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Vgr-1, a mammalian gene related to *Xenopus Vg-1*, is a member of the transforming growth factor β gene superfamily

(embryogenesis/growth factors/mouse)

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ABSTRACT **The transforming growth factor β (TGF-β)-related products of the *Xenopus Vg-1* and *Drosophila* decapentaplegic (DPP) genes have been implicated in the control of growth and differentiation during embryogenesis. We have isolated a mouse cDNA, Vgr-1, that encodes a polypeptide structurally related to *Xenopus* Vg-1. Sequence comparisons indicate that the Vgr-1 protein belongs to a family of DPP-like gene products within the TGF-β superfamily. The levels of Vgr-1 RNA were determined in embryos and tissues isolated at various stages of development. A 3.5-kilobase mRNA increases throughout development and into adulthood in many tissues and in F9 teratocarcinoma cells differentiating into endoderm in response to retinoic acid and cAMP. The amino acid homologies and patterns of expression suggest that, like the DPP gene product, Vgr-1 plays a role at various stages of development.**

Vertebrate embryogenesis proceeds through a series of induction events in which signaling molecules produced by one cell population influence the developmental fate and morphogenesis of neighboring cells (1–3). A major goal is to identify these signals and to understand how they stimulate or inhibit proliferation or differentiation of target cells. Recent work has identified growth factor-like molecules related to fibroblast growth factor, platelet-derived growth factor, and the interleukins as potential embryonic regulators (4–8). There is also evidence that proteins with structural similarity to transforming growth factor β (TGF-β) may play important roles in development, as exemplified by studies on Müllerian inhibiting substance (MIS) (9), the gene product of the *Drosophila* decapentaplegic complex (DPP) (10–12), the *Vg-1* gene product of *Xenopus laevis* (13, 14), and the activity of TGF-β2 in mesodermal induction in *Xenopus* (15, 16). Here we report the isolation and cDNA sequence of a murine member of the TGF-β superfamily, closely related to *Xenopus* Vg-1. This gene product, which we named Vgr-1 for "Vg-1-related" protein, helps to define a distinct subfamily of TGF-β-related molecules that includes Vgr-1, Vg-1, DPP, and bone morphogenic proteins (BMP-2a, -2b, -3). In contrast to *Xenopus* Vg-1 transcripts, which are maternally inherited and spatially restricted to the vegetal endoderm and decline dramatically after gastrulation (14, 17), mouse Vgr-1 RNA is present in a variety of embryonic, neonatal, and adult tissues. This suggests that, like *Drosophila* DPP, Vgr-1 plays a role at different stages throughout development.‡

METHODS

Isolation of cDNAs. Approximately 8×10^5 phage plaques of a λgt10-based cDNA library derived from 8.5-day post coitum (dpc) mouse embryos were hybridized with a ^{32}P-labeled partial *X. laevis* Vg-1 cDNA under low-stringency conditions as described (18). This cDNA corresponded to the segment from nucleotide 210 to 1755 in ref. 14. A 250-base-pair (bp) cDNA that was isolated in this way was then used to rescreen the library under high-stringency conditions (18). The cDNA fragments were subcloned into M13 derivatives (19) and were sequenced by the dideoxy sequencing methods (20).

Northern Hybridization. Embryos and tissues from mice of the outbred ICR strain were harvested at different stages of development as indicated in the legends to Figs. 3 and 4. Noon of the day on which the vaginal plug was observed was considered as 0.5 dpc. All extraembryonic membranes were removed from the embryos. F9 teratocarcinoma cells were cultured on gelatinized tissue culture dishes in Dulbecco's modified Eagle's medium with 10% fetal bovine serum. Cells were induced to differentiate into parietal endoderm as described (21). Twenty micrograms of total RNA, isolated as described (22), was electrophoresed in 1% agarose/formaldehyde gel, transferred to GeneScreen nylon membrane (NEN), and crosslinked by UV irradiation (23). An 1810-bp *Eco*RI fragment from a Vgr-1 cDNA clone, corresponding to bp 175–1985 in Fig. 1, was ^{32}P-labeled by random primer extension and used as a hybridization probe (2×10^6 cpm/ml) under the following conditions: prehybridizations and hybridizations were at 65°C in 0.5 M sodium phosphate buffer, pH 7.2/7% SDS/5 mM EDTA. The filter was washed in 0.04 M sodium phosphate buffer, pH 7.2/1% SDS (twice for 15 min at room temperature and for 30–60 min at 65°C) and exposed to Kodak XAR-5 film with an intensifying screen at −70°C. Identical results were obtained with a probe consisting of an *Eco*RI–*Sac* I fragment from a Vgr-1 cDNA clone, corresponding to bp 175–1046 in Fig. 1, and also when the final wash was carried out in 15 mM NaCl/1.5 mM sodium citrate, pH 7, at 65°C for 1 hr (24). The filters were rehybridized at 2×10^6 cpm/ml with a plasmid containing a 280-bp insert from a murine glyceraldehyde-3-phosphate dehydrogenase (G3PD) cDNA or with a 1.5-kbp α-actin cDNA. RNA size markers were from Bethesda Research Laboratories.

RESULTS AND DISCUSSION

A murine cDNA library derived from 8.5-dpc embryos was screened by hybridization at low stringency with a partial *Xenopus* Vg-1 cDNA coding sequence. This allowed the isolation of a 250-bp cDNA, which in turn was used to rescreen the same library at high stringency. Twenty-four

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Abbreviations: TGF-β, transforming growth factor β; BMP, bone morphogenic protein; DPP, decapentaplegic; MIS, Müllerian inhibiting substance; G3PD, glyceraldehyde-3-phosphate dehydrogenase; dpc, day(s) post coitum.

‡The sequence reported in this paper is being deposited in the EMBL/GenBank data base (accession no. J04566).

cDNAs were identified in this way from a total of 800,000 recombinant phage plaques. The nucleotide sequences of two cDNAs were combined to yield the sequence of the Vgr-1 cDNA shown in Fig. 1. This sequence is 1985 nucleotides long and encodes a 440-amino acid polypeptide. Although the first ATG was assigned as the putative initiator codon, it is also possible that the methionine residue at position 4 corresponds to the N terminus of the precursor. The cDNA does not include the entire 3′ untranslated region of the Vgr-1 transcript, since there is no recognizable polyadenylylation site and the sequence ends with an internal *Eco*RI recognition site. The 5′ untranslated sequence is also incomplete, but the putative initiator codon is preceded by two in-frame stop codons. The derived amino acid sequence has a recognizable N-terminal signal peptide with a hydrophobic core (26, 27), but it is not known at what residue the signal peptide is cleaved from the rest of the polypeptide. There are several interesting structural features in the polypeptide sequence.

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                                                    10                                  20
                  Met Arg Lys Met Gln Lys Glu Ile Leu Ser Val Leu Gly Pro Pro His Arg Pro Arg Pro Leu
    1 CCTTGACCGTAAGAC ATG AGG AAG ATG CAA AAG GAG ATC CTG TCG GTG CTG GGG CCC CCG CAC AGG CCG CGG CCC CTG

                                        30                                  40
     His Gly Leu Gln Gln Pro Gln Pro Pro Val Leu Pro Pro Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln Thr Ala
   79 CAC GGT CTC CAG CAG CCT CAG CCC CCG GTG CTC CCG CCA CAG CAG CAG CAG CAG CAG CAG CAG CAG ACG GCC

              50                                    60                                  70
     Arg Glu Glu Pro Pro Pro Gly Arg Leu Lys Ser Ala Pro Leu Phe Met Leu Asp Leu Tyr Asn Ala Leu Ser Asn
  154 CGC GAG GAG CCC CCT CCA GGG CGG CTG AAG TCC GCT CCA CTC TTC ATG CTG GAT CTC TAC AAC GCC CTG TCC AAT

                                        80                                  90
     Asp Asp Glu Glu Asp Gly Ala Ser Glu Gly Val Gly Gln Glu Pro Gly Ser His Gly Gly Ala Ser Ser Ser Gln
  229 GAC GAC GAA GAG GAT GGG GCA TCG GAG GGT GTG GGG CAA GAG CCT GGG TCC CAC GGA GGG GCC AGC TCG TCC CAG

              100                                   110                                 120
     Leu Arg Gln Pro Ser Pro Gly Ala Ala His Ser Leu Asn Arg Lys Ser Leu Leu Ala Pro Gly Pro Gly Gly Gly
  304 CTC AGG CAG CCG TCT CCC GGC GCT GCA CAC TCC TTG AAC CGC AAG AGT CTC CTG GCC CCG GGA CCC GGT GGC GGT

                                        130                                 140
     Ala Ser Pro Leu Thr Ser Ala Gln Asp Ser Ala Phe Leu Asn Asp Ala Asp Met Val Met Ser Phe Val Asn Leu
  379 GCG TCC CCA CTG ACT AGC GCG CAG GAC AGC GCT TTC CTC AAC GAC GCG GAC ATG GTC ATG AGC TTT GTG AAC CTG

              150                                   160                                 170
     Val Glu Tyr Asp Lys Glu Phe Ser Pro His Gln Arg His His Lys Glu Phe Lys Phe Asn Leu Ser Gln Ile Pro
  454 GTG GAG TAC GAC AAG GAG TTC TCC CCA CAT CAA CGA CAC CAC AAA GAG TTC AAG TTC AAC CTA TCC CAG ATT CCT

                                        180                                 190
     Glu Gly Glu Ala Val Thr Ala Ala Glu Phe Arg Val Tyr Lys Asp Cys Val Val Gly Ser Phe Lys Asn Gln Thr
  529 GAG GGT GAG GCG GTG ACG GCT GCT GAG TTC CGC GTC TAC AAG GAC TGT GTG GTG GGG AGT TTT AAA AAC CAA ACC

              200                                   210                                 220
     Phe Leu Ile Ser Ile Tyr Gln Val Leu Gln Glu His Gln His Arg Asp Ser Asp Leu Phe Leu Leu Asp Thr Arg
  604 TTT CTT ATC AGC ATT TAC CAA GTC TTG CAG GAG CAT CAG CAC AGA GAC TCT GAC CTA TTT TTG TTG GAC ACC CGG

                                        230                                 240
     Val Val Trp Ala Ser Glu Glu Gly Trp Leu Glu Phe Asp Ile Thr Ala Thr Ser Asn Leu Trp Val Val Thr Pro
  679 GTG GTG TGG GCC TCA GAA GAA GGT TGG CTG GAA TTT GAC ATC ACA GCA ACT AGC AAT CTG TGG GTG GTG ACA CCG

              250                                   260                                 270
     Gln His Asn Met Gly Leu Gln Leu Ser Val Val Thr Arg Asp Gly Leu His Val Asn Pro Arg Ala Ala Gly Leu
  754 CAG CAC AAC ATG GGG CTC CAG CTG AGT GTG GTG ACT CGG GAT GGA CTC CAC GTC AAC CCC CGT GCG GCG GGC CTG

                                        280                                 290
     Val Gly Arg Asp Gly Pro Tyr Asp Lys Gln Pro Phe Met Val Ala Phe Phe Lys Val Ser Glu Val His Val Arg
  829 GTG GGC AGA GAC GGC CCT TAC GAC AAG CAG CCC TTC ATG GTG GCC TTC TTC AAG GTG AGC GAG GTC CAC GTG CGC

              300              + + +                310                                 320
     Thr Thr Arg Ser Ala Ser Ser Arg Arg Arg Gln Gln Ser Arg Asn Arg Ser Thr Gln Ser Gln Asp Val Ser Arg
  904 ACC ACC AGG TCA GCC TCC AGT CGG CGG CGG CAG CAG AGT CGC AAC CGG TCC ACC CAG TCG CAG GAC GTG TCC CGG
                                  330                                 340
     Gly Ser Gly Ser Ser Asp Tyr Asn Gly Ser Glu Leu Lys Thr Ala Cys Lys Lys His Glu Leu Tyr Val Ser Phe
  979 GGC TCC GGT TCT TCA GAC TAC AAC GGC AGT GAG TTA AAA ACA GCT TGC AAG AAG CAT GAG CTC TAT GTG AGC TTC

              350                                   360                                 370
     Gln Asp Leu Gly Trp Gln Asp Trp Ile Ile Ala Pro Lys Gly Tyr Ala Ala Asn Tyr Cys Asp Gly Glu Cys Ser
 1054 CAG GAC CTG GGA TGG CAG GAC TGG ATC ATT GCA CCC AAA GGC TAC GCT GCC AAC TAC TGT GAT GGA GAG TGT TCC

                                  380                                 390
     Phe Pro Leu Asn Ala His Met Asn Ala Thr Asn His Ala Ile Val Gln Thr Leu Val His Leu Met Asn Pro Glu
 1129 TTC CCA CTC AAC GCA CAC ATG AAT GCC ACC AAC CAC GCC ATT GTA CAG ACC TTG GTC CAC CTT ATG AAT CCC GAG

              400                                   410                                 420
     Tyr Val Pro Lys Pro Cys Cys Ala Pro Thr Lys Leu Asn Ala Ile Ser Val Leu Tyr Phe Asp Asp Asn Ser Asn
 1204 TAC GTC CCC AAA CCA TGC TGC GCA CCA ACC AAA CTG AAT GCC ATC TCG GTT CTT TAC TTC GAT GAT AAC TCC AAT

                                  430
     Val Ile Leu Lys Lys Tyr Arg Asn Met Val Val Arg Ala Cys Gly Cys His
 1279 GTC ATC TTG AAA AAG TAC AGG AAT ATG GTC GTG AGA GCT TGT GGT TGC CAT TAA  GTTGAAGCTGGTGTGTGTGTGTGGGTG

 1360 GGGGCATGGTTCTGCCTTGGATTCCTAACAACAACATCTGCCTTAAACCACGAACAACAGCACAGCGAAGCGGGATGGTGACACACAGAGGGATCGTGAC

 1460 ACGCAGACACATCTCCCGCTGGTGCCTTACCCACGGAGGCTTTTATGAGGACCTTGTCAAGGGCTTTCCCAGTTCCTAACTGAGCAGTTGCTGGTCTGCA

 1560 GGAAGCTGGAAGGCTTGTAGTACAGGCCTGGAAACTGCAGTTACCTAATGTTCGCCTCCCCCAACCCCGCCCGGAGTAGTTTTAGCTTTTAGATCTAGCT

 1660 GCTTGTGGTGTAAGTAAACTTGAAGGAATATTAAATATCCCTGGGTTGAAAGACCCGGTGGTGGCTCTACAGCACCCATCCCAGGGAGATTTTTGCAGAC

 1760 ATCCGAATGGAGGGGAGAAGGGCACTCTTTCAGGTTCCATTCCCAGCAAGGGCAGCTCACACAGGACCTGCAGCCTGGCCATCAGCAGGCTCTGTGGAGG

 1860 TGCCTTCTGTCTACTGTTGTAGTTACGTGTTTTGTGTTGACTCTCGGTGGTGTGAGAATGTACTAATCTCTGTCAAGACAAACTGTAGCATTTCCACCCC

 1960 ATCCTCCTCCCTCCCTCACAGAATTC
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FIG. 1. Nucleotide sequence and deduced amino acid sequence of the mouse Vgr-1 cDNA. The 132-amino acid C-terminal segment is preceded by three arginine residues (+++) that could represent a proteolytic cleavage site. The potential sites for N-glycosylation (Asn-Xaa-Ser/Thr; ref. 25) are overlined. The cysteine residues are shaded. The sequence shown combines the sequences of λvgr14/15 and λvgr3. The cDNA sequence of the first 165 nucleotides was derived from λvgr3 only, while the rest of the coding sequence was present in both cDNAs and their sequences were in agreement.

First, the C-terminal sequence beginning at residue 338 contains seven cysteines, similar to the *Xenopus* Vg-1 sequence (14) and highly conserved among all other members of the TGF-β superfamily. The sequence of the C-terminal 120 amino acids is 59% identical with the corresponding sequence in the Vg-1 precursor polypeptide. A second feature common to many members of the TGF-β superfamily is the presence of a cluster of basic residues preceding the conserved C-terminal region. Three arginines at positions 304–306 precede the conserved C terminus of the Vgr-1 polypeptide sequence (Fig. 1), while the C-terminal third of the *Xenopus* Vg-1 gene product is preceded by four basic residues. By analogy with TGF-β (28), proteolytic cleavage of Vgr-1 may take place following these basic residues, resulting in the release of a polypeptide of 132 residues containing three potential N-glycosylation sites. However, there is at present no evidence that the *Xenopus* Vg-1 or the *Drosophila* DPP protein undergoes such proteolytic cleavage. Again by analogy with TGF-β and several other members of the family, the final and functional Vgr-1 product is probably a homodimer of the C-terminal segments of the Vgr-1 precursors, if indeed this cleavage takes place. Finally, a unique feature of the mouse Vgr-1 sequence is the stretch of 10 glutamine residues starting at position 35. These are encoded by a nucleotide sequence identical to the *opa* repeat found in a number of developmentally regulated *Drosophila* transcripts and translated into glutamine repeats in proteins such as fushi tarazu, engrailed, and Notch (29, 30). This repeat is also found in the sequence of murine interleukin 2 (31). The function of this glutamine motif is unknown.

Fig. 2 compares the C-terminal portion of the mouse Vgr-1 polypeptide with other members of the TGF-β superfamily, aligned by the seven highly conserved cysteine residues. The highest degree of similarity is with *Xenopus* Vg-1, *Drosophila* DPP, and BMP-2a, -2b, and -3 (59%, 57%, 61%, 59%, and 44%, respectively). By comparison, the C-terminal region of the Vgr-1 protein shows only 34% similarity with mature TGF-β1. The amino acid sequences in the C-terminal regions

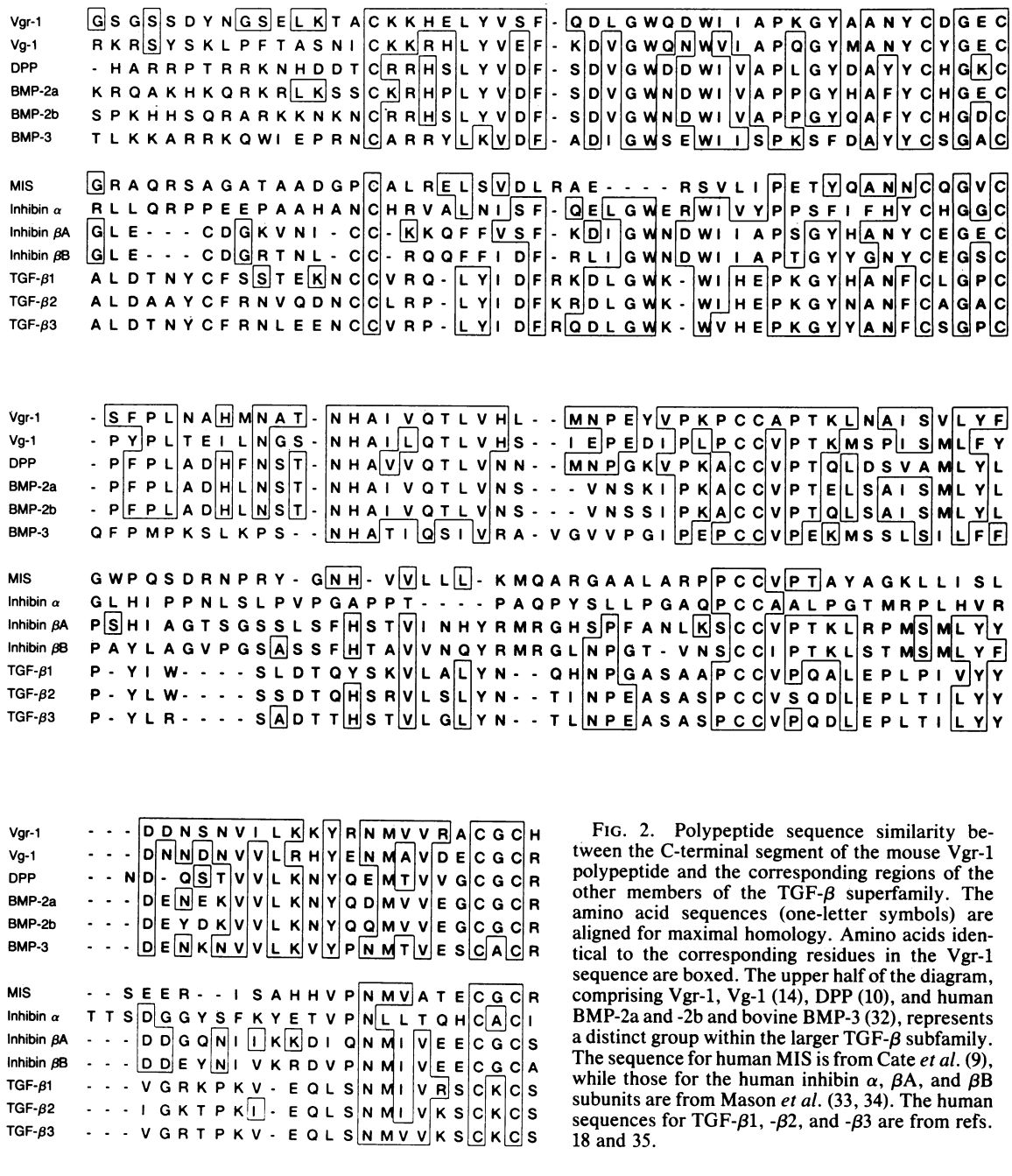

FIG. 2. Polypeptide sequence similarity between the C-terminal segment of the mouse Vgr-1 polypeptide and the corresponding regions of the other members of the TGF-β superfamily. The amino acid sequences (one-letter symbols) are aligned for maximal homology. Amino acids identical to the corresponding residues in the Vgr-1 sequence are boxed. The upper half of the diagram, comprising Vgr-1, Vg-1 (14), DPP (10), and human BMP-2a and -2b and bovine BMP-3 (32), represents a distinct group within the larger TGF-β subfamily. The sequence for human MIS is from Cate *et al.* (9), while those for the human inhibin α, βA, and βB subunits are from Mason *et al.* (33, 34). The human sequences for TGF-β1, -β2, and -β3 are from refs. 18 and 35.

of Vgr-1, Vg-1, DPP, and the BMPs that are located between the third and fourth cysteines and the sequences preceding the sixth cysteines are highly conserved when compared with the other members of the TGF-β superfamily (Fig. 2). From this comparison it appears that these proteins form a distinct subgroup within the TGF-β superfamily. The degree of divergence between the N-terminal regions of the mouse Vgr-1 and *Xenopus* Vg-1 sequences (only 16% similarity) is in contrast to the interspecies conservation seen in the N-terminal regions of TGF-β (18, 35). This suggests that Vgr-1 is not the mouse homolog of *Xenopus* Vg-1 and that other genes more closely related to Vg-1 may be present in the mammalian genome.

There is evidence that the other members of this DPP subfamily identified above play a role in embryonic development. Transcripts of the *Drosophila* DPP gene are expressed in the embryo, larva, and adult, in a variety of tissues, including mesoderm and gut endoderm as well as dorsal ectoderm (11). Both dominant haplolethal and recessive mutations have been identified with pleiotropic effects on embryo morphogenesis (12). The *Xenopus* Vg-1 gene encodes a maternally inherited mRNA that is localized to the vegetal hemisphere. This localization, and the dramatic decline in RNA abundance after gastrulation, has led to the suggestion that the Vg-1 polypeptide plays a role in mesodermal induction (14). However, there is no direct evidence as yet for such a role. BMP-2a, -2b, and -3 are thought to play a role in the differentiation of mesodermal cells during chondrogenesis and osteogenesis, but again their mode of action is not known (32). In view of presumed functions of these other members of the DPP subfamily, it seems likely that the Vgr-1 mRNA encodes a mammalian factor involved in tissue differentiation and development.

As a first step toward defining the function(s) of Vgr-1 in mouse development, we have followed the distribution of transcripts by Northern hybridization of RNA isolated from a variety of embryonic, neonatal, and adult tissues. A single mRNA species of ≈3.5 kb can be detected in mouse embryos from 8.5 dpc on (Fig. 3). It is likely that the mRNA levels increase with the age of the embryo. Vgr-1 transcripts are not, however, confined to the embryo proper but have also been found in extraembryonic tissues, including placenta and the visceral yolk sac (endoderm plus mesoderm) from 10.5 dpc onwards, and the amnion from 14.5 dpc (Fig. 4 *A* and *D* and data not shown). In addition, there are low levels of Vgr-1 expression in the pregnant uterus and the maternal decidual tissue surrounding the embryo (Fig. 4*B*). In a number of different organ systems the level of Vgr-1 RNA varies significantly with stage of development. For example, the level increases in both kidney and lung during embryogenesis and postnatally (Fig. 4*A*), and a smaller relative increase is also seen in the heart from 12.5 dpc through day 3 after birth (data not shown). Relatively high levels of Vgr-1 RNA are found in embryonic and postnatal brain (Fig. 4*B*), in adult skeletal muscle, and in developing whole limbs (Fig. 4*C*). The abundance of the Vgr-1 transcripts in isolated calvaria suggests that the gene is expressed at high levels in osteogenic cells (Fig. 4*C*). In all of these tissues and in ovary, skin, and adrenal gland (Fig. 4), only a 3.5-kb Vgr-1 transcript is seen. However, in the testis a 1.8-kb RNA is also present at about the same level as the larger transcript. Although Vgr-1 expression has a widespread distribution, some tissues [e.g., liver (Fig. 4*B*)] show only low or undetectable levels of RNA. We have also surveyed poly(A)$^+$ RNA from a wide variety of cultured normal and neoplastic mouse and human cell lines of mesodermal, ectodermal, and endodermal origin and have found significant levels of the 3.5-kb Vgr-1 mRNA in only three, namely the NRK 49F fibroblast cell line, the human fibrosarcoma HT1080, and murine PYS (parietal endoderm) cells (Fig. 5). The cell lines that scored negative for Vgr-1 mRNA were AKR-2B, BSC-1, A-431, SW480, SW620, 58MCA, 10T½, and BALB/c 3T3. Two to five micrograms of poly(A)$^+$ RNA was analyzed and autoradiography was for 7 days (data not shown). No expression was detected in mouse WEHI-3

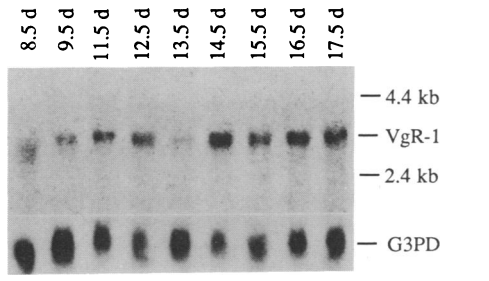

FIG. 3. Northern blot analysis of RNA from mouse embryos at different stages of development. The age of the embryos is given (dpc) above each lane. Autoradiographic exposures of the Vgr-1 and the G3PD Northern analyses were for 7 days and 1 day, respectively. The positions of RNA size markers electrophoresed in the same gel are shown.

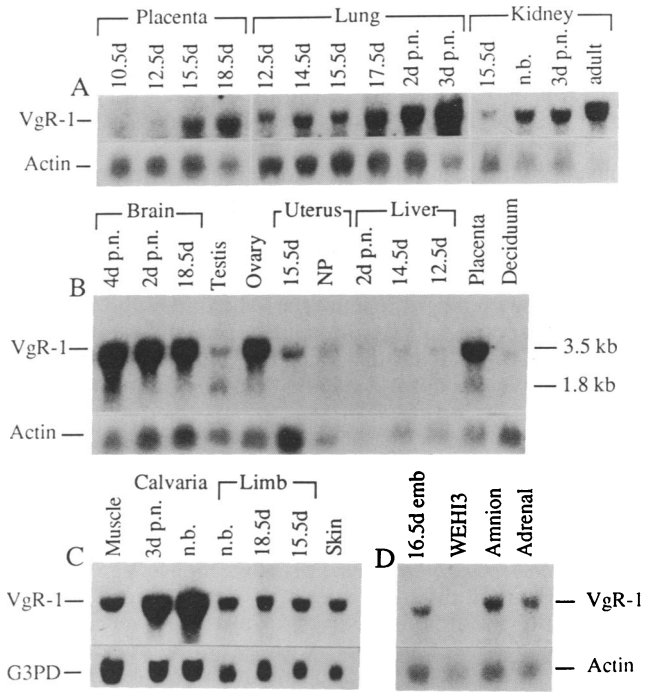

FIG. 4. Northern blot analysis of RNA from murine tissues isolated at various stages of development. Ages are given as dpc (d), days postnatal (p.n.), and newborn (n.b.). Twenty micrograms of total RNA was analyzed in each case, except for adult kidney RNA (10 μg). Unless otherwise stated, conditions were as described in *Methods*. (*A*) RNA from placenta, lung, and kidney was hybridized with the Vgr-1 probe (filter exposed with an intensifying screen for 6 days); filters were rehybridized with a mouse α-actin cDNA probe (exposure without screen for 12 hr). (*B*) RNA from brain, adult testis, adult ovary, uterus from pregnant (15.5 dpc) and nonpregnant (NP) mice, liver, 18.5-dpc placenta (see also *A*), and deciduum from 8.5-dpc pregnant mice was hybridized with Vgr-1 probe (exposure with screen for 6 days); filter was rehybridized with actin probe (exposure without screen for 8.5 hr). (*C*) RNA from adult muscle, calvaria, limb, and skin from 3-dpn pup was hybridized with Vgr-1 probe (exposure with screen for 6 days); filter was rehybridized with G3PD probe (exposure without screen for 8 hr). (*D*) RNA from 16.5-dpc embryo (see also Fig. 1), mouse WEHI-3 myelomonocytic leukemia cells, 15.5-dpc amnion, and adult adrenal was hybridized with Vgr-1 probe (exposure with two screens for 3 days); filter was rehybridized with actin probe (exposure without screen for 12 hr).

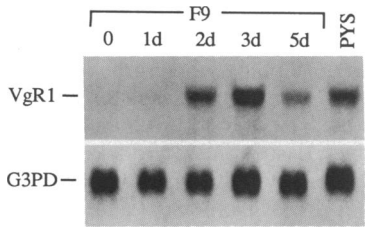

FIG. 5. Northern analysis of RNA from F9 cells differentiating into parietal endoderm. Twenty micrograms of total RNA from undifferentiated F9 cells and from F9 cells treated with 0.1 μM retinoic acid, 0.1 mM dibutyryl cAMP, and 0.1 mM isobutylmethylxanthine for 1, 2, 3, and 5 days was analyzed by Northern hybridization, together with 20 μg of total RNA from the murine parietal endoderm (PYS) cell line. (*Upper*) Vgr-1 cDNA probe. (*Lower*) Rehybridization with the G3PD probe. Autoradiographic exposures of the Vgr-1 and G3PD Northern hybridization were for 3 days and 4 hr, respectively. Longer exposures revealed the presence of a faint Vgr-1 mRNA band at days 0 and 1 (data not shown).

cells (Fig. 4*D*), which secrete factor(s) active in mesodermal induction in *Xenopus* (36).

Vg-1 was originally identified as a maternal mRNA localized in the vegetal hemisphere (presumptive endoderm) of the *Xenopus* embryo, where it may play a role in mesodermal induction (14, 17). We do not know whether the relatively high level of Vgr-1 RNA seen in the mouse ovary (Fig. 4) represents oocyte-specific transcripts. However, as shown in Fig. 5 there is a dramatic increase in Vgr-1 RNA levels when mouse F9 teratocarcinoma cells differentiate into endoderm in response to retinoic acid and cAMP (21). This induction reaches a maximum of at least 10-fold the basal level 3 days after exposure to retinoic acid and cAMP and declines thereafter as the cells progress towards the fully differentiated parietal endoderm phenotype. The differentiation of F9 cells in response to retinoic acid is thought to mimic the differentiation of inner-mass cells of the mouse blastocyst into primitive ectoderm and endoderm (21). By analogy with *Xenopus* embryogenesis, it might be expected that the first endoderm to appear in the mouse would produce factors influencing the differentiation of the ectoderm into mesoderm. The mouse Vgr-1 gene product may therefore be a candidate for such a putative mesodermal inducing factor. However, the expression seen in later embryos suggests that, like the *Drosophila* DPP gene product, mouse Vgr-1 plays a role at several different stages of development. Further insight into the function of Vgr-1 must await more precise localization of gene products with *in situ* hybridization and antibody probes, identification of the Vgr-1 receptor, and expression of biologically active protein in embryonic cells. These studies will presumably be paralleled by the definition of the biological roles of the other members of this family.

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