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Metaxin, a gene contiguous to both thrombospondin 3 and glucocerebrosidase, is required for embryonic development in the mouse: Implications for Gaucher disease

(homologous recombination/gene targeting/embryonic stem cells)

PAUL BORNSTEIN*†, CINDY E. MCKINNEY‡, MARY E. LAMARCA‡, SUZANNE WINFIELD‡, TETSUJI SHINGU*§, SREELEKHA DEVARAYALU*, HANS L. VOS¶, AND EDWARD I. GINNS‡

*Department of Biochemistry, University of Washington, Seattle, WA 98195; ‡Clinical Neuroscience Branch, National Institute of Mental Health, National Institutes of Health, Bethesda, MD 20892; and ¶Department of Tumor Biology, Netherlands Cancer Institute, 1066 CX Amsterdam, The Netherlands

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ABSTRACT **We have identified a murine gene, metaxin, that spans the 6-kb interval separating the glucocerebrosidase gene (GC) from the thrombospondin 3 gene on chromosome 3E3-F1. Metaxin and GC are transcribed convergently; their major polyadenylylation sites are only 431 bp apart. On the other hand, metaxin and the thrombospondin 3 gene are transcribed divergently and share a common promoter sequence. The cDNA for metaxin encodes a 317-aa protein, without either a signal sequence or consensus for N-linked glycosylation. Metaxin protein is expressed ubiquitously in tissues of the young adult mouse, but no close homologues have been found in the DNA or protein data bases. A targeted mutation (A → G in exon 9) was introduced into GC by homologous recombination in embryonic stem cells to establish a mouse model for a mild form of Gaucher disease. A phosphoglycerate kinase–neomycin gene cassette was also inserted into the 3′-flanking region of GC as a selectable marker, at a site later identified as the terminal exon of metaxin. Mice homozygous for the combined mutations die early in gestation. Since the same amino acid mutation in humans is associated with mild type 1 Gaucher disease, we suggest that metaxin protein is likely to be essential for embryonic development in mice. Clearly, the contiguous gene organization at this locus limits targeting strategies for the production of murine models of Gaucher disease.**

The murine thrombospondin 3 gene (*Thbs3*, here abbreviated as TSP3; refs. 1 and 2), *Muc1*, which encodes a polymorphic epithelial mucin (3, 4), and the glucocerebrosidase gene (*Gba*, here abbreviated as GC; refs. 5 and 6) are closely linked on chromosome 3E3-F1 (H.L.V. and P.B., unpublished results). Thus, *Muc1* is located just 2.3 kb 3′, and GC just 6 kb 5′, to TSP3. TSP3 protein is a member of a family of five homologous, secreted, modular glycoproteins (7, 8). In the mouse, TSP3 protein is present primarily in lung, brain, and cartilage (9), but its function is essentially unknown. Glucocerebrosidase is a lysosomal hydrolase that degrades glucosylceramide; a deficiency of the enzyme is responsible for Gaucher disease, the most common human lysosomal storage disease (10, 11). Despite the tight physical linkage of *Muc1*, TSP3, and GC, the three encoded proteins are not known to participate in common metabolic processes.

The existence of yet another transcription unit at this locus was first deduced from studies of the TSP3 promoter. TSP3–luciferase constructs were moderately active in transiently transfected rat chondrosarcoma cells, but when the orientation of the TSP3 promoter sequence was reversed, luciferase activities increased some 50-fold (P.B. and S.D., unpublished data). This finding suggested the presence of a strong promoter adjacent to the cap site of TSP3 but with a divergent transcriptional orientation. The existence of an expressed gene in the intergenic region between TSP3 and GC was supported by the finding, in the data base, of a 99-bp sequence derived from a mouse cerebellar cDNA clone, Gcap 6 (12), that matched exactly a sequence 1.3 kb upstream from the start of translation of TSP3. This gene,‖ termed metaxin from the Greek μεταξυ (in between), is transcribed in a direction opposite to that of GC, TSP3, and *Muc1*.

In an attempt to establish a mouse model for Gaucher disease, an A → G mutation (resulting in substitution of serine for asparagine) was introduced into exon 9 of murine GC by homologous recombination in embryonic stem (ES) cells. At the same time, a phosphoglycerate kinase (PGK)–neomycin gene cassette was inserted into the 3′-flanking region of GC, disrupting the coding region in the terminal exon of metaxin. Mice homozygous for the combined mutations in GC and metaxin die early during gestation. Since the analogous amino acid substitution in human GC is generally associated with a mild form of Gaucher disease (13) and since mice that lack GC do not die until after birth (14), we suggest that metaxin protein may be required for embryonic development. Metaxin and TSP3 share a common promoter sequence, and the initial nuclear transcripts of metaxin and GC may overlap. If coordinate regulation of expression of either two or all three genes can be shown, such regulation may reflect metabolic interrelationships among GC, metaxin, and TSP3. Some of the data presented in this report have been published in abstract form (15).

MATERIALS AND METHODS

Isolation and Sequence Analysis of Genomic and cDNA Clones. The isolation of genomic clones encompassing GC and metaxin has been described (2, 14). cDNA clones for metaxin were obtained by screening a C57BL/6 mouse (19 weeks) brain cDNA library (Stratagene) with a nick-translated mouse DNA sequence derived from Gcap 6 (12).

DNA sequences were determined by the dideoxy chain-termination method, both manually and by automated sequence analysis with an Applied Biosystems model 373A sequencer. Sequences were determined for both strands and

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Abbreviations: GC, glucocerebrosidase gene; TSP3, thrombospondin 3 gene; ES, embryonic stem; PGK, phosphoglycerate kinase.

†To whom reprint requests should be addressed.
§Present address: First Department of Internal Medicine, Hiroshima University School of Medicine, 1-2-3 Kasumi, Minami-ku, Hiroshima 734, Japan.

‖The sequence reported in this paper has been deposited in the GenBank data base (accession no. L36962).

were analyzed with GENEPRO (Riverside Scientific, Seattle) programs.

Northern Analysis and RNase Protection Assays. Northern blot analysis was performed according to standard procedures. Equal loading and transfer of RNA were verified by staining of the membrane with ethidium bromide. The cDNA probe for metaxin was generated by PCR and consisted of a 507-bp sequence extending from exon 3 to exon 7 (nucleotides 201–707 in Fig. 2). The probe was labeled by nick-translation. RNase protection was performed as described (16). The RNA probe consisted of a 275-bp *Sma* I/*Sma* I genomic fragment of metaxin extending from an *Sma* I site in exon 7 to an *Sma* I site in exon 8 (nucleotides 743 and 938, respectively, in Fig. 2).

Construction of a Targeting Vector. Two single-base changes were introduced into exon 9 of GC by PCR mutagenesis. An A → G transition mutates an asparagine to a serine in the protein, a change analogous to the most frequent mutation observed in patients with Gaucher disease, N370S (17). A T → G transversion 6 bp downstream causes a silent change that introduces a novel *Rsa* I site useful for diagnostic restriction analysis. The targeting vector was constructed by subcloning a 4.1-kb *Ssp* I/*Apa* I fragment containing GC exons 5–11 (with the mutations in exon 9) and the putative polyadenylylation site of GC into the *Xho* I site of pPNT (18). A 2.2-kb *Nco* I/*Bam*HI fragment, 3′ to GC and containing exons 4–7 and part of exon 8 of metaxin, was inserted into the *Bam*HI and *Xba* I sites of the above construct to produce the final targeting vector (see Fig. 4*A*). In this construct, the PGK–neomycin-resistance gene cassette is located 619 bp downstream from the translation termination codon of GC, within a 56-bp deletion in the coding region of metaxin. The construct also contains a flanking PGK–herpes simplex virus–thymidine kinase gene cassette (see Fig. 4*A*).

Transfection of ES Cells, Blastocyst Injection, and Southern Analysis. The targeting vector was linearized at a unique *Not* I site and electroporated into J1 ES cells (kindly provided by R. Jaenisch, Whitehead Institute). Correctly targeted clones were isolated from doubly selected G418-resistant and ganciclovir-resistant ES cells as described (19) and injected into C57BL/6 blastocysts (20). DNA was prepared from ES cells and mouse tails and analyzed by PCR and Southern blotting using standard protocols.

RESULTS

Characterization of Metaxin cDNA and Protein. Gcap6 cDNA (12) was used to screen a mouse brain cDNA library. DNA sequence analysis of isolated clones, in conjunction with sequence analysis of genomic DNA representing the 6-kb intergenic region between GC and TSP3, placed metaxin in the interval between GC and TSP3 (Fig. 1) and established a cDNA sequence of 1084 bp that encodes a protein of 317 aa (Fig. 2). The 3′ end of the cDNA is unambiguously defined by the presence of a consensus polyadenylylation signal, AATAAA, followed 13 nucleotides later by a poly(A) sequence (Fig. 2). However, the transcription start site of metaxin remains to be determined definitively. Although the majority of the cDNA clones extended within 10 bases of the 5′ terminus shown in Fig. 2, preliminary experiments using the 5′ rapid amplification of cDNA ends and S1 nuclease protection procedures indicate heterogeneity in transcriptional start sites for metaxin, with some transcripts containing up to an additional 100 nucleotides of 5′-untranslated region.

The ATG codon proposed for the initiating methionine in metaxin (Fig. 2) is in a relatively strong context (A at −3 and G at +4) as defined by Kozak (21). The conceptual translation product lacks a characteristic hydrophobic leader sequence or consensus sites for N-glycosylation. The amino acid sequence is unusual in that there are several stretches of charged amino acids flanked or interspersed by hydrophobic residues and that the terminal 6 aa are acidic (Fig. 2). All of these features are conserved in the human protein (S.W., E.I.G., and P.B., unpublished data). A comparison of the cDNA and amino acid sequences for metaxin with the DNA (Genbank Release 84) and protein (Genpept Release 84) data bases failed to reveal close homologies. Similarly, no significant matches with described protein structural motifs were found with Prosite (version 9). However, a distant relationship to glutathione *S*-transferases was detected.

Expression of Metaxin in Mouse Tissues. The pattern of expression of metaxin in the adult mouse was examined by both Northern and RNase protection analysis (Fig. 3). Northern analysis revealed a single mRNA of 1.3 kb, a size consistent with the cDNA sequence. Similar levels of metaxin mRNA were detected in all 10 tissues examined, although the level in kidney appeared to be higher than in other tissues (Fig. 3*A*). The results of Northern analyses were confirmed by RNase protection (Fig. 3*B*). Judging from the exon/intron structure of metaxin (Fig. 1; T.S. and P.B., unpublished data), protection of the RNA probe used in these analyses should yield fragments of 20 and 180 bp. Only the larger fragment would be visible on a 6% acrylamide gel, and this is seen in the position of the top arrow in Fig. 3*B*. Specific breakdown products of the 180-bp band are indicated by the lower two arrows. Partial degradation of protected fragments is often encountered in the RNase protection assay (16). Metaxin mRNA was also detected by RNase protection in ES cells and in some, but not all, lines of NIH 3T3 cells (data not shown).

Targeted Disruption of Metaxin. The J1 line of ES cells was electroporated with the targeting vector depicted in Fig. 4*A*. Positive/negative selection was imposed with G418 and ganciclovir. *Sph* I-digested DNA from 384 doubly resistant ES cell

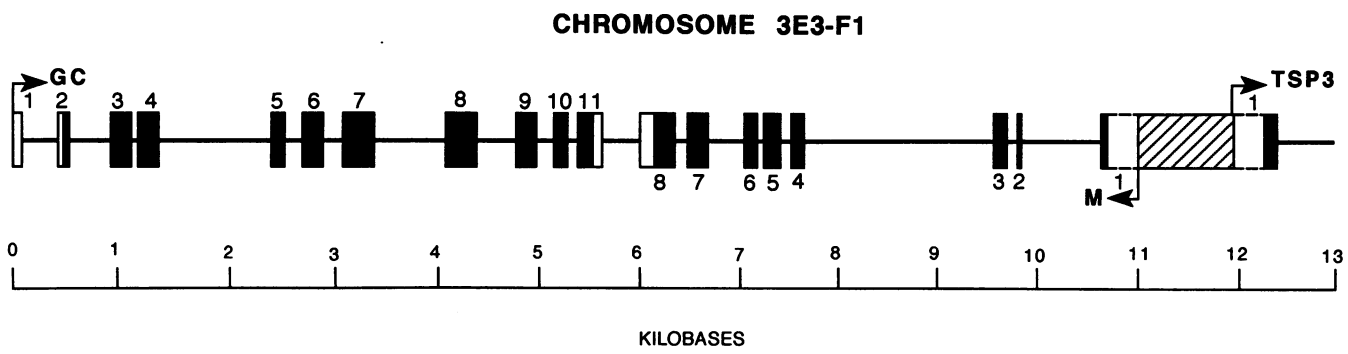

FIG. 1. Exon/intron structure of GC and metaxin (M). Open and solid rectangles depict untranslated and translated exons, respectively. Bent arrows indicate transcription start sites. As indicated by the dashed open rectangles, the precise start sites for metaxin and TSP3 have not been established. GC and metaxin are transcribed convergently. Metaxin and TSP3 are transcribed divergently and share a common promoter sequence, shown as a hatched rectangle.

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      AGA GCG TAC AAG ATG GCG GCG CCC ATG GAG CTG TTC TGC TGG TCA   45
                      M   A   A   P   M   E   L   F   C   W   S

      GGG GGC TGG GGA TTG CCG TCG GTG GAC CTG GAT AGT CTG GCC GTG   90
12    G   G   W   G   L   P   S   V   D   L   D   S   L   A   V

      CTG ACC TAT ACC AGA TTT ACA GGC GCC CCA CTG AAG ATA CAC AAG  135
27    L   T   Y   T   R   F   T   G   A   P   L   K   I   H   K

      ACC AGC AAT CCT TGG CAG AGC CCT TCA GGA ACT CTG CCT GCT CTT  180
42    T   S   N   P   W   Q   S   P   S   G   T   L   P   A   L

      CGA ACC AGT GAT GGG AAA GTC ATT ACA GTG CCA GAC AAG ATC ATC  225
57    R   T   S   D   G   K   V   I   T   V   P   D   K   I   I

      ACC CAT CTT CGT AAA GAG AAG TAT AAT GCC GAC TAC GAT CTG TCA  270
72    T   H   L   R   K   E   K   Y   N   A   D   Y   D   L   S

      GCT CGC CAA GGA GCA GAT ACC CTA GCC TTC ATG TCT CTG CTG GAG  315
87    A   R   Q   G   A   D   T   L   A   F   M   S   L   L   E

      GAG AAA CTA CTG CCT GTG CTA ATC CAT ACT TTT TGG ATA GAC GCC  360
102   E   K   L   L   P   V   L   I   H   T   F   W   I   D   A

      AAG AAC TAT GTG GAA GTG ACC CGA AAG TGG TAT GCA GAG GCT ATG  405
117   K   N   Y   V   E   V   T   R   K   W   Y   A   E   A   M

      CCC TTT CCC CTC AAC TTC TTC CTG CCC GGC CGC ATG CAG CGC CAG  450
132   P   F   P   L   N   F   F   L   P   G   R   M   Q   R   Q

      TAC ATG GAG CGG CTA CAG CTG CTG TGT GGC GAG CAC AAA TCA GAG  495
147   Y   M   E   R   L   Q   L   L   C   G   E   H   K   S   E

      AAC GAG GAG GAA CTA GAA AAA GAG CTA TAC CAA GAG GCT CGG GAG  540
162   N   E   E   E   L   E   K   E   L   Y   Q   E   A   R   E

      TGC CTA ACC CTT CTC TCT CAG CGT CTG GGC TCT CAG AAG TTC TTC  585
177   C   L   T   L   L   S   Q   R   L   G   S   Q   K   F   F

      TTT GGG GAT GCC CCT GCC TCC CTG GAC GCC TTT GTT TTT AGC CAT  630
192   F   G   D   A   P   A   S   L   D   A   F   V   F   S   H

      TTG GCC CTG CTG CTG CAG GCC AAG CTG CCC AGT GGG AAG CTG CAG  675
207   L   A   L   L   L   Q   A   K   L   P   S   G   K   L   Q

      GCC CAC CTT CGG GGG CTG CAC AAC CTC TGC GCC TAC TGC ACC CAC  720
222   A   H   L   R   G   L   H   N   L   C   A   Y   C   T   H

      ATC CTC AAC CTC TAC TTT CCC CGG GAT GGA GAT GAG GTG CCA CTT  765
237   I   L   N   L   Y   F   P   R   D   G   D   E   V   P   L

      CCA CGC CAG ACA CCA GCA GCC CCT GAG ACC GAG GAG GAG CCA TAC  810
252   P   R   Q   T   P   A   A   P   E   T   E   E   E   P   Y

      CGG CGC CGG ACC CAG ATT CTC TCT GTG CTG GCA GGG CTG GCA GCC  855
267   R   R   R   T   Q   I   L   S   V   L   A   G   L   A   A

      ATG [GTG GGC TAT GCC CTG CTC AGT GGC ATC GTT TCT ATC CAG CGG  900
282   M  [V   G   Y   A   L   L   S   G   I   V   S   I   Q   R

      ACA AGC CCT GCT CG[G GCC CCA GGC ACC CGG GCC TTG GGC CTG GCT  945
297   T   S   P   A   R] A   P   G   T   R   A   L   G   L   A

      GAA GAG GAT GAA GAG GAC TGA TGG CGT TCC TGT TCC CAG GAC TGA  990
312   E   E   D   E   E   D   *

      CTT TTC TAC TCG TGC ATT CCA GCT GTC CCT CGT CTC CCC ATG GTT 1035

      GGA GCA GCC AAA AAT GGG GCG CTG TCC TCA G[AA TAA A]CC TGT TTA 1080

      CAC TAA AAA AAA AAA AAA AAA AAA                            1104
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FIG. 2. cDNA and translated amino acid sequence for metaxin. The 5′-untranslated region is incomplete. Exon/intron junctions are indicated by open triangles, cysteine residues are underlined, and the polyadenylylation signal sequence, AATAAA, is boxed. The 56-bp deletion, which represents the site of insertion of the PGK–neomycin resistance gene (see Fig. 4*A*) is indicated by square brackets.

clones was screened by Southern analysis with probe HN (Fig. 4*A*). Twelve clones had undergone homologous recombination as judged by the presence of an additional 5.4-kb band (Fig. 4*B*), yielding a targeting efficiency of 3.1%. Of these 12 clones, 6 were found by PCR and sequence analysis to have retained a second point mutation that was present in exon 9 of GC in the targeting construct (Fig. 4*D*).

Blastocysts, obtained from C57BL/6 mice, were injected with correctly targeted ES cells and were transferred to pseudopregnant Swiss NIH or (B6 × CBA)F_1 foster mice. Injection of two of four targeted ES cell lines resulted in the germ-line transmission of the mutation. A total of 292 offspring from matings of heterozygous (G^sM^Δ/G^+M^+) animals (G^s indicates an allele of the GC gene coding for serine in place of asparagine in amino acid position 370; M^Δ indicates a disrupted metaxin allele; G^+ and M^+ are the respective wild-type alleles) were analyzed by Southern analysis of *Sph* I-digested tail DNA. Of these, 40% were wild type and 60% were heterozygous for the targeted mutation. No homozygous (G^SM^Δ/G^SM^Δ) animals were found. Caesarean section of pregnant mice 9 days postconception revealed sites of resorption interspersed among developing embryos. We conclude that disruption of metaxin leads to early fetal mortality. mRNA levels for metaxin were reduced by ≈50% in both G^+M^+/G^SM^Δ and $G^\Delta M^+/G^SM^\Delta$ mice (Table 1). Disruption of metaxin in the coding region of exon 8 (Figs. 2 and 4*A*) therefore appears to be incompatible with formation of a stable mRNA. However, metaxin mRNA levels were normal in type 2 Gaucher mice ($G^\Delta M^+/G^\Delta M^+$) generated by targeted disruption of GC (Table 1).

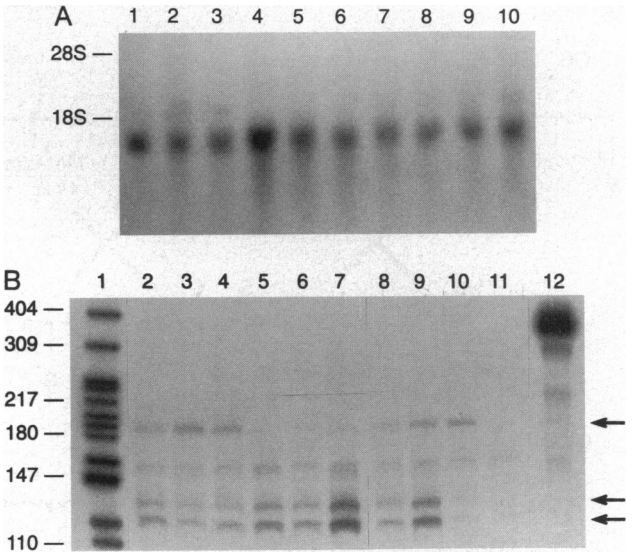

FIG. 3. Distribution of metaxin mRNA in adult mouse tissues. (*A*) Northern analysis. Twenty micrograms of total RNA was fractionated on a 1.2% agarose gel, transferred to a nitrocellulose membrane, and hybridized with a ^{32}P-labeled cDNA probe for metaxin extending from exon 3 to exon 7. Lane 1, skin; lane 2, spleen; lane 3, heart; lane 4, kidney; lane 5, brain; lane 6, liver; lane 7, lung; lane 8, muscle; lane 9, bone; lane 10, thymus. The autoradiogram was exposed for 3 days. (*B*) RNase protection analysis. Twenty micrograms of total RNA was hybridized with a ^{32}P-labeled antisense RNA probe for metaxin. After RNase digestion, ethanol-precipitable RNA was analyzed by electrophoresis in a 6% acrylamide denaturing gel. Lane 1, *Msp* I digest of pBR322 as a molecular weight standard (in bp); lane 2, bone; lane 3, skin; lane 4, tail; lane 5, spleen; lane 6, heart; lane 7, kidney; lane 8, lung; lane 9, liver; lane 10, muscle; lane 11, no RNA control; lane 12, metaxin RNA probe. The top arrow points to a protected band of 180 bp, and the lower two arrows to specific breakdown products.

The mating of $G^+M^+/G^\Delta M^+$ and G^+M^+/G^SM^Δ mice leads to the generation of $G^\Delta M^+/G^SM^\Delta$ heterozygous animals. Surprisingly, these animals die within 24 h after birth and have ≈5% of control glucocerebrosidase enzyme activity and markedly reduced protein by Western blot analysis.

DISCUSSION

We have mapped and characterized metaxin and its conceptual translation product. During the introduction of a point mutation into GC by homologous recombination in ES cells, we placed a neomycin-resistance gene in the 3′-flanking region of GC. The neomycin-resistance gene was subsequently shown to be located in the coding region of the terminal exon in metaxin and presumably interferes with the processing and/or stability of the altered metaxin transcript, since metaxin mRNA levels are reduced by 50% in mice that are heterozygous for the mutation. Mice that are homozygous for this targeted insertion die before birth. Blastocyst implantation occurs, since sites of resorption were detected in the uteri of mice examined during the 9th day of embryonic development. Presumably, G^SM^Δ/G^SM^Δ mice die between day 4 and day 9 of gestation.

The phenotype of the G^SM^Δ/G^SM^Δ mice generated in this study may be complicated by the additional presence of a point mutation (A1226G) in exon 9 of GC and by a possible effect of the insertional mutation in metaxin, which is located in the 3′ flank of GC, on the processing, stability, and/or translocation of GC pre-mRNA. Such an effect is suggested by the perinatal lethal phenotype and lower than expected glucoce-

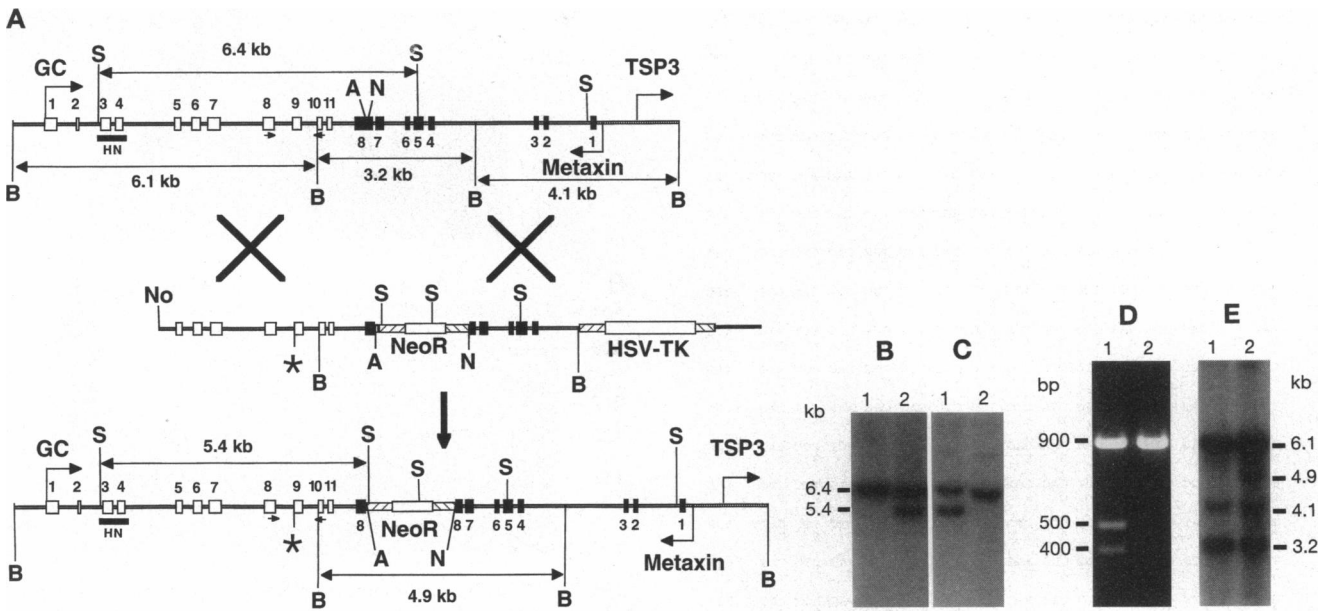

FIG. 4. Strategy for targeting GC and metaxin as well as genomic DNA analyses of resulting ES cells and mice. (*A Top*) Wild-type locus. (*A Middle*) Targeting vector linearized at a unique *Not* I (No) site. (*A Bottom*) Mutated locus. Exons 1–11 of GC are shown as open rectangles, and exons 1–8 of metaxin are shown as solid rectangles. The approximate transcription starts of GC, metaxin, and TSP3 are indicated by bent arrows. The point mutation resulting in the substitution of serine for asparagine in exon 9 of GC is indicated by an asterisk. The neomycin-resistance gene (NeoR) is inserted into exon 8 of metaxin, within a 56-bp deletion of genomic DNA between *Apa* I (A) and *Nco* I (N) sites. The bar labeled HN represents a 325-bp fragment spanning exons 3 and 4 of GC, which was used as a probe to identify correct gene targeting events. Primers in exons 8 and 10 of GC, indicated by arrows, were used to generate a 900-bp fragment that contained a new *Rsa* I site in the mutated locus (see *Materials and Methods*). Sites for *Bam*HI (B) and *Sph* I (S) are shown. Predicted sizes, in kb, of *Sph* I-digested fragments from the wild-type and mutated loci are given. HSV, herpes simplex virus; TK, thymidine kinase. (*B* and *C*) Southern blot analysis of *Sph* I-digested genomic DNA isolated from a normal and targeted ES cell clone and from the progeny of germ-line chimeras generated from a correctly targeted clone, respectively. (*B*) Lane 1, normal ES cells; lane 2, targeted ES cell line. (*C*) Lane 1, heterozygous offspring with a targeted mutation; lane 2, normal littermate. Probe HN (*A*) was used. (*D*) PCR analysis of DNA from germ-line progeny showing the presence of a new *Rsa* I site in mutant DNA. Genomic DNA was PCR-amplified using the primers shown in *A*, and the resultant 900-bp fragment was digested with *Rsa* I. Lane 1, heterozygous mutant offspring; lane 2, normal littermate. (*E*) Southern blot analysis of *Bam*HI-digested genomic DNA from progeny of chimeras. Blots were probed with both GC and metaxin cDNA probes. Lane 1, normal offspring; lane 2, heterozygous offspring. As predicted by the restriction map in *A*, the normal allele yields bands of 6.1, 4.1, and 3.2 kb, and the mutant allele yields bands of 6.1, 4.9, and 3.2 kb.

rebrosidase activity of $G^{\Delta}M^{+}/G^{S}M^{\Delta}$ animals. We believe that the point mutation, *per se*, does not contribute significantly to the embryonic lethality of these mice, since the corresponding point mutation in humans is generally associated with mild type 1 Gaucher disease (13). Furthermore, GC knockout mice ($G^{\Delta}M^{+}/G^{\Delta}M^{+}$) survive until shortly after birth (14). We have generated chimeric mice that have a disruption of metaxin but in which GC is intact, and germ-line transmission of metaxin disruption has been obtained. As mice homozygous only for metaxin disruption become available, we should be able to determine the contribution of the point mutation to the phenotype of $G^{S}M^{\Delta}/G^{S}M^{\Delta}$ mice. However, a clear demonstration of the role of metaxin in embryonic development will also require the generation of a more subtle targeted mutation that does not compromise the function of GC.

Table 1. mRNA levels for murine metaxin determined by Northern blot analysis

Genotype	No. of mice	Metaxin mRNA
$G^{+}M^{+}/G^{+}M^{+}$ (wt)	8	100
$G^{+}M^{+}/G^{\Delta}M^{+}$	7	107
$G^{\Delta}M^{+}/G^{\Delta}M^{+}$	4	94
$G^{+}M^{+}/G^{S}M^{\Delta}$	6	48
$G^{\Delta}M^{+}/G^{S}M^{\Delta}$	2	40

Metaxin mRNA levels were determined by densitometry and normalized for mRNA levels of glyceraldehyde-3-phosphate dehydrogenase, determined on the same blot. The value given is percent of wild type (wt).

The nature of the protein encoded by metaxin, its cellular location, and function remain unknown. The putative translation product of metaxin cDNA (Fig. 2) lacks a signal peptide or a consensus sequence for N-glycosylation and is therefore likely to be an intracellular protein. The protein also lacks an obvious hydrophobic transmembrane sequence, nuclear localization signal, and other consensus targeting sequences. Since metaxin mRNA is relatively abundant in most adult tissues (Fig. 3), the protein is unlikely to be a transcription factor.

The proximity of the polyadenylylation signals for GC and metaxin (Fig. 1) raises the possibility that the expression of the two genes may be regulated coordinately at the RNA level, perhaps by an antisense RNA mechanism. While the mature transcripts for GC and metaxin do not overlap, the nuclear pre-mRNAs of these convergently transcribed genes could overlap (22). Furthermore, if the two genes are transcribed concurrently, the formation of nuclear antisense RNA could lead to instability and/or targeting for degradation of one or both mRNAs (23, 24). Several examples of eukaryotic antisense transcripts have been reported (25, 26), and in some cases the formation of such RNA·RNA duplexes may have regulatory consequences (24, 27, 28).

Metaxin and TSP3 are transcribed divergently and share a common promoter sequence (Fig. 1). Experiments are needed to determine whether any transcriptional elements in this sequence function in a bidirectional manner. Bidirectional promoters are characteristically found in CpG-rich islands (29, 30). The DNA sequence of the 650 nucleotides upstream from the translation start site of metaxin is highly G+C-rich (71%) and contains 65 CpG dinucleotides (M. Collins and P.B.,

unpublished data). Furthermore, this region contains a sequence of 140 bp that is 91% identical between mouse and human. This highly conserved sequence is not present in transcripts of either metaxin or TSP3 and could represent a part of the common promoter of the two genes. Bidirectional promoters can regulate the expression of metabolically or structurally related genes (31, 32). However, metaxin is widely expressed while the distribution of TSP3 mRNA is largely limited, in the developing mouse, to brain, lung, and cartilage (9). An assessment of the metabolic interactions and function of metaxin during mouse development, as well as its implications for Gaucher disease, will require a more complete characterization of metaxin expression.

Note Added in Proof. We have now generated heterozygous mice that have an insertional mutation in exon 8 of metaxin but lack the point mutation in exon 9 of GC. Matings of these mice have yielded eight litters and 44 offspring. Southern analysis of tail DNA indicates that 19 of the animals (43%) are wild type and 25 (57%) are heterozygotes. We can therefore conclude that the point mutation in GC does not contribute to the embryonic lethal phenotype of M^{Δ}/M^{Δ} mice.

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