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Genetics

Human collagen genes encoding basement membrane α1(IV) and α2(IV) chains map to the distal long arm of chromosome 13

(coordinate expression/gene linkage/somatic cell hybrids/*in situ* hybridization/multigene family)

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ABSTRACT **At least 20 genes encode the structurally related collagen chains that comprise >10 homo- or heterotrimeric types. Six members of this multigene family have been assigned to five chromosomes in the human genome. The two type I genes, α1 and α2, are located on chromosomes 17 and 7, respectively, and the α1(II) gene is located on chromosome 12. Our recent mapping of the α1(III) and α2(V) genes to the q24.3→q31 region of chromosome 2 provided the only evidence that the collagen genes are not entirely dispersed. To further determine their organization, we and others localized the α1(IV) gene to chromosome 13 and in our experiments sublocalized the gene to band q34 by *in situ* hybridization. Here we show the presence of the α2 type IV locus also on the distal long arm of chromosome 13 by hybridizing a human α2(IV) cDNA clone to rodent–human hybrids and to metaphase chromosomes. To our knowledge, these studies represent the only demonstration of linkage between genes encoding both polypeptide chains of the same collagen type.**

Type IV collagen is exclusively located in basement membranes and associates with laminin, entactin, and heparan sulfate proteoglycans to form sheet-like structures that separate epithelium from connective tissue (1–4). Two polypeptide chains, α1(IV) and α2(IV) of M_r 185,000 and M_r 170,000, respectively (5–11), probably predominate in the molecular composition $\alpha1(IV)_2\alpha2(IV)$ (12–14), although $\alpha1(IV)_3$ homotrimers have also been identified (15). Structural analysis of the α1(IV) chain by a combination of protein and DNA sequencing has defined the location of multiple interruptions in the Gly-Xaa-Yaa region (16, 17) as well as the nature of the unprocessed noncollagenous NH_2- (7S) (18) and COOH- (NC1) terminal globular domains (19–21). The latter domain, composed of 229 residues, bears only marginal resemblance to the cleaved and highly conserved types I, II, III, and V COOH propeptides (22). In contrast to these, α1(IV) NC1 is characterized by an unusual repeat symmetry; the amino acids in the 5′ half exhibit 40% homology with those in the 3′ half including maintenance of the six cysteines (19–21).

Divergence of type IV is also manifested in the gene structure. Isolation of part of the human α1(IV) (23), mouse α1(IV) (24), and mouse α2(IV) (25) genes revealed that they lack the characteristic 54-base-pair exons which code for the uninterrupted interstitial collagenous regions (reviews, see refs. 26–28). Current knowledge of the type IV exons shows that they are irregular in size and are often separated by very large introns, resulting in a higher noncoding to coding nucleotide ratio than found in the types I–III genes (23–28).

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Using three different techniques, we and others mapped the human α1(IV) gene to chromosome 13 (21, 29, 30) and in our studies further defined the region to be at band q34 by *in situ* hybridization (30). Finding a fifth chromosome with collagen gene loci was not surprising since even the genes encoding the structurally related types I (α1 and α2), II, III, and α2(V) chains are almost entirely dispersed (29, 31–36). The type I genes, α1 and α2, are present on chromosomes 17 (32, 33) and 7 (31, 34), respectively, and the α1(II) gene is located on chromosome 12 (29, 35). Of the collagen genes mapped to date, only the α1(III) and α2(V) genes are known to share the same genomic site—i.e., the q24.3→q31 region of chromosome 2 (36).

We have recently identified a 1.7-kilobase (kb) cDNA clone coding for the 3′ part of the human α2 type IV mRNA. DNA sequencing revealed the same repeat structure in the COOH-terminal noncollagenous domain as is found in α1(IV). Alignment with the α1(IV) residues implies that these units may have evolved by means of an intra- to intergenic duplication. To determine the chromosomal location of the α2(IV) gene, the α2(IV) cDNA probe was hybridized to genomic DNA from rodent–human cell hybrids and to normal human metaphase chromosomes. Here we report the mapping of the α2(IV) collagen gene to the distal long arm of chromosome 13 and, therefore, to the same region containing the α1(IV) gene. These data suggest that the two coordinately expressed genes coding for human type IV collagen may be closely linked.

METHODS

Southern Blot Hybridization of α1(IV) and α2(IV) Clones to Rodent–Human Hybrids. Genomic DNA was isolated from each of the hybrid cell lines, digested with *Eco*RI, transferred to nylon membranes, and hybridized to the α1(IV) probe KK4 (20) or the α2(IV) probe S54 (Fig. 1), which were labeled with [^{32}P]dCTP by nick-translation (37–39). The hybrids whose designators are prefaced with GM were obtained from the Human Genetic Mutant Cell Repository (Camden, NJ), those with A_3 were from P. Pearson (University of Leiden), those with CF25 were from T. Mohandas (University of California at Los Angeles), and that with G46 was from G. A. P. Bruns (Children's Hospital, Boston).

***In Situ* Hybridization of the α2(IV) Clone to Human Metaphase Chromosomes.** The plasmid containing the α2(IV) 1.7-kb insert was ^{3}H-labeled by nick-translation to a specific activity of 2×10^7 cpm/μg. Metaphase chromosome spreads were prepared (36, 40) from two normal males (46, XY), one of whom had large satellites (a heteromorphic variant) on one

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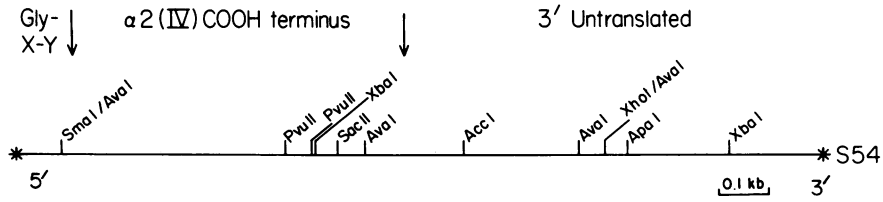

FIG. 1. Restriction map of the human α2(IV) collagen cDNA clone S54. DNA was inserted into the *Pst* I site (asterisks) of the pBR322 plasmid vector. The 1.7-kb clone codes for 35 Gly-Xaa-Yaa residues, the entire COOH-terminal noncollagenous domain, and the 3′ untranslated region (J.C.M., unpublished data).

of his chromosome 13 homologs. Hybridization and wash conditions were the same as used for previous α1(IV) *in situ* experiments (30). Chromosome staining and banding techniques have been detailed elsewhere (40).

RESULTS

Human α1(IV) and α2(IV) Collagen cDNA Clones. Isolation and characterization of the α1(IV) cDNA clone KK4 has been reported. The 1.7-kb insert codes for 182 Gly-Xaa-Yaa residues, the 229 amino acid COOH-terminal noncollagenous peptide, and 435 nucleotides of the 3′ untranslated region (20).

The α2(IV) cDNA clone S54, used for these chromosomal mapping studies, was isolated from a rhabdomyosarcoma cDNA library (41). A restriction map of the 1.7-kb insert is shown in Fig. 1. The α2(IV) clone codes for 35 Gly-Xaa-Yaa residues and the COOH-terminal noncollagenous and 3′ untranslated regions (J.C.M., unpublished data). The 5′ 126 nucleotides and derived amino acids are compared with the α1(IV) sequence in Fig. 2. These include 35 Gly-Xaa-Yaa residues and the adjacent seven amino acids of the COOH-terminal noncollagenous region. Twelve amino acids determined from a mouse α2(IV) peptide (42) showed 100% homology with the human residues obtained from DNA sequencing of the α2(IV) clone (Fig. 2).

Southern Blot Hybridization of the α1(IV) and α2(IV) Clones to Rodent–Human Hybrids. Two independent methods were employed for localization of the α2(IV) collagen gene. Our initial *in situ* studies using the ^{3}H-labeled α2(IV) clone indicated that the α2(IV) genomic locus might be present on the long arm of chromosome 13, which also contains the α1(IV) gene (30). To conclusively identify the site, subsequent chromsomal *in situ* analysis using normal human metaphase chromosomes was pursued concurrently with genomic mapping using DNA isolated from a panel of rodent–human somatic cell hybrids.

Table 1 and Fig. 3 display the results of experiments designed to determine the cosegregation frequency of the α2(IV) collagen gene locus with various human chromosomes. Parallel experiments using the α1(IV) clone were conducted to eliminate the possibility of mapping artifacts due to cross-hybridization of the α1(IV) and α2(IV) cDNA probes. Each of the rodent–human hybrid lines was examined karyologically at the time of DNA isolation and by Southern blot hybridization to probes representative of each human chromosome; the presence or absence of each is denoted with a plus or a minus. The human α1(IV) cDNA probe KK4 hybridized to a rodent *Eco*RI fragment of 14 kb (data not shown) and to human *Eco*RI fragments of 12 kb and 3.9 kb (20). The human α2(IV) cDNA probe S54 hybridized to rodent *Eco*RI fragments of 6 kb and 3 kb (mouse) or 7 kb and 5 kb (hamster) and to a human *Eco*RI fragment of 19 kb (Fig. 3). Each of the human-specific fragments at either locus segregated absolutely concordantly with only chromosome 13 in a large series of cell hybrids. Furthermore, both loci were present in a hybrid that contained the q12→qter region of chromosome 13, which was derived through translocation of chromosomes 13 and X, but were absent in similar hybrids that contained the region 13pter→13q12 or 13pter→13q22. Thus, each of the type IV collagen gene loci maps in the region 13q22→13qter (Table 1 and Fig. 3).

***In Situ* Hybridization of the α2(IV) Clone to Metaphase Chromosomes.** Three separate chromosomal *in situ* hybridization experiments were performed using the α2(IV) cDNA clone S54. Predominant hybridization in each experiment was to the distal long arm of chromosome 13 and there was no evidence of a consistent secondary site. In the 245 metaphase spreads examined, 492 grains were chromosomally located and their cumulative distribution is shown in Fig. 4. Of these grains, 11%, 14%, and 16% were found at the terminus of 13q. The 65 grains localized to this region represented at least six times the number on any other chromosomal segment of the same length. Therefore, the

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α1(IV)   CAG AAA      G ATG      T GCC   G  CT ACT   T   A  GA   A TTT CC      C
α2(IV)   TTC CGG GGA GAT GAA GGA CCC ATA GGC CAC CAG GGG CCG ATT GGC CAA GAA GGT GCA CCA GGC
α2(IV)   Phe Arg Gly Asp Glu Gly Pro Ile Gly His Gln Gly Pro Ile Gly Gln Glu Gly Ala Pro Gly
α1(IV)   Gln Lys     Glu Met         Ala     Pro Thr         Arg     Phe Pro     Pro

α1(IV)    CC GAT     TTG   A   A TCC AT    G CCT        AC  CCA TCT   T GAT CA       T    T G
α2(IV)   CGT CCA GGG AGC CCG GGC CTG CCG GGT ATG CCA GGC CGC AGC --- GTC AGC ATC GGC TAC CTC CTG
α2(IV)   Arg Pro Gly Ser Pro Gly Leu Pro Gly Met Pro Gly Arg Ser  -  Val Ser Ile Gly Tyr Leu Leu
α1(IV)   Pro Asp     Leu         Ser Met     Pro         Thr Pro Ser     Asp His     Phe     Val

α2(IV)   Gly Met Hyp Gly Arg Ser - Val X Ile Gly Tyr Leu Leu
Peptide
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FIG. 2. Amino acids derived from the 5′ nucleotides in the human α2(IV) cDNA clone. The 126 5′ nucleotides in the α2(IV) clone S54 (Fig. 1) and derived amino acids are aligned with the corresponding ones in human α1(IV) (20, 21). Only differences in the α1(IV) nucleotides and amino acids are shown. Dashes indicate lack of a nucleotide or amino acid at that position. The human α2(IV) clone was identified by comparing 12 residues with those obtained from protein sequencing of a mouse α2(IV) peptide (42) shown in italics. The X denotes an unidentified amino acid in the protein sequencing and the arrow designates the junction of the collagenous and noncollagenous regions.

Table 1. Segregation of proα1(IV) and proα2(IV) collagen loci in somatic cell hybrids

Hybrid	KK4 or S54*	Chromosome 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	Y	X/13†	13/X‡	13/X§
34-2-3	+	−	−	−	−	−	+	−	−	−	−	−	+	+	−	−	−	−	−	−	−	−	−	−	−			
WC-H27	+	+	+	−	+	−	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	−			
WC-H29A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	+	+	+	+	+	+	+	+	−			
WC-H30A	−	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	−			
WC-H36B	−	+	−	+	+	+	+	+	+	−	−	−	+	−	+	+	+	+	−	+	−	+	+	+	−			
GM7300	−	−	−	−	−	−	+	−	+	−	−	+	−	−	−	−	−	−	−	−	−	−	−	+	−			
GM6318B	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−			
A_3ADA_1-D12	+	+	−	+	+	+	−	−	+	−	−	−	+	+	+	−	+	−	−	−	+	+	−	−	+			
A_3ADA_6-F5	+	+	+	−	−	+	−	+	+	−	−	−	+	+	+	+	−	+	+	+	−	+	+	+	−			
A_3ADA_{13}	+	+	−	+	+	−	+	+	+	−	−	+	+	+	+	+	+	−	−	+	−	+	−	+	−			
A_3ADA_{14}	+	−	−	+	+	+	+	−	+	−	+	+	+	+	+	+	−	−	−	+	+	+	+	−	+			
A_3G1	−	−	−	+	+	−	−	−	+	+	+	−	−	−	−	+	−	+	−	+	+	−	+	+	+			
A_3G14	−	−	−	+	+	+	+	+	+	+	−	+	+	−	+	+	−	+	+	+	+	−	+	−	−			
A_3HR20	−	−	−	+	+	+	+	−	−	+	−	+	−	−	+	−	−	+	−	−	−	−	+	−	−			
PgMe4	+	−	+	+	−	−	+	+	+	−	−	+	+	+	+	+	−	+	−	−	+	+	−	+	+			
CF25-8/15	+	−	−	−	−	−	−	+	−	−	−	−	−	†	−	−	−	−	−	−	+	+	−	†	−	+	−	−
CF25-1R20	−	+	−	−	+	+	−	+	−	−	−	−	+	‡	−	−	−	+	+	+	+	+	−	‡	−	−	+	−
G46-C2	−	−	−	−	−	−	−	−	−	−	+	−	−	§	−	−	−	−	−	−	−	+	+	§	−	−	−	+
% discordancy¶		39	28	50	56	56	44	39	44	61	56	44	28	0	39	44	39	61	50	50	39	28	61	53	39			

*KK4 [α1(IV)]; S54 [α2(IV)]. The presence or absence of human-specific hybridization is denoted as a "+" or "−." KK4 and S54: 13q22→13qter.
†Translocation chromosome Xqter→Xp22::13q12→13qter, not included in 13 or X discordancy calculation.
‡Translocation chromosome 13pter→13q12::Xp22→Xpter, not included in 13 or X discordancy calculation.
§Translocation chromosome 13pter→13q22::Xq21→Xqter, not included in 13 or X discordancy calculation.
¶The correlation of α1(IV) and α2(IV) hybridization with the presence or absence of each chromosome is indicated as % discordancy.

results show that a single α2(IV) collagen locus is present at the distal q33→q34 region of chromosome 13 and are consistent with the position derived from the analyses of somatic cell hybrids shown in Table 1 and Fig. 3.

DISCUSSION

In the experiments presented here, we have conclusively localized the human α2 type IV collagen gene to the distal long arm of chromosome 13 by two independent methods. Hybridization to DNA from rodent–human hybrids with different deletions of chromosome 13 assigned the α2(IV) locus to the segment 13q22→terminus. Mapping by the chromosomal *in situ* technique allowed more refined sublocalization to the distal q33→q34 region, which also contains the α1(IV) locus (30), as shown diagramatically in Fig. 5. These results represent the second example of synteny for two members of this large multigene family and, to our knowledge, the only demonstration of linkage between genes encoding both chains of a single collagen type. This finding lends further credence to our earlier suggestion (36) that one might expect to find clustering of several collagen members and dispersion of others in a fashion analogous to the globin pattern. Two separate multigene clusters containing the α and β globin genes are present on chromosomes 16 and 11, respectively (43–45). At both loci, the genes are tightly linked and contiguous. The arrangement of the collagen genes is also reminiscent of the histones since clusters of different histone genes map to at least three human chromosomes (46). For this gene family, we hypothesized that there presumably existed

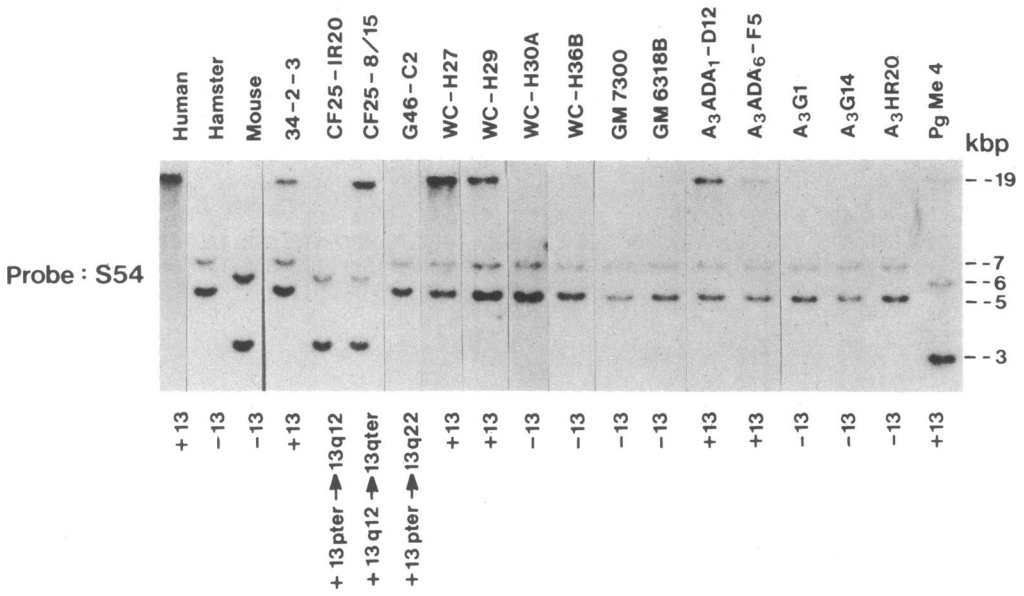

FIG. 3. Hybridization of the α2(IV) collagen clone to genomic DNA from rodent–human cell hybrids: segregation of the proα2(IV) collagen locus in somatic cell hybrids. Ten micrograms of genomic DNA was cleaved with *Eco*RI, electrophoresed in an agarose gel, and transferred to nylon membranes. The genomic DNAs were hybridized to the ^{32}P-labeled, nick-translated α2(IV) plasmid DNA S54. The 19-kb α2(IV) *Eco*RI fragment is of human origin. kbp, Kilobase pairs.

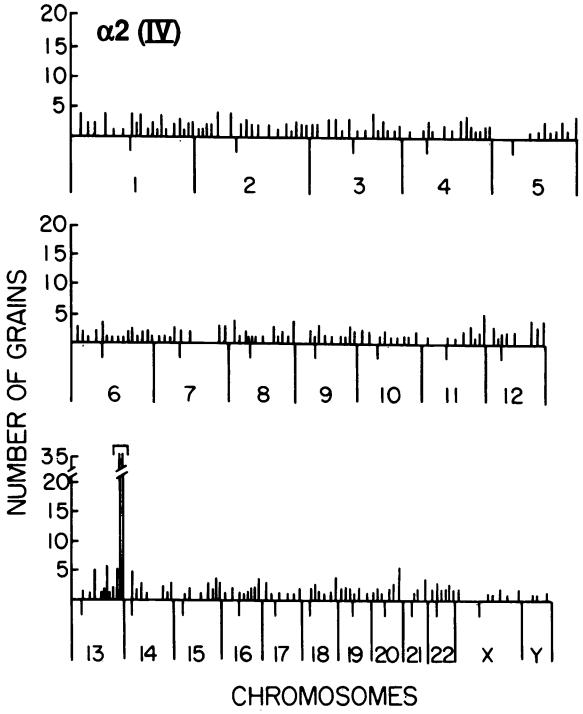

FIG. 4. Grain distribution from the α2(IV) *in situ* hybridizations. The histogram shows the cumulative grain distribution in 245 metaphase spreads using the α2(IV) clone as a probe. The abscissa represents the chromosomes in their relative size proportions and the ordinate shows the number of silver grains.

an ancestral site that gave rise to the present clusters distributed among multiple chromosomes by means of mechanisms involving reduplication, sequence modification, and recombination. A similar situation now emerges for the collagen gene family.

These chromosomal assignment studies are also important for continuing efforts toward generation of a human genetic map. There is a relative paucity of loci assigned to the distal portion of chromosome 13; only the loci for coagulation factors VII and X (47) and the random restriction fragment length polymorphism p9A7 (37) are known to occupy this location. The utility of genetic markers with defined map positions on chromosome 13 has been demonstrated in studies of retinoblastoma (38) and osteosarcoma (39), for which the definition of chromosomal breakpoints was critical.

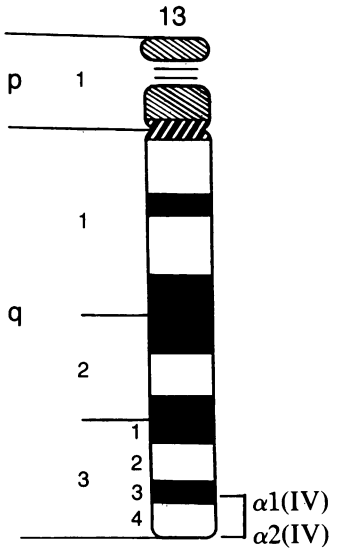

FIG. 5. Idiogram of chromosome 13. The bracket shows the region of chromosome 13 (distal q33→qter) to which the human α1(IV) and α2(IV) collagen genes have been localized.

Close linkage of the α1(IV) and α2(IV) collagen genes at the distal terminus of chromosome 13 appears to be a viable arrangement when one considers the structure of the coding units and polypeptide chains. Information generated from DNA and protein analyses implies that the chance of unequal crossing-over between the type IV genes is significantly less likely than would be expected for the more closely related interstitial collagen genes (26–28), whose chromosomal dispersion serves to prevent intergenic recombination (29, 31–36). Unlike the conserved types I (α1 and α2), II, and III collagen chains, Kühn *et al.* (48) state that the mouse α1(IV) and α2(IV) collagenous regions are highly divergent. As seen in Fig. 2, the 35 human α2(IV) Gly-Xaa-Yaa amino acids can be aligned with those in the α1(IV) chain only because of the presence of glycine as every third residue and the frequent occurrence of proline. In contrast, the α1(IV) and α2(IV) COOH-terminal noncollagenous domains are about 63% homologous (J.C.M., unpublished data). However, the longest contiguous stretch of identical nucleotides numbers 13 and occurs just twice, due primarily to the much greater preference for guanine and cytosine in the α2(IV) codons. A high G/C content is also found in the long α2(IV) 3′ untranslated region (J.C.M., unpublished results), unlike the (A + T)-rich α1(IV) counterpart (19, 21). Moreover, the type IV exons examined so far are irregular in size (23–25) and do not conform to the 54-base-pair pattern prevalent in the interstitial collagen genes, which exhibit an almost identical intron/exon distribution (reviews, see refs. 26–28).

The chromosomal pattern encountered for the type IV genes leads us to speculate that if additional type IV collagen chains exist (49), the corresponding genes will also be clustered in the 13q33→q34 region. Interestingly, the B_1 and B_2 laminin genes, which are coordinately regulated with α1(IV) and α2(IV), have been shown to be tightly linked on mouse chromosome 1 (50). The physical proximity of these coding units probably reflects the mechanism of their genetic evolution and may influence their exclusive expression in basement membranes.

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