Gene Expression, Vol. 6, pp. 301-314, 1997
Printed in the USA.

1052-2166/97 $10.00 + .00

Temporal and Spatial Specificity of PDGF α Receptor Promoter in Transgenic Mice

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Aberrant expression of the platelet-derived growth factor α receptor (PDGFαR) has been linked to developmental abnormalities in vertebrate models, and has been implicated in multiple disease states in humans. To identify *cis*-acting regulatory elements that dictate expression of this receptor, we generated transgenic mice bearing the reporter gene β-galactosidase (*lacZ*) under the control of a 6-kb promoter sequence. Expression of *lacZ* was monitored throughout embryonic development, with special focus on nervous tissue, skeleton, and several organ systems wherein PDGFαR expression is thought to play a pivotal role. In several independent transgenic mouse strains, *lacZ* expression recapitulated predominant features of PDGFαR gene expression during mouse development. These results demonstrate that critical tissue-specific regulatory elements for PDGFαR expression are located within a 6-kb upstream region of the PDGFαR gene.

PDGF α receptor Gene expression Transgenic mice *lacZ* staining

PLATELET-DERIVED growth factor (PDGF) is a mitotic factor for primarily mesodermally derived cells (3,5,11,29,30,59) and for a restricted set of ectodermally derived cell types (31,35,36). It exists as either a homodimer or a heterodimer of two gene products, polypeptide subunits A and B. PDGF elicits its biological activity through binding to a specific cell surface receptor. Like the ligand to which it binds, the receptor for PDGF is comprised of two subunits, each encoded by different genes that are differentially regulated. The receptor subunits, identified as α (PDGFαR) and β (PDGFβR), are characterized on the basis of differences in ligand binding affinity (5,18,47). The two unique PDGF receptor subunits function as a noncovalently linked dimeric unit, and are capable of dimerizing to themselves, in the αα or ββ homodimeric state, or to each other as the αβ heterodimer (8,9,14,15,60). The β-subunit of the PDGF receptor is capable of binding PDGF BB homodimers with high affinity and PDGF AB heterodimers with moderate affinity, but is unable to specifically associate with AA homodimers. In contrast, the α-subunit binds all three isoforms of PDGF with high affinity.

Identification of the PDGF A gene transcripts in the early *Xenopus laevis* and mouse embryos was the first implication of the participation of PDGF in early embryogenesis (29,41). Research focused on the delineation of the respective pattern of expression of the PDGF ligands and receptors during embryogenesis provided the critical finding that the PDGF A chain and α receptor subunit are selectively expressed in separate but adjacent cell layers during embryogenesis (30, 36,37). The potential for PDGF A to function in

Received May 28, 1997; revision accepted July 2, 1997.
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early development through a paracrine mechanism, on the basis of selective affinity of the α receptor for the PDGF A chain ligand, therefore exists.

The strongest evidence for a direct role for PDGF in early development comes from characterization of the mouse mutation *Patch* (*Ph*). *Ph* arose as a spontaneous mutation in a C57Bl/6 colony, and was originally described for its characteristic variable dorsal white spotting pattern in heterozygotes. Homozygous *Ph* is an embryonic lethal mutation exhibiting numerous morphological defects, including malformations of the spinal cord, a variable pattern of fluid-filled blebs, abnormal development of the heart, head, and neck structures, defects in heart outflow tract, cleft face, and irregular somites (16). We and others have determined that the *Ph* mutation carries a large deletion in the genetic sequence encompassing the entire coding region of the PDGFαR gene (35,50). As virtually all the defective cell types in *Ph* are sites where PDGFαR is normally expressed, there appears to be a very close correlation between PDGFαR dysfunction and the defects observed (31,35). Recent descriptions of targeted disruption of the PDGF A, B, and βR genes have thus far supported the role of the PDGF ligand–receptor system in early development as predicted on the basis of in vitro expression data. Knockout mouse embryos, bearing null mutations in the PDGF B and PDGFβR genes, display major defects in the renal and circulatory systems (25,49). Homologous recombination at the murine PDGF A locus is described as causing severe developmental defects in the respiratory system, where the PDGF A/α signaling system is known to be critical (4).

Collectively, the expression data and mouse mutation studies support the notion that expression of PDGFαR is important for the correct development of vertebrate embryos. Because the initial observation of the proliferative effect of PDGF on smooth muscle cells, a potential role for this mitogen in stimulating the proliferation of subendothelial cells following injury has been postulated. Recent characterization of the role of PDGF has contributed to a greater understanding of the importance of this signaling system in numerous disease states, as aberrant expression of the PDGFαR receptor has been implicated in a variety of pathological consequences. In malignant astrocytoma, constitutive expression of PDGF receptors serves as a molecular marker, and perhaps an underlying cause of the hyperproliferative state (19,28,33,34). Comparable links have been documented for PDGF A and α receptor expression in such diseases as atherosclerosis [(43,51) for reviews], mesangial nephritis (23), pulmonary fibrosis (1), and bone marrow fibrosis (27).

The objective of this study has been the characterization of the molecular mechanism that results in the unique and critical pattern of restricted PDGFαR expression. We have previously isolated and characterized a 6-kb upstream fragment of the murine PDGFαR promoter (57). As a first step in addressing the control elements and their cognate binding proteins necessary for PDGFαR expression in vivo, we have generated transgenic mice carrying the reporter gene β-galactosidase (*lacZ*) under control of this 6-kb regulatory domain. Results from whole and sectioned embryo staining reveal a pattern of expression dictated by this promoter within cells of mesenchymal and ectodermal origins that is consistent with an early and restricted role in development.

MATERIALS AND METHODS

*Construction of PDGFαR-*lacZ *Transgene Vector*

The PDGFαR-*lacZ* transgene vector was constructed by cloning the 6-kb regulatory region of the PDGFαR promoter upstream of the bacterial reporter β-galactosidase gene *lacZ* into the pSA plasmid vector. The pSA vector contains a 150-bp SV40 intron splice sequence (*Pst*I site) and a 210-bp SV40 polyadenylation sequence (*Hin*cII site) in a pKS$^+$ Bluescript plasmid backbone (Stratagene). The 6-kb PDGFαR promoter (R^1-H^1 fragment, see Fig. 1) was isolated from the pKS$^+$ 58/05 construct (57) with *Not*I (5′) and *Cla*I (3′) double digestion and cloned into pSA at the *Sma*I site upstream of the SV40 splice sequence by blunt end ligation. The *lacZ* cDNA was isolated from pKS$^+$ Bluescript β-galactosidase construct with *Xba*I (5′) and *Xho*I (3′) double digestion and cloned into pSA at *Cla*I site between the SV40 intron splice and polyadenylation sequences by blunt end ligation. All constructs were verified by extensive restriction endonuclease analysis and DNA sequencing at the cloning junctions.

For microinjection purposes, the PDGFαR-*lacZ* transgene was isolated from the vector sequence after digestion with *Not*I and *Sal*I and separation by agarose gel electrophoresis. Transgene DNA was recovered from gel slices after treatment with Gelase (Epicentre Technologies) under conditions recommended by the manufacturer for high-efficiency yields.

Generation and Identification of Transgenic Animals

Transgenic mice were derived in accordance with Dana-Farber Cancer Institute ARD Guidelines (Protocol #91-091) following standard embryonic manipulation techniques (21). Positive founders were identified by Southern analysis of a tail biopsy. Each tail sample was digested with 0.5 mg/ml Proteinase K in a digestion buffer (100 mM NaCl, 10 mM Tris, pH 7.6, 10 mM EDTA, pH 8.0, and 0.5% SDS) at 55°C overnight. Genomic DNA was prepared from the digested samples utilizing phenol and chloroform extraction, followed by ethanol precipitation. Incorporation of the PDGFαR-*lacZ* transgene was confirmed in these samples by Southern analysis. Genomic DNA (10 μg) from each tail sample was digested to completion with *Hin*dIII restriction endonuclease for at least 12 h at 37°C. Genomic DNA samples were resolved by agarose gel electrophoresis, transferred to nylon membrane (Duralon), and hybridized with probes corresponding to 2.1 kb of the *Cla*I-*Eco*RI DNA fragment encoding the 3′ end of the *lacZ* cDNA and a 1-kb PDGF A chain as a internal control (see Fig. 2). Radiolabeled probes were prepared using random prime techniques. Exposure and quantitation of gels for all experiments were performed on a PhosphorImager (Molecular Dynamics).

Timed matings were established between founder lines back crossed to C57Bl/6 animals. Plug dates were noted, and embryonic day 0 was determined to be midnight of the evening before a vaginal plug was observed. Animals were sacrificed at various stages for analysis of embryonic transgenic offspring. Gestational age of the embryos was confirmed as established by Theiler (52). Dissected embryos and newborn pups were collected for fixation and *lacZ* staining.

Whole Mount lacZ *Staining*

For whole embryo staining, embryos were first fixed in 1% paraformaldehyde/0.5% glutaldehyde in a buffer of 0.1 M PIPES, pH 7.0, 2 mM $MgCl_2$, 2 mM EDTA, and 0.2% NP-40 at room temperature with gentle rocking for a period of 30 min (for E11.5 embryos and younger) or 1 h (for embryos between E11.5 and 13.5) or 3 h (for embryos older than E13.5). Following fixation, embryos were rinsed for three periods of 30 min each in a solution of 0.01% sodium deoxycholate, 0.02% NP-40, and 2 mM $MgCl_2$ in 0.1 M phosphate buffer, pH 7.3. Whole embryos were transferred to the staining reaction containing 1 mg/ml 4-chloro-5-bromo-3-indolyl-β-galactosidase (X-gal), 5 mM ferricynide, 5 mM ferrocyanide, 0.01% sodium deoxycholate, 0.02% NP-40, and 2 mM $MgCl_2$ in 0.1 M phosphate buffer, pH 7.3. Samples were incubated at 37°C in a humidified incubator for a period that ranged from 1 h to overnight (embryos older than E14.5 or intact adult organs) depending on the intensity of the *lacZ* staining. After detection of the *lacZ* staining, animals were rinsed in PBS, postfixed in 4% paraformaldehyde for 2 h, and stored in 30% sucrose for subsequent observation and photography. Nontransgenic embryos in the same litter were treated and stained in the same manner, and served as negative controls for detection of nonspecific background staining; there was no discernible staining in these negative controls for all animals stained.

LacZ *Staining of Sectioned Embryos*

All embryos used for sectioning were first stained in X-gal solution under the condition described above. Following incubation in sucrose solution, embryos were towel dried, frozen with the aid of Histo Freeze-2000 (Fisher brand), and embedded in OCT solution (Tissue-Tek, Miles Inc.). The frozen blocks were stored at −70°C until use. Sections were cut on a cryostat at approximately 10 μm thickness and placed on poly-L-lysine-coated microscopy slides. The slides were air dried overnight before being further processed. For detecting *lacZ* activity, sections were first equilibrated in staining solution without X-gal for 30 min before the addition of X-gal substrate for an additional 1-3 h at 37°C. Sections were then counterstained with eosin and mounted for photography. Alternate sections were later counterstained with hematoxylin for clearer confirmation of the tissue histology.

RESULTS

*Expression of PDGFαR-*lacZ *Gene in Gastrulation*

We generated transgenic mouse lines to examine the temporal and spatial specificity of the PDGFαR promoter in vivo. The transgenic mice were made to express the *lacZ* reporter gene under the control of a 6-kb fragment of the PDGFαR promoter. A map of the PDGFαR-*lacZ* transgene vector is shown in Fig. 1. Positive founders were identified by Southern blot analysis for the presence of a 4.6-kb *lacZ*-containing DNA fragment

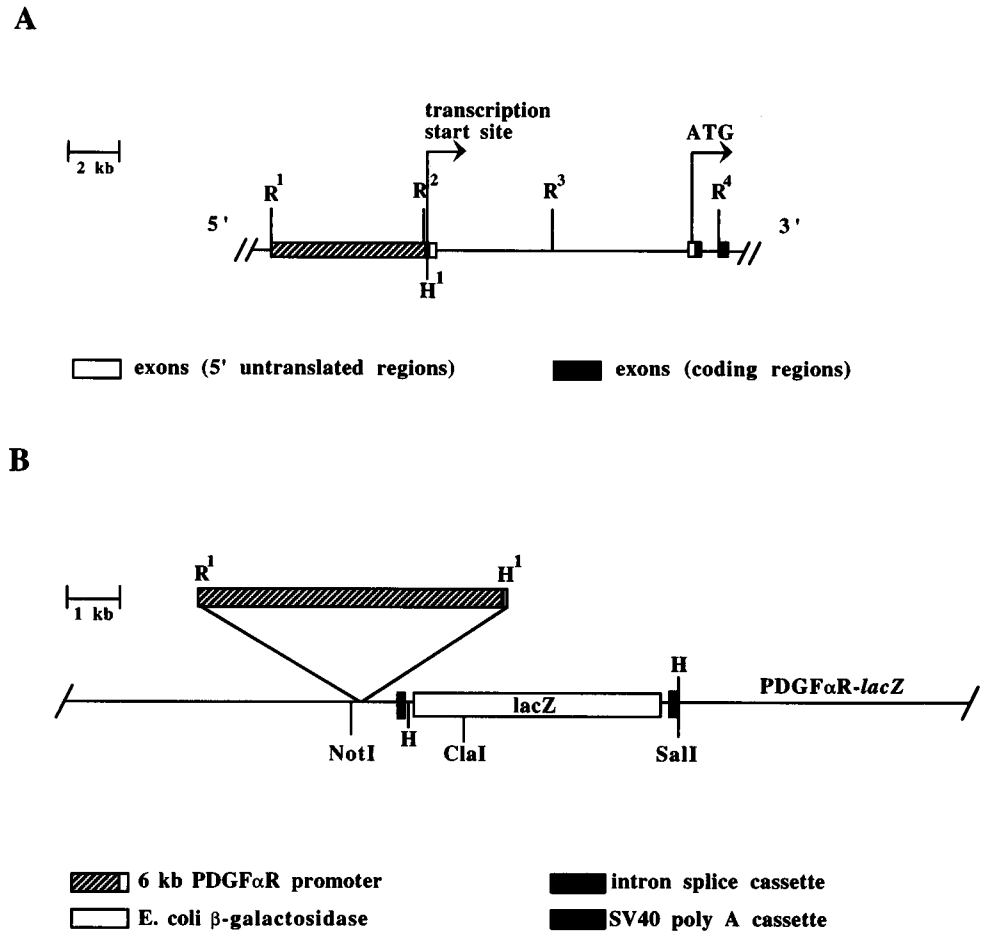

FIG. 1. Construction of the PDGFαR-*lacZ* transgene vector. (A) The partial genomic organization of the PDGFαR gene including the 6-kb promoter sequence that was used to generate the transgene DNA. (B) The map of the pKS$^+$ Bluescript plasmid vector that contains the PDGFαR-*lacZ* transgene. The PDGFαR-*lacZ* transgene vector was further modified with the addition of a SV40 intron splice signal between the promoter and *lacZ* sequences, and a SV40 polyadenylation signal at the 3′ end of the *lacZ* sequence. Both *Not*I and *Sal*I restriction sites are unique within the transgene vector to allow excision of the PDGFαR-*lacZ* DNA for microinjection. R and H stand for *Eco*RI and *Hin*dIII restriction enzyme sites.

upon *Hin*dIII restriction enzyme digestion (Fig. 2). In the same Southern analysis, PDGF A-specific probe was included as an internal positive control. Six founders were identified by Southern blot analysis as positive for transgene incorporation. Out of the six founders that we analyzed, one line (T-16) alone bore the transgene as demonstrated by Southern analysis but failed to show substantial *lacZ* expression. This may be attributed to rearrangement within the transgene or repressor activity near the site of transgene integration. The other five founders all demonstrated *lacZ* staining in the same pattern. Detailed *lacZ* expression analysis was performed in all five lines and the results from the highest expressing line (T2) were used to exemplify our findings in this report.

The resulting *lacZ* expression pattern, as determined by whole mount embryo staining, revealed an overall similarity to the in situ localization of the endogenous PDGFαR expression pattern during early embryogenesis (Fig. 3). *LacZ* activity was first observed as an intense pattern of staining in the intraembryonic mesoderm of the E7.5-day primitive streak stage mouse embryo (data not shown). One day later, the *lacZ* staining remained most pronounced in cells of mesenchymal origin, with striking staining observed in the somites and head mesenchyme (Fig. 3, E8.5). At this stage, significant *lacZ* expression was also detected in the extraembryonic yolk sac. We and others have previously shown that PDGFαR is expressed in both visceral and parietal extraembryonic endoderms and, thus, is likely to play an important role

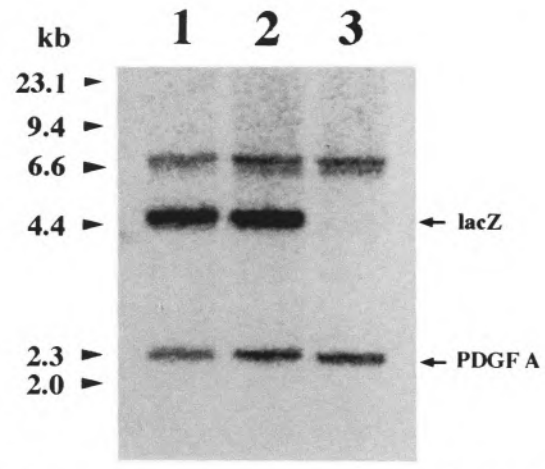

FIG. 2. Identification of transgenic animals by Southern blot analysis. Positive transgenics were identified by the presence of *lacZ* gene in the genomic DNA samples prepared from tail biopsy. Lanes 1-3 represent tail DNA samples prepared from the F_1 littermates of T2 founder. For Southern blot analysis, genomic samples were digested with *Hin*dIII restriction enzyme to completion, and subsequently size fractionated on 1% agarose gels and transferred onto nylon membranes. Positive identification of *lacZ* gene expression was measured by the detection of a 4.4-kb DNA fragment that corresponded to the entire coding region of *lacZ* cDNA and the SV40 polyadenylation signal sequence. As an internal control, blots were simultaneously hybridized with a DNA probe that specifically recognizes a 2.3-kb *Hin*dIII fragment of the PDGF A gene. The 7.0-kb signal is specific for PDGF A probe, which represents partial digested DNA fragment that contains the PDGF A sequence corresponding to the probe used.

in the development of the extraembryonic structure (36,45,57).

PDGFαR-lacZ *Expression in the Developing Skeleton*

Prior to cell differentiation, the *lacZ* staining in the somites was uniformly distributed throughout the entire blocks. This homogenous appearance of *lacZ* staining pattern, however, slowly dissipated into defined structures as somitic cells began migration and differentiation to establish different lineages. From among the three somite derivatives (sclerotome, dermatome, myotome), we were able to follow the PDGFαR promoter activity in only two of the three lineages, namely the dermatome, which forms the dermis layer of the skin (as exemplified in Fig. 5A), and the sclerotome, which contributes to the formation of cartilage and bone of the body (Figs. 3 and 4). Myotome remained negative throughout its development into the muscle (data not shown).

As demonstrated in the whole mount embryo staining, the sclerotome-specific PDGFαR promoter activity was reflected by the intense *lacZ* staining in the developing primordia of axial skeleton (vertebral column), radius bones of the limbs, and ribs (Fig. 3, E10.5–14.5). However, the specific activity of the PDGFαR promoter in the skeletal component was not confined to that derived from mesoderm only, as it was also functional in the visceral skeleton, a derivative of cranial neural crest cells. Prior to day 14.5, the *lacZ* staining was localized to the mass of neural crest-derived mesenchyme found throughout the cephalic region and within the first and second branchial arches. As the cartilaginous component in the cranium continued to form, some of this broad *lacZ* staining pattern observed in younger embryos was redistributed and localized to confined areas as shown in the 14.5-day-old embryo (Fig. 3). The dark markation of stains behind the eye extending to the lower jaw corresponded to the mandibular arch from which the upper and lower parts later differentiated into the quadrate and Meckel's cartilage, respectively. The pattern of staining near the base of the skull corresponded to the clavicle and parts of the scapula, which together function to provide the supporting skeleton to the head. Specific *lacZ* staining was also found in the medial nasal process and the orbital plate of frontal bone above the eye (Fig. 3, E14.5).

The activity of the PDGFαR promoter, like the endogenous PDGFαR gene, was transient in the developing skeletal system. The initial high level of *lacZ* expression in the precartilaginous cells was greatly diminished to an eventual undetectable amount, as chondrofication and ossification of the respective cartilage and bone cells took place. For example, in the cross section of a growing rib, as shown Fig. 4A, the *lacZ* staining was most intense in the actively proliferating mesenchyme and precartilaginous cells located near the growing tip of the rib cage. By contrast, *lacZ* expression at the base of rib cage was restricted only to the dense connective tissue perichondrium membrane surrounding the already ossified rib center. A similar situation was observed in the developing limbs (Fig. 4B) and vertebral column (Fig. 4C) of the 14.5-day embryo. In both cases, the ossified centers of the phalangeal bones and vertebra were completely devoid of PDGFαR promoter activity but the surrounding perichondrium membrane remained positive for the promoter activity. The coordinated suppression of the PDGFαR promoter activity with the onset of chondrofication was also seen in the neural crest-derived skeletal components. This is illustrated in Fig. 4C, where in the cranial vault strong *lacZ* staining remained in the pericardial membrane surrounding the petrous

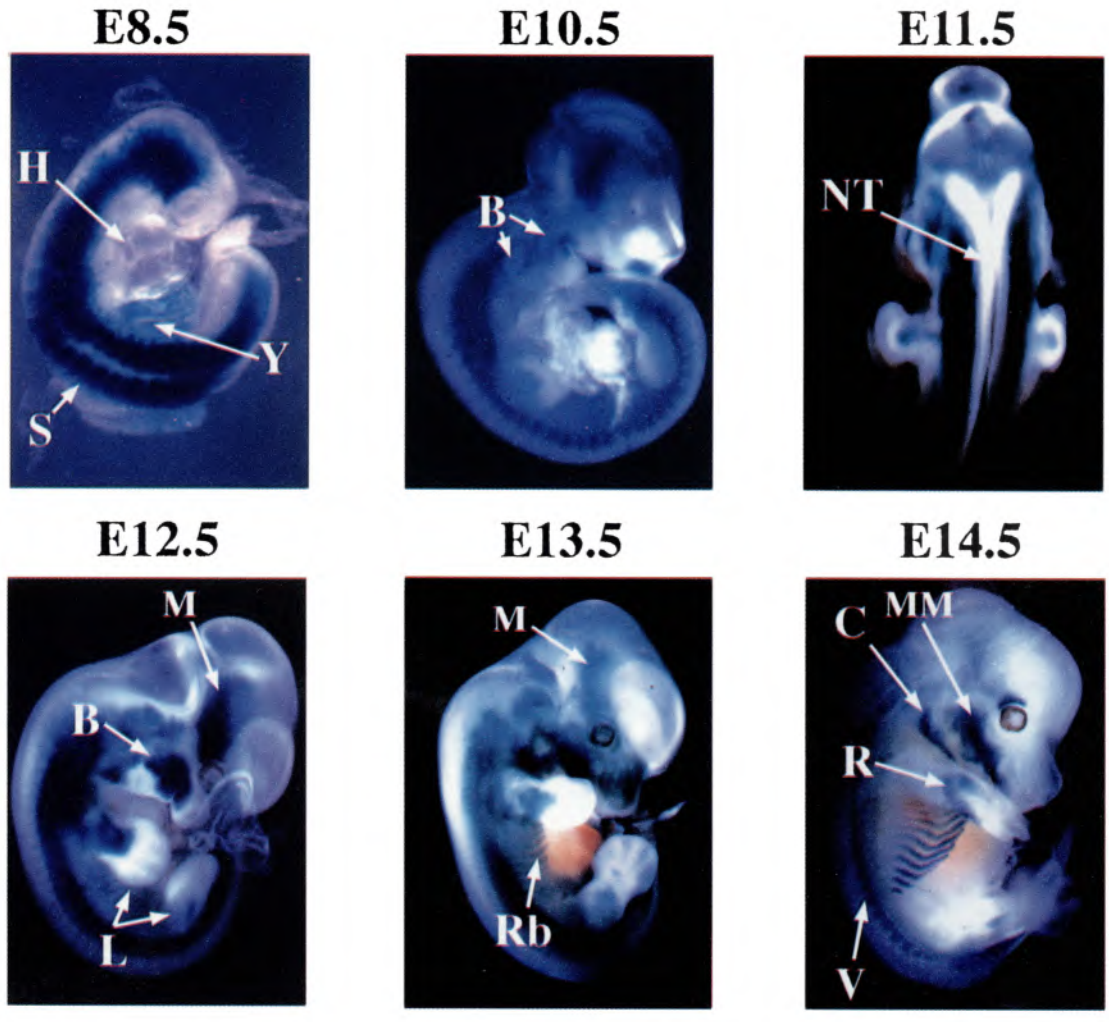

FIG. 3. Expression of PDGFαR-*lacZ* transgene expression during early embryogenesis. The six panels represent whole mount *lacZ* staining of E8.5-, E10.5-, E11.5-, E12.5-, E13.5-, and E14.5-day embryos. *LacZ* staining was carried out at 37°C for 1 h for E8.5–12.5 embryos and 3 h for E13.5–14.5 embryos. Abbreviations: B, branchial arches; C, clavicle; H, heart; L, limbs; M, head mesenchyme; MM, maxilla and Meckel's cartilage; NT, neural tube; R, radius bones; Rb, ribs; S, somites; V, vertebrae; Y, yolk sac.

part of the temporal, basioccipital, and basisphenoid bones that form the various bony elements of the cranial base.

*Restriction of PDGFαR-*lacZ *Expression in the Developing Nervous System*

A number of studies have shown that transcripts of PDGFαR are detected in the progenitor and/or mature forms of neurons (12,22,40,48,56) and glial cells (12,39,40,42,44,61) of the central and peripheral nervous tissues. In our transgenic lines, we were able to show selective PDGFαR promoter activity in the nervous system in a pattern similar to that of the endogenous gene. There was no evidence of PDGFαR promoter activity in the spinal neural tube, either before or after tube closure (Fig. 3). The initial *lacZ* expression in the neural lineage was detected in the roof of neopallial cortex, a region that will later develop into cerebral cortex, of an E12.5-day embryo (Fig. 5A). It is known that throughout the development of the embryonic brain, neurons initially appear within the ventricular zone and, as they mature, migrate outwards along radial glial fibers to form the cortical plate. The fibrous pattern of *lacZ* staining observed (Fig. 5A) suggests that this *lacZ* expression may be associated with the radial glial cells that provide a matrix for neuronal migration during development of the cortex. However, the staining at the ventricular layer at later times (E14.5 day, Fig. 5B), with its more punctate localization, suggests a pattern of transient expression

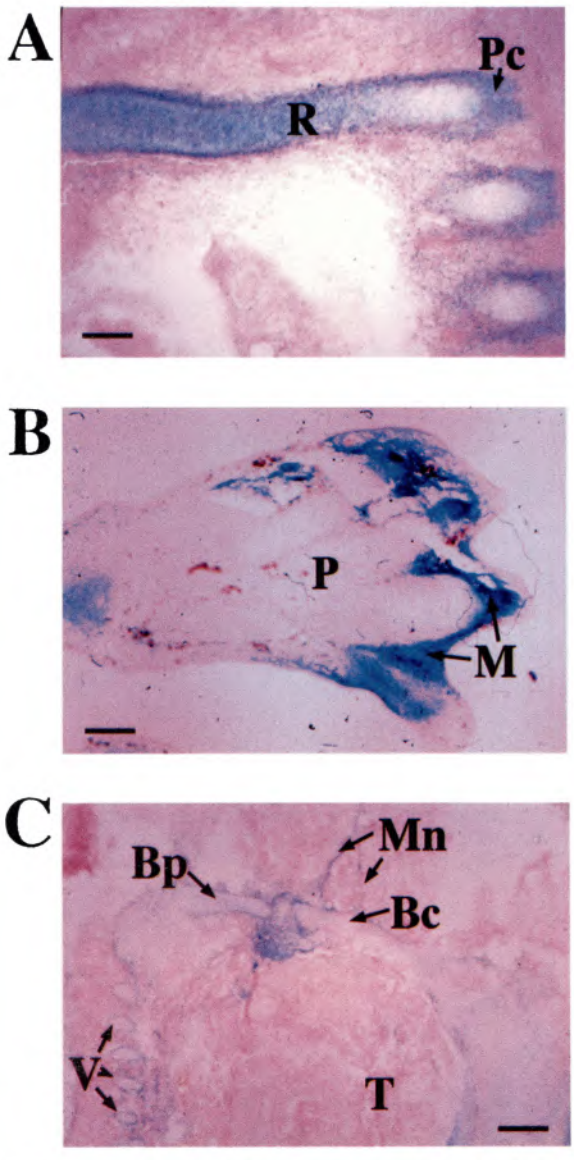

FIG. 4. Expression of PDGFαR-*lacZ* transgene in developing cartilage and bone. *LacZ* staining (1 h at 37°C) of the parasagittal sections of the ribs (A), hind limb (B), and the vertebral column and base of the skull (C) of an E13.5-day embryo. Abbreviations: Bc, basicocceptal bone; Bp, basisphenoid bone; M, mesenchyme; Mn, meninges; P, phalangeal bone; Pc, perichondrium; T, tongue; V, vertebrae. Bar = 400 mm (A), 1 mm (B, C).

within newly differentiated neurons. In contrast to embryonic brain, there was limited *lacZ* staining in the postnatal or adult brain, found primarily as punctate and low-level staining scattered sparsely in the cerebral cortex and thalamus (data not shown). Meninges, on the other hand, were stained positive throughout brain development into adulthood (as exemplified in Fig. 4C). Concomitant with the onset of *lacZ* expression in the cerebral cortex of the central nervous system, we began to observe strong *lacZ* staining in both the trigeminal (data not shown) and dorsal root ganglia at day E14.5 (Fig. 5D), both of which were completely negative prior to this stage (Fig. 5C). Under higher magnification, this *lacZ* staining could be identified within satellite neuroglia cells surrounding the ganglial cell bodies. A detailed immunohistological analysis will be required in the future to confirm the nature of these *lacZ*-positive cells in the ganglions and brain cortex.

Within the central nervous system, we also examined the PDGFαR-*lacZ* expression pattern in the optic system, a neurogenic organ where PDGFαR-mediated signaling pathway is thought to play an important role. As shown in Fig. 5E, the PDGFαR promoter activity in the eye was detected primarily in the lens, retina, and the surrounding sclera. The *lacZ* staining in the lens was confined to the outer layer of lens epithelium, which has been shown to express high level of PDGFαR transcripts (36,45). Analysis of the effect of PDGF AA on cultured lens organ study has implicated that presence of PDGF receptor, presumably the α receptor, in lens epithelium might be important for maintaining the translucent property of the lens (6). Most of the *lacZ* staining in the retina was found in the granular cell layer of the retina (Fig. 5F), which contains mainly the bipolar neurons and glia (Müller glia). It has been shown previously that both retinal ganglion neurons and glia respond to PDGF or produce PDGFαR (13,32,55). No *lacZ* staining was detected in the optic nerve at any time during eye development.

Expression of PDGFαR-lacZ *Transgene During Organogenesis*

Next, we examined the validity of the PDGFαR promoter activity in governing gene expression during early organogenesis. Our special focus was placed on the kidney, heart, and lungs where PDGFαR transcripts are known to be selectively expressed. Defects in the heart and lung tissues have also been described as major deformities in the PDGFαR minus *Ph* embryo.

Unlike the *lacZ* staining in most of the gut region (Fig. 6A), which was only transient, the staining observed in the kidney, heart, and lungs was continuous throughout the embryonic stages and into adulthood, such as the case in the kidney. The staining in the nephrotome lineage was visible very early on in the metanephros of E9.5 embryos (Fig. 6A). As the metanephros differentiate and regions of outer cortex and inner medulla of the kidney

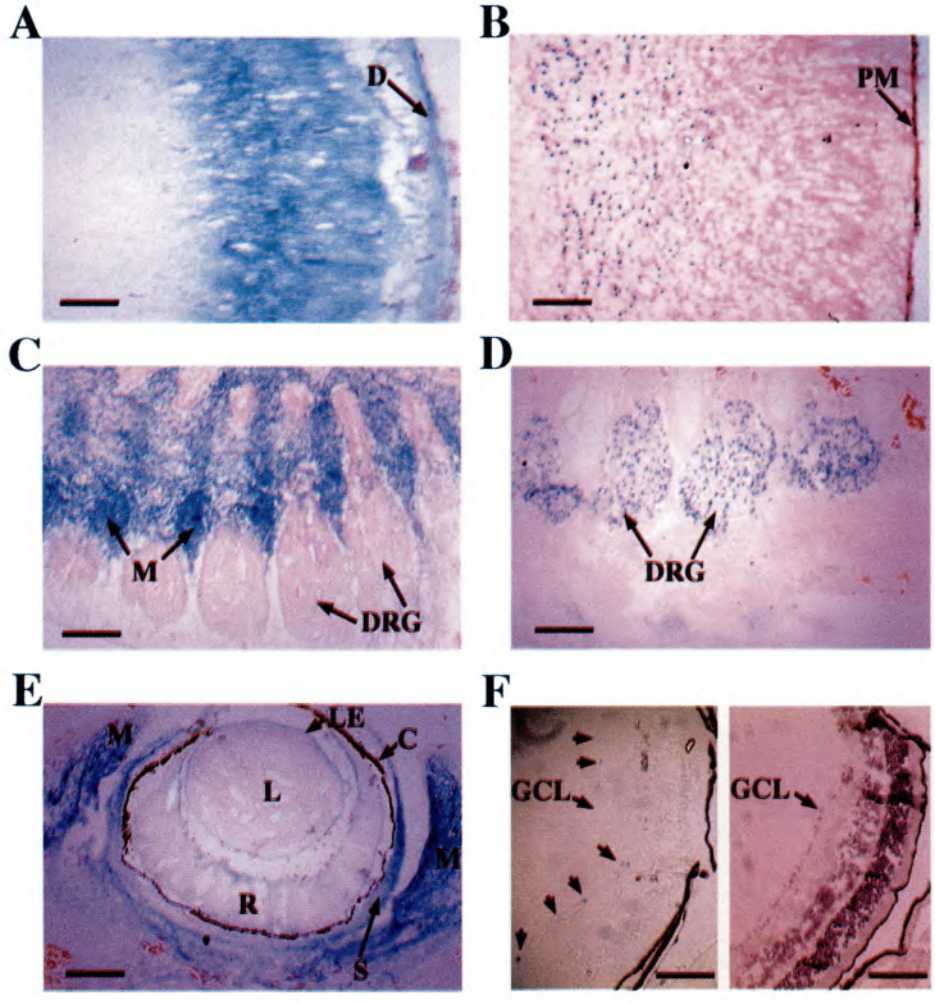

FIG. 5. Expression of PDGFαR-*lacZ* transgene in the nervous tissue. PDGFαR promoter activity was detected in the neurogenic lineage as displayed by the *lacZ* expression in the parasagittal sections of the cerebral cortex (A, B), dorsal root ganglia (C, D), and eye (E, F, left inset). (F, right inset) Eosin staining of a parallel eye section that is immediately adjacent to the *lacZ*-stained eye section shown in the left inset of the same panel. The arrowheads point to the *lacZ*-positive cells located within the ganglion cell layer of the retina. Developmental stages: E12.5-day embryo (A, C), E14.5-day embryo (B, D, E), P14 mouse (F). Abbreviations: C, choroid; D, dermis; DRG, dorsal root ganglion, posterior; GCL, ganglion cell layer; L, lens; LE, lens epithelium; M, mesenchyme; PM, pia mater; R, retina; S, sclera. *lacZ* staining was carried out at 37°C for 1 h. Bar = 100 mm (A, B), 400 mm (C, D, E), 200 mm (F).

are delineated, an organized *lacZ* staining in a ring-like structure in the outer cortex of the embryonic kidney was established (Fig. 6B). At high magnification, these sites resemble the interstitial and mesengial structures of early glomeruli. This pattern persisted in mature kidney as exemplified in a P14-day mouse (Fig. 6C). We also observed strong *lacZ* staining in the collecting tubules within the medulla (Fig. 6C). There was no detectable PDGFαR-*lacZ* expression in the blood vessels that enter or leave the kidney at any developmental stages.

The *lacZ* staining pattern in the heart corresponded to, but was not identical to, the pattern of the endogenous receptor transcripts, suggesting additional genetic information and/or control mechanism is involved in the regulation of the receptor expression in this organ. For example, PDGFαR transcript is first detected in cardiac mesenchyme tissue in the heart at day E8.5. At later stage of development, the PDGFαR transcripts became confined mostly to the pericardium, the endocardial cushions that contribute to the formation of cardiac valves, and the primitive

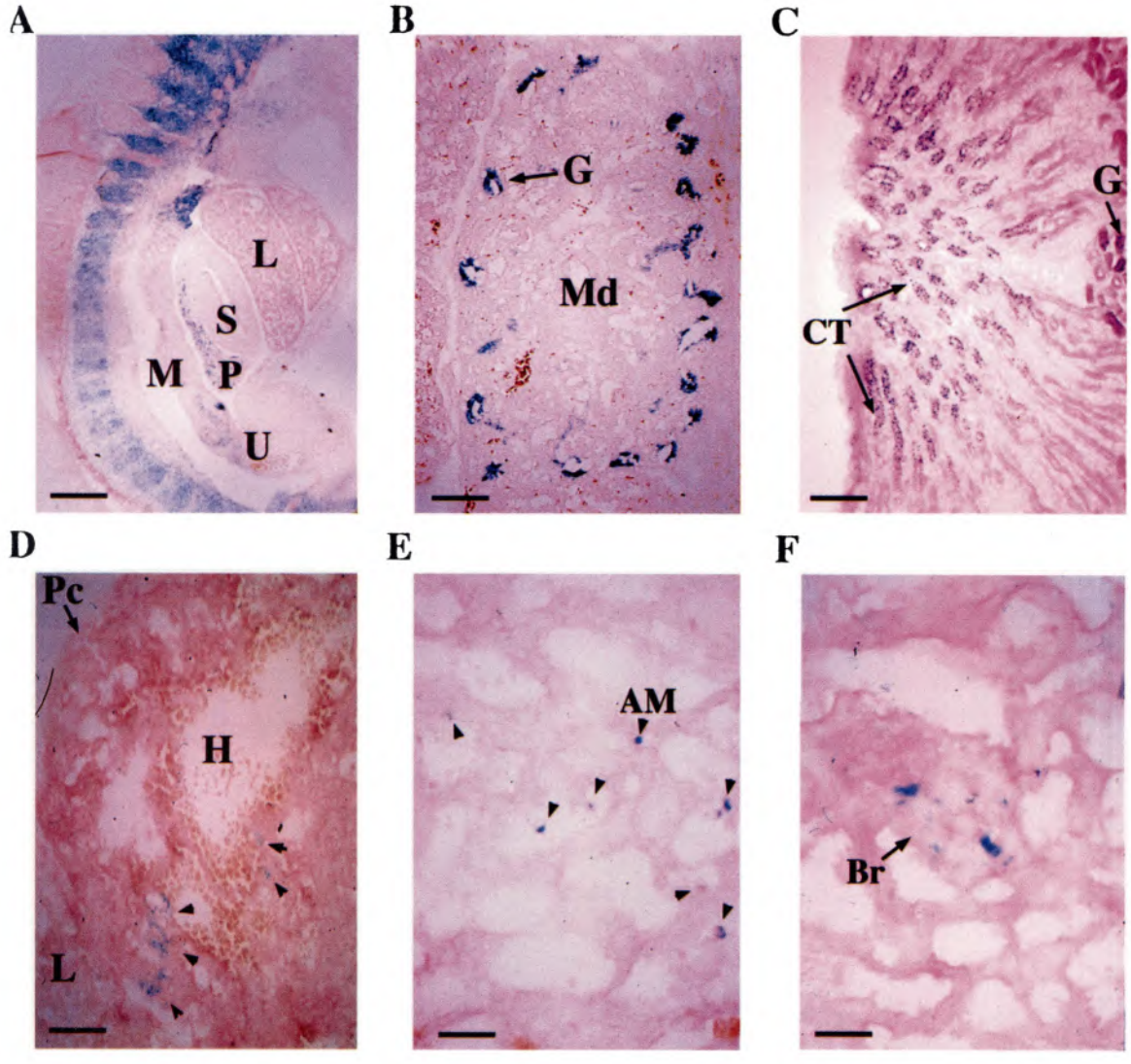

FIG. 6. Expression of PDGFαR-*lacZ* transgene in the gut, circulatory, and respiratory systems. Representatives of each system are included in parasagittal sections of the gut region (A), kidney (B, C), heart (D), and lung (E, F). Developmental stages: E12.5-day embryo (A), E14.5-day embryo (B), E13.5-day embryo (D), P10 mouse (C, E, F). Abbreviations: AM, alveolar myofibroblasts; Br, bronchus; CT, collecting tubules; G, glomeruli; L, liver; M, metanephros; Md, medulla; P, pancreas; Pc, pericardium; S, stomach; U, urogenital ridge. *LacZ* staining was carried out at 37°C for 3 h. Bar = 1 mm (A), 400 mm (B, C, D), 100 mm (E, F).

trabeculae (31,36,45). With our transgenic animals, we did not observe significant *lacZ* staining in the heart until day E12.5. Nor did we detect *lacZ* staining in the pericardium (Fig. 6D) and the atrio-ventricular valves. However, we did observe sporadic *lacZ* staining in the atrio-ventricular cushion tissue, implicating the PDGFαR promoter activity was active only in a small cell population within the cushion tissue (data not shown). Most consistently detected in the heart sections were pockets of staining corresponding to the truncus and trabeculae of the ventricle as shown in Fig. 6D.

The pattern expression of PDGFαR-*lacZ* in the lung is closely correlated with that of the endogenous gene the expression pattern, though the relative level of lung-specific *lacZ* staining in all of the transgenic lines was weaker than anticipated. Restricted staining was localized to the alveolar myofibroblasts (Fig. 6E) and the smooth muscle cells surrounding the bronchiole columnar epithelium (Fig. 6F). The specificity of the PDGFαR promoter function in the lung myofibroblasts echoes the importance of this receptor in lung development as suggested by the recent study on the PDGF A knockout mouse model (4). Targeted

disruption of the PDGF A gene generates major defects in the lung alveolar myofibroblast development and alveogenesis.

DISCUSSION

A broad body of literature indicates that activation of the PDGFαR plays a pivotal role in early development of the vertebrate embryo. Aberrant expression of PDGFαR has been linked to numerous developmental defects and proliferative disease states. We have set out to identify the genetic information needed to generate a temporal- and spatial-specific expression pattern of PDGFαR gene. In this study, we show that crucial *cis*-acting regulatory elements, necessary for directing expression of PDGFαR during embryogenesis, are largely contained within a 6-kb fragment of DNA from the 5′ flanking region of the mouse PDGFαR gene.

The activity of this PDGFαR promoter is most prominent in mesoderm and neural crest cell derivatives. With the exceptions of lens epithelium and neurogenic lineage, the promoter is generally not active in ectodermally and endodermally derived tissues. Furthermore, expression of the *lacZ* reporter protein is strongest at the time of mesoderm formation and becomes greatly diminished or totally absent in many differentiated mesoderm derivatives (e.g., muscle). This temporal specificity of the promoter activity is best illustrated in the developing skeletal structures, where expression of the *lacZ* reporter recapitulates timed expression of the endogenous PDGFαR gene. *LacZ* activity is transient during the formation of cartilage and bone elements, with the highest expression level localized to the mesenchyme within the region of the developing cartilage primordia. As these cells differentiate further, and gradually become more separated as a result of the continuous accumulation of matrix around them, they acquire the characteristics of mature cartilage cells (chondrocytes) and no longer express *lacZ* gene (Fig. 4). A similar pattern of PDGFαR promoter activity—high throughout early mesodermal proliferation and low at later stages of cell differentiation—is also observed during formation of neural crest-derived craniofacial structures. These studies are consistent with the view that activation of PDGFαR functions as an inducer of cell proliferation, rather than cell differentiation. Continuous expression of PDGF receptor in cells that normally express the receptor transiently has been shown to block the transition of cell proliferation to cell differentiation, in some cases resulting in cell transformation. In vitro, factors such as TGF-β, basic FGF, interleukins, and all *trans*-retinoic acid have been shown to modulate PDGF receptor expression in different cell lines (2,15,24,38,46, 53,54,62). It is therefore possible that some of these molecules could serve as the biological factors for modulating PDGFαR-mediated signaling pathway during tissue and organ formation in vivo. The specificity of PDGFαR promoter activity in the skeletal components may shed light on the anatomical defects seen in *Ph* embryos, which develop severe craniofacial (cleft face) and vertebral (spina bifida) abnormality upon disruption of signaling via the PDGFαR (16).

Studies summarized here show that the 6-kb 5′ flanking DNA fragment of mouse PDGFαR gene contains crucial genetic elements that recapitulate the predominant PDGFαR expression pattern during early embryogenesis. The noted deviation of the observed *lacZ* expression pattern at a few sites such as the heart valves and pericardium, from that of PDGFαR in situ hybridization signal, suggests that additional DNA sequences outside the 6-kb promoter may also be needed to direct or enhance PDGFαR expression in vivo. Our recent discovery of a tissue-specific activation element within the intron of the PDGFαR gene could support this possibility. Inclusion of the intronic sequence to the PDGFαR-*lacZ* transgene is shown to greatly increase *lacZ* expression in a cell type-specific manner in vitro (C. Wang, unpublished data). Additional transgenic lines have now been made to investigate the effect of this intronic sequence on the specificity of PDGFαR promoter. Alternatively, differential usage of transcription start sites of the PDGFαR gene may occur within a specific tissue type. It has recently been shown that there exists a new PDGFαR transcript that is encoded by a growth arrest-specific gene (*gas*) in fibroblast (26). The *gas* transcript shares the same nucleotide sequence composition as the originally identified PDGFαR; the noted exception between these two transcripts is found in the first exon, which in *gas* is replaced by a 111-nucleotide sequence that is previously specified as part of the intron one of PDGFαR gene. We have preliminary evidence to suggest that DNA sequence 5′ proximal to the 111-nucleotide sequence has the capacity to initiate gene transcription in vitro.

The temporal- and spatial-specific pattern of PDGFαR expression suggests that the PDGFαR gene is subjected to modulation by a number of

developmentally regulated transcription factors. We have recently identified a member of the *GATA* transcription factor family, *GATA-4*, as an essential component of the transcription activation complex responsible for PDGFαR expression in the visceral and parietal extraembryonic endoderm (58). Additional members of the *GATA* family, such as *GATA-5* and *GATA-6*, are also known to play an important role in regulating heart and other organ development. Thus, our finding that PDGFαR promoter contains biologically functional *GATA*-responsive elements offers the opportunity to assess the participation of different members of *GATA* transcription factors in the control of PDGFαR expression during organogenesis. Other transcription factors that may interact with the PDGFαR promoter are the homeobox proteins such as the *Pax*. *Pax* proteins contain a large superfamily of homeobox-like transcription factors that are developmentally regulated (17). There is a striking overlap between the distribution of different *Pax* members with the expression pattern of PDGFαR throughout embryogenesis. For example, expression of the *Pax1* gene coincides with PDGFαR expression in the intervertebral discs. Mouse mutants carrying a defective *Pax1* gene, with greatly reduced transcriptional activity, develop a less severe but similar abnormality to that observed in the spinal column of the *Ph* embryo. When the two mutant mice are bred, the compounded defects are characteristic of the human neural tube birth defect spina bifida (20). We have recently identified a putative *Pax3* recognition site within the 6-kb PDGFαR promoter. The *Pax3* gene has been suggested to play an important role in the development of central nervous system and dermomyotome cell lineages (7,10,17). We are currently investigating the role of *Pax3* and other *Pax* gene members in regulating PDGFαR expression through this recognition site in development. It will not be surprising to see that other members of the homeobox gene family may also be involved in the control of the expression of this receptor.

In conclusion, PDGFαR is expressed conditionally in response to physiologic, environmental, and temporal cues. Inappropriate expression of PDGFαR is known to serve as a molecular marker, and perhaps an underlying cause of a wide range of hyperproliferative disease states. We have demonstrated in this study that, under normal condition, transcriptional control plays a primary role in dictating the specific pattern of PDGFαR expression in development. Studies are now ongoing to identify the *cis*- and *trans*-acting regulatory factors involved for the complex pattern of PDGFαR gene expression and to utilize this knowledge to define the roles that PDGFαR plays in the normal or pathological development of the many tissues in which it is expressed. Furthermore, the ability of the 6-kb promoter sequence to recapitulate predominant feature of the endogenous gene makes it an extremely useful tool for analyzing different roles of PDGFαR-mediated signal transduction pathways that play in the development of specific tissue/organ forming events. This can be achieved by expressing, under the control of our promoter, the different mutant forms of PDGFαR that lack specific signaling property (e.g., mitogenic versus chemotactic) into PDGFαR null mouse background.

ACKNOWLEDGMENTS

We would like to thank Dr. Joel Lawitts and the Beth Israel Transgenic Facility for their assistance throughout this project. We are grateful to Mr. Baoliang Song, Ms. Betsy Grimm, and Ms. Abha Chandra for their technical contributions. We also thank Dr. Mark Mercola for his helpful discussions and critical reading of this manuscript. This work was supported by NIH grant HD 24926 to C.D.S. and by AICR grant 96A015 to C.W.

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