Structure, Chromosomal Localization, and Expression Pattern of the Murine *Magp* Gene*

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The microfibril-associated glycoprotein (MAGP) was recently established as a discrete constituent of 10-nm microfibrils. We have characterized the primary structure of the mouse transcript, the structure and chromosomal localization of the murine gene, and the developmental pattern of gene expression. The transcript consists of 1,037 base pairs as determined by cDNA cloning, Northern blot analysis, S1 nuclease mapping, and primer extension mapping. Using a cDNA fragment as a probe, we isolated a single genomic clone that contained the entire mouse gene. Analysis of this clone indicated that *Magp* is fragmented into 9 exons, with the initiator Met codon located in exon 2. As determined by analysis of somatic cell hybrid lines and by fluorescence in situ hybridization, the mouse gene was mapped to chromosome 4 at a location corresponding to region D3-E1. Genomic sequence immediately upstream of the transcription start site was found to be GC-rich but lacked TATA or CCAAT boxes as well as other cis-acting motifs known to regulate transcription. Promoters of this type are usually found in genes that exhibit broad temporal and spatial patterns of expression. Consistent with this idea, the *Magp* transcript appeared to be the widespread product of mesenchymal/connective tissue cells throughout mouse development. This study presents the first comprehensive evaluation of microfibril gene expression during mammalian development.

The extracellular matrix contains a heterogeneous population of 3–20-nm filaments that Low (1962) termed microfibrils. Microfibrils 10 nm in diameter are present in elastic and non-elastic tissues and have a similar, if not identical, structure and composition (Cleary and Gibson, 1983). An acidic glycoprotein of 31 kDa termed the microfibril-associated glycoprotein (MAGP), was shown to be a major antigen of elastin-associated microfibrils (Gibson et al., 1986). More recently, an effort to purify microfibril components identified protein bands of 340, 78, 70, 31, and 25 kDa, plus the cross-linking enzyme lysyl oxidase (Gibson et al., 1988). Several other elastin-associated microfibril components (either integral or associated), including fibrillin (Sakai et al., 1986), thrombospondin (Arbeille et al., 1991), and 36-kDa microfibril-associated glycoprotein (Kobayashi et al., 1989), have now been characterized.

The normal role of 10-nm microfibrils in the extracellular matrix is only partly understood. In elastic tissues, microfibrils serve as templates for the assembly of tropoelastin molecules into elastic fibers (Ross et al., 1977). In non-elastic tissues, on the other hand, microfibrils may serve an anchoring function (Cleary and Gibson, 1983). Microfibrils may also have a role in tissue remodeling and cell migration following myocardial necrosis (Vracko et al., 1990), and general interest in extracellular microfibrils was created recently because of the observation that mutations in microfibril components cause heritable connective tissue disease. Naturally occurring mutations in the fibrillin gene on chromosome 15 (designated *FBN-1*) are linked with Marfan syndrome and with dominantly inherited ectopia lentis (Lee et al., 1991; Dietz et al., 1991; Maslen et al., 1991; Tsipouras et al., 1992; Kainulainen et al., 1992; Dietz et al., 1992; Dietz et al., 1993). Cloning experiments also revealed a second fibrillin-like transcript whose gene (designated *FBN-2*) resides on chromosome 5 (Lee et al., 1991). *FBN-2* was genetically linked to congenital contractural arachnodactyly, a rare disorder that shares some of the skeletal manifestations of Marfan syndrome (Lee et al., 1991; Tsipouras et al., 1992).

MAGP was recently established as a discrete microfibril component by molecular cloning of the bovine cDNA (Gibson et al., 1991), but little is known about its contribution to normal microfibril structure and function. Questions of this type can be investigated in animal models such as the mouse following the expression of specific mutations. Toward this end, we have characterized the structure and chromosomal map position of the mouse *Magp* gene and determined its expression pattern during development.

**EXPERIMENTAL PROCEDURES**

*Polymerase Chain Reaction.*—Day 13.5 embryos were obtained from timed pregnant CD-1 mice (Charles River). Poly(A)' RNA was prepared using commercially available reagents (Fast Track, In-Vitrogen). cDNA synthesis was performed as described (cDNA Synthesis System Plus kit protocol, Amersham Corp.), except that the reverse transcriptase enzyme was purchased separately (Seikagaku).
Aliquots of cDNA (~10%) were PCR amplified (Fig. 1) using commercially available reagents (Perkin-Elmer Cetus Instruments). Amplification occurred through 30 cycles of denaturation, annealing, and elongation to obtain a 487-bp fragment. The annealing temperature of the reaction was determined by the equation 4(G+C) + 2(A+T) = 6 = Ta (Patterson et al., 1989).

**Rapid Amplification of cDNA Ends—**cDNA single strands were synthesized as described above. To obtain the 5' end, cDNA synthesis was initiated using oligonucleotide primer 1515 (Fig. 1). A poly(A) tail was added to single strands according to standard protocols (Frohman, 1990) using terminal transferase (Boehringer Mannheim). Nested PCR was then carried out as previously described (Patterson et al., 1989). For the 5' PCR reaction, the following primers were employed:

**Amp 1:** upstream, 5' CCA TTC ATT AGT GCA GCC GTC TTT TTT TTT TTT TT T3' (primer 2264)

**Amp 2:** downstream, 5' AGC CGG AGG GCA CAC GGA GCT CTT CT3' (primer 2173)

To obtain the 3' end of the MAGP transcript, cDNA synthesis was initiated using an oligo(dT) primer. For the 3' PCR reaction, the following PCR primers were employed:

**Amp 1:** upstream, 5' GCC AAT TCG ACT GTC TGC CAC GAG GAA CTT CT3' (primer 2264)

**Amp 2:** downstream, 5' AGC CGG AGG GCA CAC GGA GCT CTT CT3' (primer 2173)

PCR products were purified and cloned into the TA cloning vector (InVitrogen). The subcloned insert was sequenced using the Sequenase version 2.0 sequencing kit according to the manufacturer's recommendations. DNA sequence was analyzed with the MacVector software program.

**Northern Blot Analysis—**Poly(A)* RNA was prepared from day 13.5 mouse embryos as described above. Samples of 2–10 μg were electrophoresed on a 2.5% agarose/2.2 M formaldehyde gel and then transferred to a nylon membrane (Hybond-N, Amersham). The RNA was cross-linked to the membrane by exposure to a UV light source for 30 sec. Hybridization was performed in a 5% dextran sulfate, 5× SSPE buffer containing 1× Denhardt's solution, 0.5% SDS, and 40 μg/ml denatured, degraded salmon sperm DNA. The 487-bp PCR product was labeled by random priming, and hybridization was performed in fresh buffer for 16 h at 65 °C. Blots were washed progressively to high stringency (0.1× SSPE/0.1% SDS at 65 °C) and then placed against x-ray film with intensifying screens (XAR, Kodak) at −86 °C.

**Finger Print Mapping—**Poly(A)* RNA (2.5 μg aliquots) isolated from day 13.5 mouse embryos was incubated with 0.07–0.9 ng of 32P-labeled oligonucleotide primer in buffer (900 mM NaCl, 5× Denhardt's solution, 0.5% SDS, and 40 μg/ml denatured, degraded salmon sperm DNA) for 2.5 h at 65 °C. After hybridization, the blot was washed with 0.1× SSPE/0.1% SDS at 65 °C and then placed against x-ray film to detect autoradiographic bands. Autoradiographs were developed with intensifying screens (XAR, Kodak) at −86 °C.

**Tissue in Situ Hybridization—**The 487-bp PCR fragment was subcloned into the pBluescript KS+ plasmid (Stratagene). Template DNA was linearized with either EcoRI or SalI, extracted, and precipitated with ethanol. Sense and antisense transcripts were generated from 1 μg of template with T3 and T7 polymerases in the presence of [32P]UTP at >6 μCi/μl (Amersham, >1200 Ci/mmol) and 1.6 units/μl RNasin (Promega), with the remaining in vitro transcription reagents provided in a kit (SureSite, Novagen Inc.). After transcription at 37 °C for 1 h, DNA templates were removed by a 15-min digestion at 37 °C with 0.5 unit/μl RNase-free Dnase I, extracted, and precipitated with ethanol. According to the formula determined by Cox et al. (1984), riboprobes were hydrolyzed to an average final length of 150 bp by incubating in 40 μM NaHCO3, 60 μM Na2CO3, 80 μM DTT for 42 min at 65 °C. Hydrolysis was terminated by washing the membrane with 0.1× SSPE/0.1% SDS at room temperature and 0.56% (v/v), respectively, and the probes were then ethanol-precipitated, dissolved in 0.1 M DTT, counted, and stored at −20 °C until use.

Paraffin-embedded tissue sections (7 μm) from NIH Swiss mouse embryos at 8.5–9.0, 13.5, and 16.5 days post coitus were obtained from Novagen, Inc. All tissues were fixed in 4% paraformaldehyde. Day 8.5–9.0 sections often contained embryos surrounded by intact membranes and uterine decidual epithelium, and they frequently contained the placental disk. They were cut in randomly oriented sections, and paraffin sections were used to determine its exact size. Separate protection assays with RNA as the template were used as negative controls.

**RNAse Precautions—**Tissue sections were heated to 65 °C for 10 min, deparaffinized in three changes of xylene for 5 min, and rehydrated in a descending ethanol series (that included phosphate-buffered saline (PBS)) as the final step. Slides were soaked in 0.2 N HCl for 5 min, rinsed in PBS, digested with 0.0002% proteinase K in PBS for 30 min at 37 °C, and rinsed briefly with diethylpyrocarbonate-treated water. After equilibrating for 3 min in 0.1 M triethanolamine-HCl, pH 8.0, sections were acetylated in 0.25% (v/v) acetic anhydride in 0.1 M triethanolamine-HCl for 10 min at room temperature, rinsed in PBS, and dehydrated in an ascending ethanol series. Each section received 100–200 μl of prehybridization solution (0.5 mg/ml denatured RNAse-free tRNA (Boehringer-Mannheim), 10 mM DTT, 5 μg/ml denatured, sulfurylated salmon sperm DNA, 50% formamide, 10% dextran sulfate, 300 mM NaCl, 1× RNAse-free Denhardt's solution (made with RNAse)
free bovine serum albumin, Sigma), 10 mM Tris-HCl, pH 7.4, 1 mM EDTA) and was incubated on a 50 °C slide warmer in a humidified enclosure for 2 h. The sulfonylated salmon sperm DNA blocking reagent was used in both prehybridization and hybridization solutions to help reduce nonspecific binding to tissue by 35SH groups on the probe. It was prepared by labeling RNase-free salmon sperm DNA (Sigma) with nonradioactive α-thio-dCTP and α-thio-dATP (Amer-
signet, 1989). Sense and antisense probes were applied to adjacent serial sections from each embryo. Slides were rinsed 3 times in 4 × SSC, washed with 2 × SSC, 1 mM DTT for 30 min at 50 °C, digested with RNase A (20 µg/ml RNase A in a buffer containing 0.5 M NaCl, 10 mM Tris, pH 8.0, 1 mM EDTA, pH 8.0) for 30 min at 37 °C, and rinsed briefly with 2 × SSC, 1 mM DTT. Three additional washes were performed, each at 50 °C for 30 min, as follows: once in 2 × SSC, 50% formamid, and 1 mM DTT, and twice in 1 × SSC, 0.13% (w/v) sodium pyrophosphate, 1 mM DTT. Slides were dehydrated and then exposed to x-ray film (Hyperfilm-βmax, Amersham) for 60–132 h to visualize overall hybridization patterns, dipped in autoradiographic emulsion (Kodak NTB-2, diluted to 50% with 0.3 M ammonium acetate), slowly dried for 2 h, and exposed for 2 weeks at room temperature. After developing the emulsion, sections were counterstained with hematoxylin and eosin, dehydrated, mounted with xylene-based medium, and the hybridization signal was visualized under darkfield microscopy.

Hybrid Panel Mapping—A panel of Chinese hamster x mouse and rat x mouse somatic cell hybrids was produced and characterized as

**Fig. 1.** PCR cloning strategy. Oligonucleotide primer sequences were based on the published bovine MACP cDNA sequence (Gibson et al., 1991). The 5'- and 3'-untranslated regions of the transcript appear single spaced and in smaller type. The 5' boundary of exon 1 and 3' boundary of exon 9 have yet to be determined). The 5'- and 3'-untranslated regions of the transcript appear single spaced and in smaller type. Excess prehybridization solution was removed with a brief rinse in 4 × SSC before application of probe. Riboprobes, fresh tRNA, and sulfonylated salmon sperm DNA were denatured for 10 min at 70 °C and chilled on ice. Fresh buffer with denatured probe added to 1 × 106 CPM/ml was applied and slides incubated at 50 °C for 18.5 h in sealed humidified chambers on a slide warmer (Simmons et al., 1989). Sense and antisense probes were applied to adjacent serial sections from each embryo. Slides were rinsed 3 times in 4 × SSC, washed with 2 × SSC, 1 mM DTT for 30 min at 50 °C, digested with RNase A (20 µg/ml RNase A in a buffer containing 0.5 M NaCl, 10 mM Tris, pH 8.0, 1 mM EDTA, pH 8.0) for 30 min at 37 °C, and rinsed briefly with 2 × SSC, 1 mM DTT. Three additional washes were performed, each at 50 °C for 30 min, as follows: once in 2 × SSC, 50% formamid, and 1 mM DTT, and twice in 1 × SSC, 0.13% (w/v) sodium pyrophosphate, 1 mM DTT. Slides were dehydrated and then exposed to x-ray film (Hyperfilm-βmax, Amersham) for 60–132 h to visualize overall hybridization patterns, dipped in autoradiographic emulsion (Kodak NTB-2, diluted to 50% with 0.3 M ammonium acetate), slowly dried for 2 h, and exposed for 2 weeks at room temperature. After developing the emulsion, sections were counterstained with hematoxylin and eosin, dehydrated, mounted with xylene-based medium, and the hybridization signal was visualized under darkfield microscopy.

Hybrid Panel Mapping—A panel of Chinese hamster x mouse and rat x mouse somatic cell hybrids was produced and characterized as

**Fig. 2.** Mouse MAGP amino acid sequence. The deduced primary sequence is shown. Bovine amino acids that differ from mouse are shown in boldface type. Dotted lines show regions of the mouse and bovine primary sequence that fail to align.

**Fig. 3.** Mouse Magp transcript, A, mouse Magp cDNA sequence. The Met to Stop nucleotide sequence is shown in large type. The 5' and 3'-untranslated regions of the transcript appear single spaced and in smaller type at the ends of the coding sequence. Nucleotide numbers are given at the right. Arrowheads point to exon/intron boundaries as deduced from DNA sequence analysis (the 5' boundary of exon 1 and 3' boundary of exon 9 have yet to be determined). Note the in-frame ATG codon (underlined) just upstream of the designated initiator Met codon. We could not distinguish between these two ATG codons on the basis of context, i.e. they both contribute to moderately faithful Kozak consensus sequences. Nevertheless, we have chosen the downstream ATG codon as the initiator because of its homology with the previously published bovine cDNA sequence (Gibson et al., 1991) and because of the results of the primer extension and S1 nuclease mapping experiments (see Fig. 4). B, Northern blot analysis of day 13.5 mouse embryo mRNA.
reported previously (Yang-Feng et al., 1986). PCR studies were performed on a set of 13 hybrids and on mouse, Chinese hamster, and rat parental control lines. PCR primers were designed to amplify a 250-bp portion of the Magp intron 1 sequence as follows: forward primer (with EcoRI tail) 5'-GCG AAT TCG GAT CCA GGA GCT GGA GAG ACT-3'; reverse primer (with SaI tail) 5'-AT GCT GAC CAT CCT CGG GTC TCT CTT TGT TCT CTC-3'. Each 50-μl reaction contained in excess of 250 ng of template DNA, 15 pmol of each primer, and 0.25 units of Taq polymerase (Promega) in the buffer provided by the manufacturer, supplemented with MgCl₂ to a final concentration of 1.5 mM. Amplification consisted of 35 cycles of 94°C (1 min), annealing (62°C, 1 min), and extension (72°C, 1 min). In addition, one initial delay cycle at 95°C for 5 min and a final delay at 72°C for 5 min were included. Reaction products were analyzed on a 1.5% agarose gel. Reaction products from mouse and rat parental lines and rat x mouse somatic cell hybrid RTM 9 were additionally analyzed on an 8% polyacrylamide gel to clearly distinguish the size difference in products.

Fluorescence Chromosomal in Situ Hybridization—A mouse adrenocorticotoid tumor cell line, referred to here as Y1-HSR, was grown and harvested as previously described (George and Francke, 1980) with the exception of a longer colcemid treatment (2-16 h) and a 40-min hypotonic exposure using 0.7% sodium citrate. Slides were made immediately after harvesting using standard cytogenetic methods. Fluorescence chromosomal in situ hybridization was carried out using nick-translated biotinylated DNA from the YC-129 m genomic clone. Whole phage DNA was biotin labeled (biotin-11-dUTP, Sigma) by nick translation using a kit (Life Technologies, Inc.). Metaphase spreads prepared from Y1-HSR cells were hybridized with the biotin-labeled probe as described elsewhere (Milatovich et al., 1991) with the exception that the probe and competitor DNAs were desiccated rather than precipitated. The DNAs were suspended in hybridization solution to give a final concentration of 60 ng/μl probe and 200 ng/μl each of salmon sperm DNA and mouse competitor DNA. Chromosomes were stained with propidium iodide. Hybridization sites on the chromosomes were detected with fluorescein isothiocyanate-conjugated avidin, and signals were counted as specific only when they were present on both chromatids of a chromosome. A Zeiss Axiphot microscope equipped with epifluorescence and a cooled charge coupled device camera (Photometrics PMS12/Macintosh computer system was used for imaging. Software was supplied by T. Rand (Yale University).

RESULTS

Molecular Cloning of the Mouse Magp Transcript and Gene—We assumed that there would be significant homology between the mouse and bovine coding sequences and therefore used a PCR strategy to obtain most of the mouse cDNA sequence (Fig. 1). We then used a rapid amplification of cDNA ends protocol (Frohman, 1990) to obtain the 5' and 3' ends of the transcript.

The deduced MAGP molecule is a polypeptide of 185 amino acids, with a calculated pl of 4.86 and a predicted molecular mass of 20.6 kDa. As shown in Fig. 2, the mouse and bovine coding sequences are highly homologous, showing greater than 85% identity at the amino acid level. Predictably, mouse MAGP appears to contain two structurally distinct regions. The amino-terminal half (amino acids 1-96) of the primary sequence contains a large number of glutamine, proline, aspartic acid, and glutamic acid residues (>43%), while the carboxyl-terminal half (amino acids 97-183) contains 13 cysteines in conserved positions. The odd number of cysteines

### TABLE I

Comparison of Magp sequences and mouse chromosomes in rodent x mouse somatic cell hybrids: discordancy analysis of the Magp PCR product and somatic cell hybrids

| MAGP/chromosome | Mouse chromosome |
|-----------------|-----------------|
|                 | 1    2    3    4    5    6    7    8    9    10    11    12    13    14    15    16    17    18    19    X |
| Concordant hybrids | 4   6   4   6   3   4   5   4   2   2   0   4   3   2   5   2   5   4   6   3 |
| +/+              |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
| +/-             | 2   3   5   7   5   3   3   3   5   5   5   6   4   5   5   3   3   6   4   2 |
| Discordant hybrids | 2   0   2   0   2   2   1   2   4   3   6   2   3   4   1   3   1   2   0   2 |
| +/-             |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
| +/-             | 5   4   2   0   1   3   3   2   2   2   1   3   2   2   4   4   4   0   3   5 |
| Total discordant hybrids | 7   4   4   0   3   5   4   4   6   5   7   5   5   6   5   7   5   2   3   7 |
| Total informative hybrids | 13  13  13  13  11  12  12  13  12  12  13  13  13  13  13  13  12  12  12  |
| Percent discordant hybrids | 54  31  31  0  27  42  33  31  46  42  54  38  38  46  38  38  17  23  58 |
has suggested that MAGP can form intermolecular disulfide bonds, an observation consistent with biochemical studies that indicated that MAGP was covalently bound within microfibrils by disulfide linkages (Gibson et al., 1986). Murine MAGP contains no known cell attachment sequence (e.g. RGD, YIGSR, GPEILDVPST, KQAGDV, GRGD, LDV, RYVVLPR, GWSPW, and WSXWS were not found) and no consensus acceptor site for N-linked glycosylation. Therefore, carbohydrate component(s) are most likely O-linked through serine or threonine residues. Oligosaccharide moieties of this type, e.g. N-acetylgalactosamine and galactosamine, are the major sugars found in hydrolysates of bovine MAGP (Gibson et al., 1986).

Altogether, we obtained 1037 bp of cDNA sequence, including the 5'- and 3'-untranslated regions of the mouse transcript (Fig. 3A). Consistent with this observation, the murine message migrated as a transcript of ~1.2 kb in a Northern analysis (Fig. 3B). Primer extension and S1 nuclease mapping were used to characterize the 5' end of the mouse transcript (Fig. 4, A and B). The results of both experiments indicated that the 5'-untranslated region is 80 nucleotides in length.

We isolated a 19-kb genomic clone from a murine SV129 phage library and determined the genomic organization of Magp. The 10-kb genomic clone appeared to contain the entire gene, which was fragmented into nine exons (Fig. 3A, arrowheads). Sequence analysis demonstrated that the 5'-untranslated region was found within the first 2 exons of the mouse gene. The 2nd exon also coded for the putative initiator Met residue, which occurred in the context of a sequence (ATGGAAATGA) that was homologous to the Kozak consensus (Kozak, 1991). Introns/exon boundaries were also determined by DNA sequence analysis, which demonstrated GT and AG dinucleotides at all upstream and downstream intron junctions, respectively (data not shown). Additionally, all coding sequence exons began and ended with split codons. Beginning with the 3' boundary of exon 2, the pattern of codon splitting was 1/2, the single exception being the exon 7-8 boundary where the codon was split 2/1. Finally, the nucleotide sequence of all 9 exons in the mouse genomic clone was determined. This effort eliminated the possibility of including bovine sequence in Fig. 3A, a potential consequence of the PCR cloning strategy.

Chromosomal Localization of Magp—Magp-specific oligonucleotide primers directed the amplification of a 250-bp DNA fragment from mouse genomic DNA and from the DNA of hybrid cell lines that contain mouse chromosome 4. A 270-bp fragment was amplified from rat DNA, but no fragment was amplified from Chinese hamster DNA. In relation to the mouse chromosome content of the hybrid cell panel, discordancy analysis of the PCR results revealed at least two discordancies with all mouse chromosomes except chromosome 4 (Table I). This result indicated that Magp resides on this chromosome.

Fluorescence in situ hybridization was performed using the

![Fluorescence chromosomal in situ hybridization using a biotinylated Magp probe on two independent mouse Y1-HSR metaphase chromosome preparations.](image)

![Partial characterization of the Magp promoter region.](image)
Murine Magp Gene Structure and Developmental Expression

Fig. 7. Temporal and spatial expression of Magp during murine development. A, overview of mRNA expression as determined by in situ hybridization. Day 8.5–9.0 sections contained embryos surrounded by intact membranes, uterine tissues, and the placental disk, cut in random planes. Day 13.5 and 16.5 sections contain isolated whole embryos sectioned in the sagittal plane near or about the midline. Identical conditions were maintained throughout autoradiography and photography, thereby allowing a comparison of the overall strength of hybridization in all tissue sections. The Magp transcript appears to be the widespread product of mesenchymal/connective tissue cells. However, the brain, spinal cord, heart, and liver in the day 13.5 and 16.5 tissue sections (arrowheads) do not show significant hybridization. B, detailed analysis of the mouse embryo at day 8.5–9.0 of development. The cephalic neural tube is shown at the top, and the caudal neural tube is shown at the bottom. At day 8.5–9.0 of development, the hybridization signal appears to be concentrated in the mesenchyme. Heart muscle,
endocardial tissue, and the neural epithelium do not show significant hybridization. C

3, detailed analysis of the mouse embryo at day 13.5 of development. C

presents the heart and surrounding structures, including a branch of the pulmonary artery, liver, lung, and the central tendon of the diaphragm. The hybridization signal appears to be concentrated in the wall of the pulmonary artery, the central tendon of the diaphragm, and the atrio-ventricular bulbar cushion tissue. A line

drawing of structures shown in C has been included. Abbreviations used in the line drawing include: PA, pulmonary artery branch; L, lung; Li, liver; D, central tendon of the diaphragm; At, atrium; V, ventricle; AVBC, atrio-ventricular bulbar cushion tissue. (Note that the central tendon of the diaphragm has been exaggerated somewhat for clarity.) The cardiac atrium, cardiac ventricle, and the liver parenchyma do not show significant hybridization. C presents vertebral cartilage near the spinal cord. The hybridization signal appears to be concentrated in the pericartilaginous connective tissue. There is a lesser, but nevertheless significant, degree of hybridization in the developing vertebral cartilage body (arrows are used to outline the vertebral body). C presents the lung. The hybridization signal appears to be concentrated in developing alveolar tissue. Developing large airways (arrows) do not show significant hybridization. D, detailed analysis of the mouse embryo at day 16.5 of development. The figure shows representative brightfield and darkfield sections of the skin, testis, skeletal muscle, and liver (top to bottom). The hybridization signal appears to be concentrated in the interstitial connective tissues of the skin (i.e. skin dermis), skeletal muscle, and testis. In all darkfield photographs (B-D), red blood cell and other plasma membranes give a faint white signal that contributes to the background of the experiment. Bar, 20 μm.
Mercurine Magp Gene Structure and Developmental Expression

YC129 m phage clone that was biotin-labeled and hybridized to Y1-HSR metaphase chromosomes. Twenty-three of twenty-nine analyzed metaphase cells had fluorescent staining on both chromatids (at least one chromosome/cell) at a location corresponding to the D3-E1 region of chromosome 4 (Fig. 5). The physical mapping results were consistent with mapping results for the human MAGP locus, which lies in a region of conserved synten on chromosome 1, bands p36.1–p38 (Faraco et al., 1993).

Temporal and Spatial Expression of MAGP During Mouse Development—As shown in Fig. 6, the genomic clone included ∼0.96 kb of sequence contiguous with the 5′-untranslated region of the transcript. The sequence immediately upstream of the transcription start site was GC-rich but lacked TATA and CCAAT boxes. Promoters of this type are usually found in genes that exhibit broad patterns of expression (Dypan, 1986). To examine this question further, tissue in situ hybridization was performed to evaluate the temporal and spatial expression of Magp transcripts during mouse development. Fig. 7A presents an overview of mRNA expression. In general, expression was widespread at all time points, with the strongest hybridization occurring at day 13.5. In day 8.5–9.0 tissues, Magp was strongly expressed in several embryo tissues but was less abundant in placenta, placental membranes, eoptalcental cone, and maternal (decidual) tissues. In day 13.5 and 16.5 tissues, Magp expression again was widespread and strong in many embryo tissues, with the possible exception of the liver, heart muscle (i.e., atria and ventricles), brain, and spinal cord.

Microscopically, the Magp transcript was synthesized by mesenchyme of day 8.5–9.0 embryos (Fig. 7B). At day 13.5, Magp expression was observed in several loose connective tissues as well as the interstitium of the wall of the pulmonary artery and aorta, lung, central tendon of the diaphragm, atrio-ventricular bulbar cushion tissue, and peripheral cartilage (Fig. 7C,D). At day 16.5, Magp expression was again observed in several loose connective tissues and the skin dermis. The perichondrium and periosteum of endochondral bone, the endoskeleton of skeletal muscle, and the interstitial connective tissue of the aorta, kidney, gut, testis, and lung were also positive (Fig. 7D). We failed to detect Magp expression in the interstitial connective tissue of the liver (Fig. 7, C, and D), heart (atria and ventricles, Fig. 7C) brain, and spinal cord. Expression also was not detected in the following parenchymal cell types: neuronal (ganglion) cells of the central and peripheral nervous system; skeletal and cardiac muscle cells; keratinocytes; mucosal epithelial cells of the lung, gut, pancreas, and salivary gland; adipocytes; hepatocytes, hypertrophic chondrocytes; osteoblasts; adrenal cortical epithelial cells; epithelial cells of renal glomeruli, tubules, and collecting ducts; or lens epithelial cells.

DISCUSSION

This study has characterized the mouse Magp gene and transcript. The gene is localized in the D3-E1 region of mouse chromosome 4, is less than 10 kb, and is interrupted by 8 introns. All 9 exons are bounded by split codons. The DNA sequence upstream of the transcription start site is GC-rich but lacks TATA and CCAAT boxes and does not show homology to other known transcriptional control motifs. The promoter region of the human FBN-1 gene has a similar primary structure (Pereira et al., 1993).

The deduced mouse MAGP molecule is a polypeptide of 185 amino acids that shows greater than 85% identity with bovine MAGP at the mRNA and (conceptual) protein sequence levels. We find two areas of relative nonhomology, nucleotides 240–258 and nucleotides 165–178 (Fig 3A), between the mouse and bovine cDNA sequence. In particular, a portion of the sequence between nucleotides 240–258 provides the conceptual translation AAAGDGL, which is only GTV in the bovine sequence (Gibson et al., 1991) and GNA in the human sequence. An affinity-purified anti-peptide antibody against the bovine amino acid sequence from this region (VIPATTLEPGT) completely failed in our hands to immuno-stain normal mouse tissues (data not shown). Including a three-amino acid (AAA) insertion, there are a total of five differences between the sequence of the bovine peptide and the conceptual sequence encoded by the mouse cDNA (VIPATTPPEAGD). These differences likely account for the inability of the bovine antibody to stain mouse tissues. Perhaps the two regions of greatest divergence are less important for MAGP function, at least in the small mammal.

The mouse Magp transcript appears to be the widespread product of mesenchymal and connective tissue cells. Moreover, our results provide the first evidence of Magp gene expression in atrio-ventricular bulbar cushion tissue, vertebral cartilage, perichondrium and periosteum of endochondral bone, central tendon of the diaphragm, and interstitial connective tissues of the gut and testis. It should be noted, however, that Magp expression may actually be more widespread than we have indicated. Although several tissues were negative after a 2-week exposure to the photographic emulsion (e.g., liver, heart, pericardium, epicardium, spinal cord, and peryventricular brain tissue), they appeared to be positive after a 5-day exposure to Hyperfilm (compare the level of hybridization achieved with anti-sense and sense probes after a 5-day exposure to the film in Fig. 7A). A similar point can be made regarding the placenta and its membranes at days 8.5–9.0 of development. While this experience suggests that direct exposure to Hyperfilm is a sensitive method of transcript detection, the significance is somewhat uncertain, because the results could not be corroborated microscopically. Although the issue should remain open, we have chosen to report all tissues that were negative by microscopic evaluation as negative for Magp expression.

We recently evaluated the expression of the mouse Fbn-1 gene at similar time points in development. Unlike Magp, Fbn-1 gene expression was not significantly above the experimental background in day 9 embryo tissues. The placenta, eoptalcental cone, or placental membranes were also negative. However, the Fbn-1 gene was strongly expressed in maternal tissues just beneath and lateral to the placental implantation site. After day 9, the expression pattern of Fbn-1 resembled that of Magp. Taken together, the tissue in situ hybridization results suggest that both microfibril genes are coordinately expressed in several mouse embryo tissues, but clear differences in tissue-specific expression also exist. These differences in gene expression are of interest, because they suggest that the composition of 10-11nm microfibrils may vary in time and space. This, in turn, may have important implications for microfibril structure and function in normal tissues.

Native microfibrils often appear in the electron microscope as unbranched tubular filaments. Under denaturing conditions, however, a "beads on a string" appearance is observed (Keene et al., 1991). Immunoelectron microscopy studies indicate that the human FBN-1 gene product forms multiple thread-like filaments that contribute to the "string" substructure identified under denaturing conditions. Recent studies
have shown that the FBN-2 gene product co-localizes with the FBN1 gene product in bovine microfibril preparations and that MAGP is located in the "bead" substructure (Gibson, 1993). Given that MAGP is a component of 10-nm microfibrils, it is reasonable to speculate that naturally occurring mutations in MAGP would be associated with a Marfan-like phenotype involving connective tissues such as the dermis, skeletal tissues, and heart valve. In this regard, the identification of MAGP transcripts in atrio-ventricular bulbar cushion tissue is noteworthy. This tissue arises early in heart development through the proliferation and migration of endocardial mesenchyme toward the center of the primitive atrio-ventricular canal, events that signal the first step in the formation of the atrio-ventricular septae and valves. These observations suggest that naturally occurring mutations in MAGP may be associated with alterations in both of these structures.

Our survey of known mouse mutations mapping to the distal portion of chromosome 4 did not reveal any phenotypes affecting the cardiovascular and skeletal systems. The possibility remains, however, that two mouse mutations, Er, repeated epilation, and pf, pupoid fetus (Holbrook et al., 1982; Fisher et al., 1984), could be caused by mutations in the Magp gene. Er and pf have a general and profound influence on epidermal differentiation, including the metabolism of two proteins, filaggrin and keratin, which are important to normal cellular differentiation and tissue architecture. Er/+ heterozygous animals grow a normal appearing first coat until the age of about 13 days, when hair loss begins; repeated growth and re-epilation follow without a definite pattern. In addition, there is a high incidence of multiple cutaneous papillomata and the development of squamous cell carcinoma (Lutzen et al., 1985). Er/Er homozygous animals, on the other hand, die at birth from an inability to breathe because of a closed oral cavity. In day 17 mutant embryos, the skin is extremely thin and smooth with few vibrissae and hair follicles, the snout is truncated, the limbs and tail are greatly shortened and held close to the trunk, the anal and urogenital orifices are closed, and there is cleft palate. Thus, the phenotypes of Er/Er and pf/pf homozygotes are similar, which suggests that Er and pf may be related genes or, perhaps, different mutations in the same gene.

Depending on their nature and location, mutations in Magp that alter the structure or expression of the molecule should have a dominant effect on the organization of microfibrils and the function of the extracellular matrix. A long-term goal is to understand how the MAGP molecule contributes to normal microfibril structure and function. This goal can be accomplished by establishing established mutant mouse strains such as Er and pf as well as mouse strains with targeted null and structural mutations at the Magp locus.

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