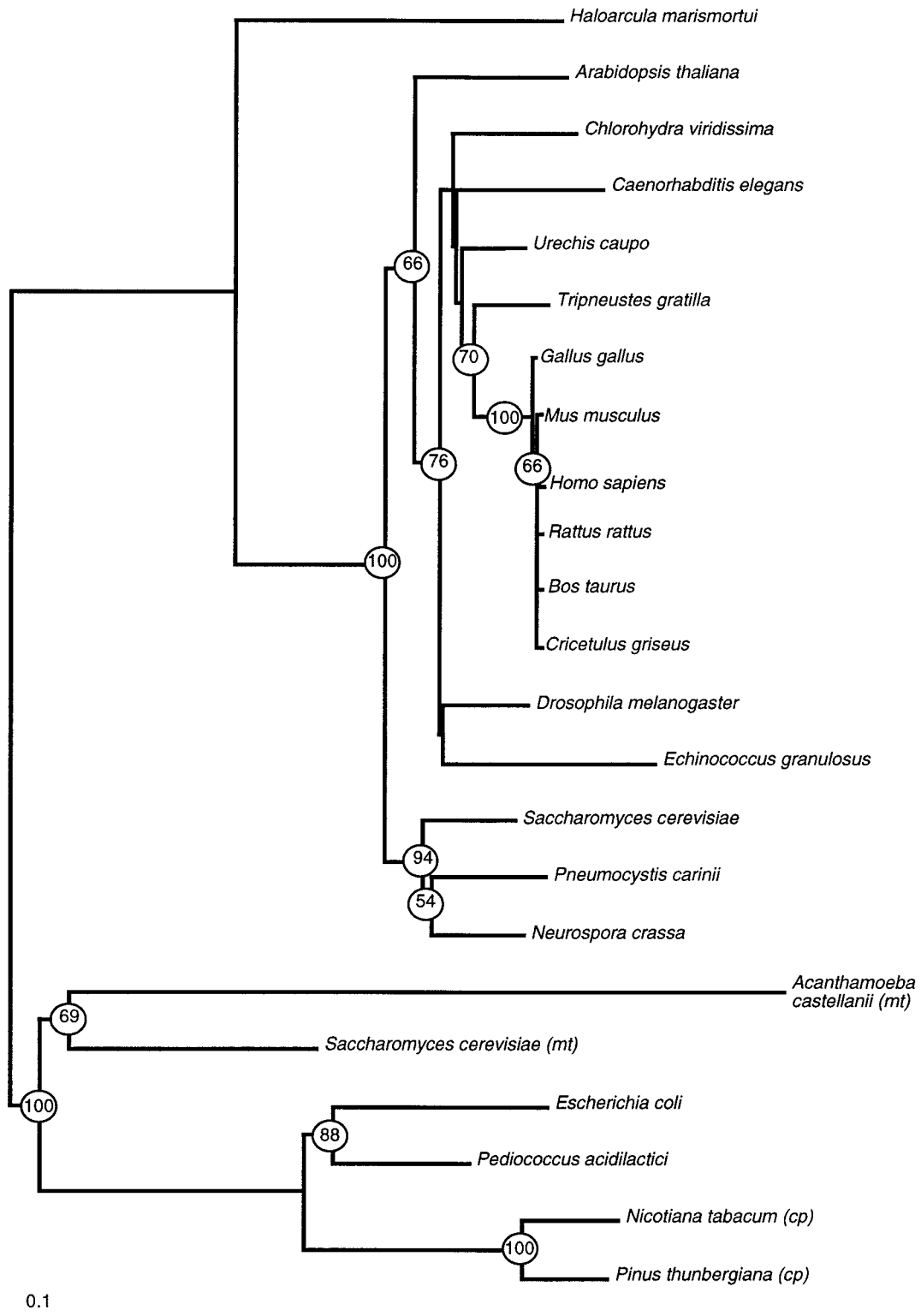


FIG. 2.—Phylogenetic relationships between the 23 37LRP/p40 protein sequences determined using the neighbor-joining method (Saitou and Nei 1987) on a distance matrix constructed with a Poisson correction (Galtier, Gouy, and Gautier 1996) and global gap removal option. A total of 161 sites were analyzed. Bootstrap values above 50% (based on 1,000 replicates) are given on the relevant nodes of the tree. Nodes supported by bootstrap values below 50% are collapsed into polytomies.

Table 2
Multiple Alignment of the Region Spanning Amino Acids 161–180 (numbering refers to the human sequence),
Representing the Laminin-Binding Site of the 67 LR

Organisms	Laminin-Binding Site	Laminin
<i>Saccharomyces cerevisiae</i> (mt).....	I P C N N R G K H S I G L I W Y L L A R	—
<i>Acanthamoeba castellanii</i> (mt).....	I P G N S K S L K S L F F Y M V I A K	—
<i>Pinus thunbergiana</i> (cp).....	T D C D P D L V D I P I P A N — D D G I	—
<i>Nicotiana tabacum</i> (cp).....	T N C D P D L A D I S I P A N — D D A I	—
<i>Pediococcus acidilactici</i>	I P S N D D A I R A V R L I T S K M A D	—
<i>Escherichia coli</i>	I P G N D D A I R A V T L Y L G A V A A	—
<i>Haloarcula marismortui</i>	V P T N N K G R K A L S V V Y W L L A N	—
<i>Saccharomyces cerevisiae</i> ♦.....	I P C N N R G K H S I G L I W Y L L A R	—
<i>Arabidopsis thaliana</i>	I P A N N K G K H S I G C L F W L L A R	—
<i>Drosophila melanogaster</i>	I P C N N K S A H S I G L M W W L L A R	+ ^a
<i>Caenorhabditis elegans</i> ♦.....	V P C N N K G E R S I G L M W W M L A R	+ ^b
<i>Urechis caupo</i>	I P C N N K S I H S V G L M W W M L A R	+ ^c
<i>Tripneustes gratilla</i>	I P C N N K S I H S I G L M W W M L A R	+ ^c
<i>Gallus gallus</i>	I P C N N K G A H S V G L M W W M L A R	+
<i>Cricetulus griseus</i>	I P C N N K G A H S V G L M W W M L A R	+
<i>Rattus rattus</i>	I P C N N K G A H S V G L M W W M L A R	+
<i>Mus musculus</i>	I P C N N K G A H S V G L M W W M L A R	+
<i>Bos taurus</i>	I P C N N K G A H S V G L M W W M L A R	+
<i>Homo sapiens</i> ♦.....	I P C N N K G A H S V G L M W W M L A R	+

NOTE.—Laminin or laminin-related protein expression is indicated. Arrows indicate the three peptides used for the experiments reported in figures 3, 4, and 5. Mitochondrial (mt) and chloroplast (cp) proteins are also indicated.

^a Yarnitzky and Volk (1995).

^b Rogalski et al. (1993).

^c Hawkins, Fan, and Hille (1995).

lutionary history of the molecule, we investigated the laminin-binding ability of the putative laminin-binding site deduced from the 37LRP/p40 protein sequences of three different organisms. Two new peptides were also synthesized: peptide Gc was deduced from the 37LRP/p40 protein sequence of *C. elegans*, which expresses two laminin-related proteins, unc-52 and unc-6 (Ishii et al. 1992; Rogalski et al. 1993); and peptide Gs was derived from the 37LRP/p40 sequence of *S. cerevisiae*, which does not express laminin. As a negative control, a scrambled peptide of the human sequence, peptide Gx, was used. Solid-phase binding assay of the binding of

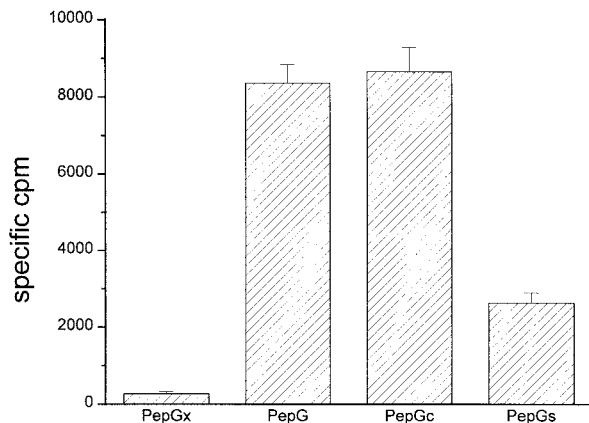


FIG. 3.—Binding of synthetic peptides to immobilized laminin. Microwells of a 96-well polyvinyl chloride plate were coated with 100 μ l of human laminin (10 μ g/ml) in PBS for 2 h at 37°C. After washing and blocking of nonspecific binding sites with 1% BSA in PBS, N-terminal-biotinylated peptide G, Gx, Gc, or Gs was added for 2 h at 4°C at 1 μ g/ml in 100 μ l PBS supplemented with 0.05% Tween 20 and 1% BSA. Peptide binding was monitored with ¹²⁵I-avidin (1 \times 10⁵ cpm/well).

N-terminal biotinylated peptides to human laminin showed that the laminin-binding capability of peptide Gc was similar to that of peptide G, whereas a low binding capability for peptide Gs and no binding at all for the scrambled peptide Gx, respectively, were found (fig. 3). Furthermore, cytofluorimetric analysis of the binding of the four biotinylated peptides on A431 tumor cells previously coated with exogenous human laminin revealed comparable amounts of peptide G and peptide Gc bound on the cell surface (fig. 4B and C), whereas

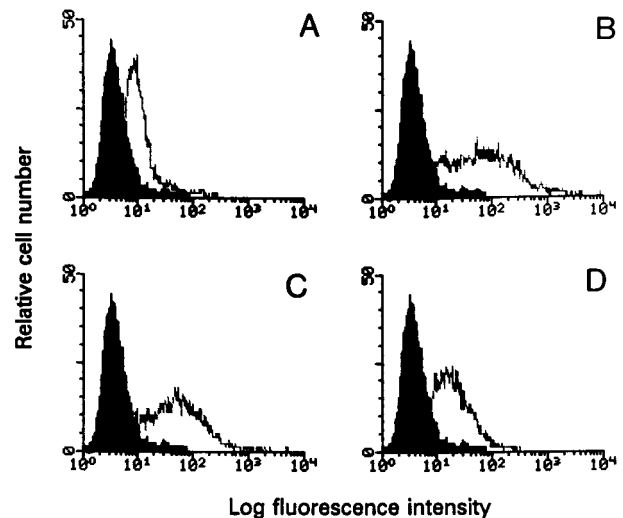


FIG. 4.—Peptide binding to tumor cell lines. A431 cells (3×10^5 /sample) treated with human laminin (50 μ g/ml) were incubated with biotinylated peptide Gx (A), peptide G (B), peptide Gc (C), or peptide Gs (D) for 30 min at 37°C. Bound peptides were monitored with fluorescein-labeled streptavidin (5 μ g/ml). Solid areas show background values.

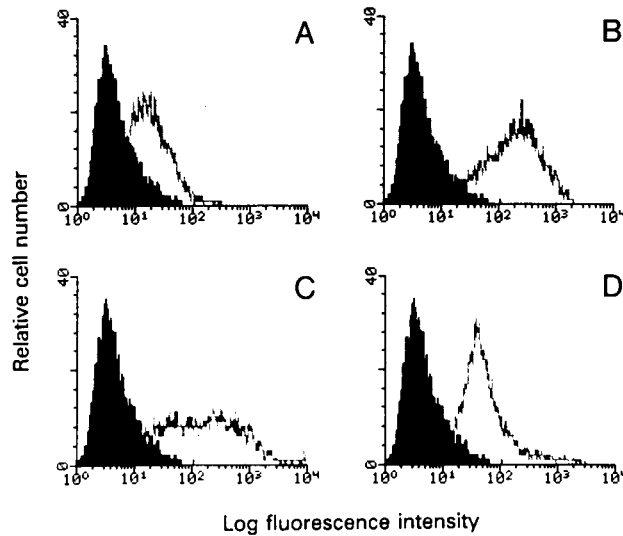


FIG. 5.—Increased cell surface binding of exogenous laminin after incubation with different peptides. A431 cells were treated for 30 min at 37°C with human laminin preincubated with peptide Gx (A), peptide G (B), peptide Gc (C), or peptide Gs (D). Human laminin bound on the cell surface was detected by indirect immunofluorescence using a rabbit polyclonal serum (1:100) and fluorescein isothiocyanate goat anti-rabbit Ig. Solid areas show background values.

lower binding levels were detected for peptide Gs (fig. 4D) and for scrambled peptide Gx (fig. 4A).

Analysis to determine whether the peptides induce conformational changes that increase the affinity of laminin for integrin receptors showed that pretreatment of exogenous human laminin (before coating to the cells) with peptide G and peptide Gc increased laminin binding to the cell surface by 15- and 10-fold, respectively, as compared with peptide-Gx-treated laminin, whereas laminin binding was only increased twofold by peptide Gs (fig. 5).

Discussion

The phylogenetic tree we constructed strongly suggests that the 37LRP, p40, and S2 proteins are encoded by orthologous genes and, thus, that the 37LRP is indeed a ribosomal protein that acquired the additional novel function of laminin receptor protein during evolution. The acquisition of this novel function is probably linked to the fixation of the palindromic sequence LMWWML, which appears to be critical for the laminin-binding activity (Castronovo, Tarabozetti and Sobel 1992). Indeed, peptide Gs, which shares 14 out of 20 amino acids with peptide G but contains only 3 of the 6 members of the palindromic sequence and lacks the double tryptophan, had little or no laminin-binding activity, whereas peptide Gc, sharing 16 out of 20 peptide G amino acids but displaying the palindromic sequence, bound laminin with the same activity as peptide G.

The acquisition of laminin-binding properties in vertebrates also produced a strong increase in the functional constraints that act on this molecule, producing a dramatic slowdown in the evolutionary process (see fig. 2). All vertebrate proteins are nearly identical along their

complete length, including the C-terminal region, which is the most divergent in other organisms (see fig. 1). In particular, we observed an average sequence divergence of only 1.7% between chicken and other mammalian proteins, which diverged about 250 MYA (Benton 1993), whereas average sequence divergence between vertebrate and invertebrate sequences, which diverged about 600 MYA (Lipps and Signor 1992), is 28.6%. This observation suggests that the new laminin-binding function, essential for survival, requires a protein conformation that negatively selects most of the diverging sequences, and that the C-terminal sequence is involved in maintaining this conformation. Moreover, the extraordinary conservation among vertebrates in the C-terminal region of the 37LRP/p40 suggests that a specific function might be localized in this domain. The mature membrane form of the 67-kDa laminin receptor related to the 37LRP/p40 has been described only for vertebrates. Although the mechanism by which this mature form, located on the membrane, is synthesized from the 37-kDa precursor remains unclear, we speculate that the C-terminal portion contains elements responsible for the incorporation of the 37LRP into the mature 67LR. Note that the C-terminal region in vertebrate proteins contains a five-time repeat motif of Glu, Asp, and Trp residues (DWS X₁₄ TEDWSA X₃ TEDWSA X₆ TEW X₄ T[E/D]W) and that remnants of this pattern are observed in other eukaryotic proteins, despite the high divergence between nonvertebrate protein sequences in this region. The sequence spanning sites 67–309 of the multialignment (about the first 200 amino acids, excluding gaps, in fig. 1), which confers the phylogenetic relationship of the 37LRP molecule with the S2 procaryotic ribosomal protein, is more likely related to the activity of the molecule in the translational machinery. This region contains the only eight amino acid residues conserved over all protein sequences considered here.

The *P. carinii* (Ascomycotina) protein, which has been found to bind both fibronectin and laminin in vitro (Narasimhan et al. 1994), is also more closely related to other fungal ribosomal proteins than to vertebrate 37LRP/p40 proteins, suggesting that the primary role of the *P. carinii* protein is in the translation machinery. It is possible to speculate that the acquisition of a specific sequence conferring laminin-binding capability is favorable to pathogenic microorganisms, enhancing their ability to attach to host cells and invade host tissues. In *P. carinii*, the palindromic sequence differs from that of the human gene by only one amino acid (Ile instead of Met), but the double tryptophan is present. The relevance of the two consecutive tryptophans for laminin binding is supported by the finding that a similar combination is the main feature of a protein module (the WW domain) which is present in a variety of cytoskeletal and signal-transducing proteins (Macias et al. 1997).

In conclusion, the 37LRP/p40 appears to be a ribosomal protein which is essential for protein synthesis. This explains why transfection of antisense gene or antisense oligonucleotides is lethal for all cells (unpublished observations). Concurrent with the appearance of the extracellular matrix and, therefore, of the laminin

molecule during evolution, the sequence of the gene gained a palindromic sequence which apparently confers laminin-binding capability to the molecule. Like the collagen-binding stress protein HSP47, which is a receptor for procollagen (Natsume et al. 1994), the 37LRP/p40 might play the role of chaperonin in the folding of the complex laminin molecule and, after posttranscriptional modification leading to a 67-kDa receptor located on the membrane, might play a role in the interaction of cells with the extracellular matrix. Recent analysis demonstrating high similarity between the N-terminal region of the 37LRP/p40 and various members of the HSP70 family (Chen, Fornace, and Laszlo 1996) strongly supports this hypothesis.

Our conclusions about the acquisition of a dual role for a ribosomal protein during evolution are in accord with evidence that many ribosomal proteins are bifunctional molecules, acting in diverse cellular processes in addition to protein synthesis (Starkey et al. 1996; Wool 1996). Some well-characterized ribosomal proteins have been implicated in DNA replication, control of transcription, protein transport, and cell transformation (Wool 1993).

The up-modulation of 37LRP/p40 gene expression in tumors might be related not only to laminin-binding activity (Sobel 1993), but also to the requirement of tumor cells for increased protein synthesis to face the new growth needs of these cells. Increased levels of mRNAs encoding several ribosomal proteins have been found in colon carcinoma cells (Pogue-Geile et al. 1991; Kondoh et al. 1992; Barnard et al. 1993), demonstrating that although ribosomal proteins are considered housekeeping genes, their expression levels are subject to precise control mechanisms and closely related to cell proliferation rate.

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