Platelet-derived growth factor-A and its receptor are expressed in separate, but adjacent cell layers of the mouse embryo

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Summary

The localized developmental expression of murine platelet-derived growth factor A (PDGF-A) was compared to that of its receptor (Pdgfra). Our in situ hybridization study included germ layers of primitive streak embryos, early axial structures (dermatome, myotome, sclerotome, floor plate), the skin and some of its derivatives (hair and mammary gland), the developing forelimb, the branchial arches and various sense organs (otic vesicle, olfactory epithelium and the eye). We report that PDGF-A and Pdgfra are expressed in separate, but adjacent cell layers in these structures and that in most, the ligand is expressed in the epithelium, whereas the receptor is in the mesenchyme. This localization corresponds to classical experimental evidence for developmental interactions across cell layers. We suggest that the spatio-temporal regulation of PDGF-A and Pdgfra, and other related systems, represents one model for the spatial regulation of receptor-ligand interactions.

Key words: PDGF-A, Pdgfra, developmental localization, receptor-ligand interaction.

Introduction

The PDGF receptor-ligand system is involved in the control of cell differentiation. This assumption is supported by their mitogenicity in cultured connective tissue cell lines (Ross et al., 1986), by the effect of PDGF-specific antibodies on the smooth muscle of regenerating blood vessels (Ferns et al., 1991), as well as by the role of PDGF in the timing of glial cell differentiation (Noble et al., 1988; Raff et al., 1988). Its contribution to cell and tissue homeostasis is emphasized by the malignant potential, acquired when PDGF is incorporated into retroviruses, or when it is overexpressed (Doolittle et al., 1983; Heldin et al., 1987).

A widely held assumption suggests that genes that are involved in cell differentiation also contribute to embryonic development. Indeed both PDGF isoforms (A and B), and both PDGF receptors (α and β), are expressed at different stages of murine embryogenesis (Mercola et al., 1990). Nevertheless little is known about their developmental function, possibly because PDGF has no effect on one of the most studied developmental systems, the Xenopus animal cap model (Cooke and Wang, 1991).

Recently it has been shown that Patch (Ph), a recessive embryonic lethal mutation of the mouse, is associated with a genomic deletion in the gene encoding the α receptor for PDGF (abbreviated as Pdgfra; see Stephenson et al., 1991). This finding renewed the interest in the possible role of PDGF in mammalian development. We have recently compared the mutant phenotype of Ph with the developmental expression of Pdgfra and found that Ph interferes with morphogenesis in homozygous mutant presomitic embryos (Orr-Urtreger et al., 1992). To extend these observations we have now compared the developmental expression of Pdgfra with that of its specific ligand, PDGF-A (Heldin et al., 1987; Seifert et al., 1989) in simultaneous in situ hybridization experiments.

The central observation of the study to be described here is that PDGF-A and Pdgfra are expressed in separate, but adjacent, sheets of cells at many locations, from gastrulation till late embryogenesis, including primary germ layers and the epithelium and mesenchyme of various organs. Topographically similar interactions, denominated as ‘appositional’, characterize numerous classical developmental models (for general information, see Gilbert, 1991 and Slack, 1991). The appositional transcripational localization displayed by Pdgfra and PDGF-A will be compared with the localized expression of different polypeptide growth factor-tyrosine kinase receptor systems.

Materials and methods

Embryos

C57BL/6J mice were kept under a 14 hours light, 10 hours dark (from 7.30 p.m. to 5.30 a.m.) regime. The time of pregnancy was established by vaginal plugging the morning following mating.
This was regarded as day 0.5 post coitum (p.c.). Embryos were prepared and processed for fixation as described previously (Orr-Urtreger et al., 1991).

Probes
For the detection of Pdgfra transcripts, a 359 bp long cDNA fragment was isolated from the interkinase region of the murine Pdgfra cDNA as described previously (Do et al., 1992). A probe for PDGF-A was isolated by PCR, using first strand cDNA prepared from poly-A rich RNA isolated from embryos 12.5 days (p.c.) (Todd et al., 1987) as template. The PCR primers used were synthesized from the fourth exon of murine PDGF-A, on the basis of the cDNA sequence of Mercola et al. (1990). They were: 5'-AGGAAGCCATTCCTGCA-3' and 5'-CTTGACACTGCCTG-GTG-3'. A 189 bp long DNA fragment synthesized by PCR was cloned into Bluescript II KS+ and the identity of its sequence was confirmed. T3 and T7 polymerase-catalyzed transcripts were synthesized in the presence of 35S-UTP for in situ hybridization. Both anti-sense and sense (control) transcripts were used for hybridization.

In situ hybridization
Previously described standard techniques were used (Orr-Urtreger et al., 1990). Exposition was 1-4 weeks, the PDGF-A probe requiring the longer period.

Results
PDGF-A and its receptor are localized in reciprocal germ layers
In situ hybridization results shown in Fig. 1 depict the relative expression of PDGF-A and Pdgfra in pre-somitic embryos at 7.5 days of gestation. In the embryonic part (Fig. 1B,C,E and F) Pdgfra (receptor) transcripts were mostly expressed in the mesoderm, whereas those of PDGF-A (ligand) occupied cells of the primitive ectoderm and visceral endoderm. Thus a sandwich-like arrangement was formed with the germ layers expressing alternatively Pdgfra or PDGF-A transcripts. With the ligand as probe very little signal could be seen above the mesoderm (Fig. 1C and F), suggesting that the expression domains of the receptor and the ligand were well separated.

Additional observations could be made in sections which traversed the primitive streak (Fig. 1D-F). In this area both Pdgfra and PDGF-A expression was limited or absent. It is therefore possible that this receptor-ligand pair is not involved in the development of the axial mesoderm as was suggested in an analysis of the receptor (Orr-Urtreger et al., 1992).

PDGF-A and Pdgfra were expressed also in the extraembryonic part of the embryo. The pattern of expression in this area seemed to be different, even at low magnification, from the expression pattern characteristic for the embryonic part. Hybridization signals of the receptor, in the vicinity of the cephalic end, localized to a cell layer close to the yolk cavity (Fig. 1B), whereas cells expressing the ligand were situated more centrally and closer to the ectoplacental cavity (Fig. 1C). At higher magnification it could be seen that along the cephalic endoderm and those of the ligand in the chorionic ectoderm (Fig. 1G-I). It follows that in the embryonic part, visceral endoderm cells expressed PDGF-A, whereas cells of the same germ layer in the extraembryonic part expressed the receptor, Pdgfra (see also schematic Fig. 9A).

This switch between ligand and receptor expression took place at the level of the exocoelomic cavity of the 7.5 day-embryo (Fig. 1B,C, H and I). The exact position of the inter-face between receptor-expressing and ligand-expressing visceral endoderm cells was however difficult to determine. Strong ligand expression ceased above the headfold area and beyond the posterior end of the primitive ectoderm, at or close to the area where the flattened squamous embryonic phenotype changes into the more columnar extraembryonic cell type (Snell and Stevens, 1966). The inner cell layer of the exocoelomic cavity, the extraembryonic mesoderm, besides receptor expression in the allantois (Orr-Urtreger et al., 1992), contained both receptor- and ligand-expressing cells.

Taken together, the result of this expression pattern was that along the triple layered embryonic half and along much of the double layered extraembryonic half of the embryo, sheets of cells transcribing the growth factor became apposed to sheets of cells which transcribe the receptor (see schematic Fig. 9A). Whether the spatio-temporal relationship, whereby the transcription of Pdgfra and PDGF-A are localized to separate but adjacent cell sheets, is also prevalent in other structures during later stages of development, is the subject of the following sections.

Reciprocal expression of PDGF-A and Pdgfra during early axial development
During early somitogenesis, at 8.0 days p.c., mesodermal aggregates representing the first somites mainly expressed the receptor, and to a much lesser extent the ligand, which was transcribed at high levels in the epithelial monolayer, representing the surface ectoderm. Neither Pdgfra nor PDGF-A were expressed at this stage in the definitive, embryonic endoderm (Fig. 2A-D).

At 10.5 and 11.5 days of gestation, transverse sections across the trunk revealed Pdgfra expression in the dermomyotome and in the migrating sclerotome (Fig. 2F and I), whereas PDGF-A was expressed in the surface ectoderm, in the myotome and in the floor plate of the neural tube (Fig. 2G and J). An expression pattern, essentially similar to that of presomitic embryos, became established by this arrangement. Layer to layer, this arrangement consisted of the surface ectoderm expressing the ligand, the dermomyotome under it expressing the receptor, followed by the myotome expressing the ligand, which is then succeeded by the sclerotome and loose mesenchyme expressing the receptor (Fig. 2F-J; see also schematic Fig. 9B). This sandwich-like arrangement in the mid-gestation embryo was connected with a developmental change in Pdgfra expression. The early somite, as shown in Fig. 2B, mainly expressed the receptor (Pdgfra), whereas later, the myotome part of the dermomyotome almost exclusively expressed PDGF-A (Fig. 2G and J).
Fig. 1. Expression of Pdgfra, and its ligand PDGF-A (PDGF, A chain) in the primitive streak-embryo (7.5 days p.c.). (A, D and G) Bright-field illumination, (C, F and I) dark-field illumination of the same sections, hybridized with the PDGF-A probe. (B, E and H) adjacent sections hybridized with the Pdgfra probe. am, amnion; ee, embryonic ectoderm; ecc, exocoelomic cavity; eme, extraembryonic mesoderm; epc, ectoplacental cavity; me, mesoderm; pe, parietal endoderm; ps, primitive streak; te, trophectoderm; tg, trophoblastic giant cells; ve, visceral endoderm. Bars, (A-C) 150 µm; (D-I) 60 µm
Fig. 2. Expression of PDGF-A and its receptor in somitic and axial structures. (A-D) Midsection of a 8.0 day-embryo, somites 2-4. (A and B) Bright- and dark-field views of the same section, hybridized with the Pdgfra probe. (C and D) Hybridization with PDGF-A. (E-G) 10.5 day-embryos; (H-J) 11.5 day-embryos, transverse sections. (E and H) bright-field; (F, G, I and J) dark-field. (F and I) Hybridization with the receptor, Pdgfra. (G and J) Hybridization with the ligand, PDGF-A. dt, dermatome; en, endoderm; fp, floor plate; mt, myotome; nt, neural tube; se, surface ectoderm; so, somite; st, sclerotome. Bars, (A-D) 30 μm; (E-G) 100 μm; (H-J) 150 μm.
Appositional localization of PDGF-A and its receptor during muscle and limb development

Transverse sections across the limb bud of an 11.5 day-embryo revealed that PDGF-A expression in the myotome continues into skeletal muscle primordia and into the surface ectoderm of the developing limb (Fig. 3C), whereas that of its receptor displays a separate, but more diffuse pattern of expression in the limb bud mesenchyme (Fig. 3B). 

*Pdgfra* expression did not follow the differentiation of the sclerotome into advanced osteogenesis. It was however present in the perichondrium of the developing bone (Fig. 4B and F).

We previously observed that *Pdgfra* was expressed in the apical ectodermal ridge (AER) of the limb bud (Orr-Urtreger et al., 1992). In this study we compared the expression of PDGF-A with that of its receptor in this structure. Fig. 3E and F shows that both the growth factor and its receptor are transcribed in the AER ectoderm. Save for the very area of the AER, transcripts of the ligand and the receptor were well separated. PDGF-A transcripts were

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**Fig. 3.** Expression of *Pdgfra* and PDGF-A in the developing forelimb (11.5 days p.c.). (A-C) Transverse sections, (D-F) distal part of the forelimb (high magnification). (A and D) Bright-field illumination. (B and E) Hybridization with *Pdgfra*. (C and F) Hybridization with PDGF-A. AER, apical ectodermal ridge; exm, extensor muscles; flm, flexor muscles; fp, floor plate; mt, myotome; sc, spinal cord; se, surface ectoderm. Bars, (A-C) 500 µm; (D-F) 60 µm.
localized in the surface ectoderm and those of *Pdgfra* in the mesenchyme. Whether PDGF-A and *Pdgfra* are expressed in the same, or in different, but closely situated cells of the AER, could not be defined unequivocally by this method.

PDGF-A was clearly detectable in the various muscle groups of the forelimb and shoulder of a 14.5 day-embryo, whereas transcripts of the receptor surrounded the developing bone and appeared in the prospective joints (Fig. 4A-C). It is possible that PDGF-A in the muscles interacts with its receptor in the perichondrium and joints (Orr-Urtreger et al., 1992). They together then could contribute to the formation of specific muscle-bone attachment sites. Similar reciprocal localization could be seen between PDGF-A expression in the intercostal muscles and ribs. *Pdgfra* was expressed in the perichondrium of the developing rib and in the mesenchyme of the intercostal space (Fig. 4F), whereas PDGF-A was expressed in three distinct areas, corresponding to the intercostal muscles (Fig. 4G). It is worth mentioning that since the first separation of the myotome and dermatome, prospective myoblasts consistently expressed PDGF-A throughout muscle development.

**During organogenesis PDGF-A is expressed in the epithelium and Pdgfra in the mesenchyme**

Morphogenetic cell interactions between epithelia and mesenchymes are important for organogenesis (Saxen et al., 1980). We therefore assumed that various developing organs could be most appropriate to test how general the appositional regulation of PDGF-A and *Pdgfra* is during later development.

Fig. 5A-H illustrates patterns of expression in the embryonic integument and its derivatives. The integument of the embryo displayed PDGF-A transcripts at most sites, as early as 8 days p.c. (Figs 2D and 5C,F and K), whereas *Pdgfra* was expressed beneath it, in the mesenchyme and in the prospective dermis (Fig. 5B, E, H and K). Similarly the epidermal bud of the developing mammary gland expressed the ligand (Fig. 5C), whereas the receptor was expressed in cells of the surrounding mesenchyme (Fig. 5A and B). In
Fig. 5. Expression of Pdgfra and PDGF-A in the embryonic integument and its derivatives. (A-C) A developing mammary gland (14.5 days p.c.). (A) Bright-field, (B) dark-field illumination of the same section, hybridized with the Pdgfra probe. (C) Adjacent section hybridized with the PDGF-A probe. (D-H) Developing hair (vibrissa) (14.5 days p.c.). (D and G) Bright-field illumination, (E and H) dark-field illumination of sections that were hybridized with Pdgfra, and (F) dark-field illumination of the section in D that was hybridized with PDGF-A probe. (I-K) Fusion of the mandibular arches (10.5 days p.c.). (I) Bright-field and (K) dark-field illumination of the same section that was hybridized with PDGF-A probe. (J) Dark-field illumination of a consecutive section hybridized with Pdgfra. dr, dermis; eb, epidermal (mammary) bud; ed, epidermis; hb, epithelial hair bulb; hp, mesenchymal hair papilla; ma, mandibular arch; mc, mesenchyme; my, myelocoel; ov, otic vesicle; ph, pharynx; se, surface ectoderm. Bars, (A-F) 50 µm; (G and H) 130 µm; (I-K) 500 µm.
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Fig. 6. Pdgfra and PDGF-A expression in salivary gland (A–C) and lung development (D–H). (A and D) Bright-field illumination, (B and E) dark-field illumination of the same sections (14.5 days p.c.) hybridized with Pdgfra. (C and F) Dark-field illumination of adjacent serial sections, hybridized with PDGF-A. (G and H) High power magnifications through crossections of bronchi, that were hybridized with Pdgfra (G) and PDGF-A (H). br, bronchi; eb, epithelial branch; ep, epithelium of the bronchi; mc, mesenchyme. Bars, (A–C) 200 µm; (D–F) 250 µm; (G and H) 40 µm.

Fig. 5I–K demonstrates the expression of Pdgfra and PDGF-A in transverse sections across the pharyngeal area of a 10.5 day-embryo, and shows transcripts of PDGF-A in the surface ectoderm of the mandibular arch and in the endothelium of the pharynx, whereas those of the receptor could be found in the underlying branchial arch mesenchyme. PDGF-A expression prevails along the entire surface ectoderm, but it stops where fusion of the mandibular arches takes place. This raises the possibility that PDGF-A and its receptor could be involved in branchial arch fusion and in the formation of the face. Fig. 5I–K also demonstrates in situ hybridization in the otic vesicle, from which the inner ear develops. Its lining expresses PDGF-A, whereas the mesenchyme surrounding it contains transcripts of the receptor, Pdgfra.

Similar observations were made in two derivatives of the another skin derivative, the sensory hair of the snout, the ligand was transcribed in the surface ectoderm and in the developing epithelial hair bud (Fig. 5D and F), whereas the receptor was transcribed in the surrounding mesenchyme including the hair root papilla (Fig. 5E,G and H).
foregut and pharynx. The branching epithelial buds of the submandibular salivary gland expressed the ligand, PDGF-A (Fig. 6C), whereas the surrounding mesenchyme expressed the receptor, Pdgfra (Fig. 6B). In the developing lung, the bronchial epithelium expressed PDGF-A (Fig. 6F and H), whereas the surrounding mesenchyme expressed Pdgfra (Fig. 6E and G).

Two structures formed at the junction of the central nervous system and the oral epithelium, Rathke’s pouch and the nasal cavity, complete this series of examples (Fig. 7). Rathke’s pouch gives rise to the adenohypophysis, whereas the neurohypophysis is formed by the neighbouring neuroectoderm. Fig. 7A-C demonstrates Pdgfra transcripts in the surrounding mesenchyme and PDGF-A transcripts in the presumptive adenohypophysis. In the nasal cavity transcripts of the ligand occupy the olfactory epithelium, whereas those of the receptor the surrounding mesenchyme (Fig. 7D-F).

Taken together our results suggest that in most organs the spatial regulation of the PDGF system is executed in concert, by localizing the ligand to the epithelium or endothelium and the receptor to the adjacent mesenchyme, in a mode similar to their expression during gastrulation and other early stages of development.

Fig. 7. Expression of Pdgfra and PDGF-A in the Rathke’s pouch and nasal epithelium. (A-C) Transverse sections through Rathke’s pouch in a 11.5 day-embryo. (D-F) Sagittal sections through the nasal cavity (14.5 days p.c.). (A and D) Bright-field illumination, (B and E) dark-field illumination of the same sections hybridized with Pdgfra. (C and F) Dark-field, hybridization of adjacent sections with the PDGF-A probe. dc, diocoel; fb, forebrain; if, infundibulum; mc, head mesenchyme; ne, nasal epithelium; Rp, Rathke’s pouch. Bars, 200 µm.
Fig. 8. Expression of *Pdgfra* and PDGF-A in the developing eye. (A-E) 10.5, (F-H) 11.5 and (I-K) 14.5-day embryos. (A-H) Transverse sections; (I-K) parasagittal sections.


Sections D, G, and J were hybridized with *Pdgfra*; sections B, E, H, and K were hybridized with PDGF-A. el, eyelid; le, lens epithelium; lf, lens fibers; lv, lens vesicle; mc, mesenchyme; os, optic stalk; pl, pigment layer of retina; nr, neural layer of retina; se, surface ectoderm; sl, sclera. Bars, 160 μm. Open arrows in J and K point to light-scattering artifacts deriving from the pigment layer and the lens fibers and not from autoradiographic grains.
**Appositional localization of PDGF-A and its receptor**

The eye develops through a series of inductive interactions. Lens induction takes place between the predisposed neuroectoderm and the head ectoderm, which is made competent for lens vesicle formation by previous contacts with the mesenchyme. As a result, the surface ectoderm invaginates and becomes the lens vesicle, which then forms the lens. Additional interactions between the developing lens and the surface ectoderm covering it are responsible for the development of the cornea (Henry and Grainger, 1990).

Fig. 8A, B and E illustrates the expression of the ligand in the optic stalk neuroectoderm and in the posterior part of the optic cup, as well as in the surface ectoderm of the head of a 10.5 day-embryo. The receptor, as seen in a successive section, was expressed in the ectodermal lens vesicle and in the surrounding mesenchyme of the head (Fig. 8C, D). Similar relative expression patterns were also observed a day later. In the prospective pigment layer PDGF-A transcripts were detected (Fig. 8H), whereas Pdgfra was expressed in the lens, which by now has separated from the surface ectoderm (Fig. 8G). The surface ectoderm, from which the lens develops, expressed the ligand, whereas its derivatives the lens vesicle and the lens, expressed the receptor. Hence at a certain stage of lens induction and in connection with it, the lens ectoderm switched from ligand to receptor expression.

Later during development (14.5 days p.c.), Pdgfra was found to be expressed in the sclera and choroid, in the lens epithelium and in the mesenchyme of the developing eyelid (Fig. 8J), whereas transcripts of PDGF-A were displayed in the surface ectoderm of the eyelid and sclera (Fig. 8K). (The signals in the pigment layer and lens fibers of Fig. 8J and K are due to light scattering). We conclude that during eye development, as throughout embryogenesis, Pdgfra and PDGF-A expression is restricted to separate, but adjacent cell sheets.

**Discussion**

The mode of PDGF-A and Pdgfra expression

In general and at the anatomical level, PDGF-A and Pdgfra expression were localized to multiple sites and cell lineages throughout development in a similar way to other growth factors (Lyons et al., 1991; Haub and Goldfarb, 1991; Hebert et al., 1991; Niswander and Martin, 1992) and growth factor receptors (Orr-Urtreger et al., 1990, 1991, 1992). The spatial regulation of Pdgfra and PDGF-A was however distinguished at a fine histological level. The present report demonstrates this characteristic transcriptional localization.

Our data, summarized in Table 1 and Fig. 9, show that the two genes are transcribed in separate, but adjacent sheets of cells as early as the late primitive streak stage and as late as lens development, or the development of skeletal muscles. To designate this characteristic expression pattern we suggest the term ‘appositional’ localization, or ‘appositional’ expression. This designation derives from a recent critical discussion of early development, where it was used to define embryonic induction across adjacent cell sheets (Slack, 1991).

The term, appositional expression, is related to autocrine and paracrine, which are frequently used terms in connection with the localization of polypeptide growth factors and receptors and their role in cancer (Sporn and Todaro, 1980; Heldin et al., 1987). In a minority of our material, cells expressing the receptor and cells expressing the ligand could not be clearly distinguished (around the exocoelome of early embryos, in early somites, in the AER and in the intercostal area). This usually coincided with simultaneous changes in cell fate and expression pattern. Whether this was due to autocrine expression, or to insufficient resolution afforded by the in situ hybridization technique, remains to be determined.

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<th>Area</th>
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<th>Pdgfra (receptor)</th>
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<tr>
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<td>Primitive ectoderm</td>
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<td>Perichondral mesenchyme</td>
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<td>Skin and derivatives</td>
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<td>Lung</td>
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Our observations suggest that the appositional pattern of PDGF-A and Pdgfra expression is connected to developmentally regulated changes in cell type. The pattern also corresponds to the epithelio-mesenchymal interactions of organogenesis. These two questions and the applicability of appositional regulation to other polypeptide growth factor-growth factor receptor systems will be discussed below.

**Correlation between changes in cell type and the expression of PDGF-A and Pdgfra**

We report that along the germ layers of the presomitic embryo, cells expressing Pdgfra transcripts face cells which contain PDGF-A transcripts, and vice versa (see schematic Fig. 9A). This reciprocal localization could contribute to morphogenic interactions between germ layers, like those described in the chicken embryo (Waddington, 1932; Azar and Eyal-Giladi, 1981).

The visceral endoderm, in contrast to the primitive ectoderm and the mesoderm, expressed both receptor and ligand transcripts, in correspondence with the developmentally defined morphology of its cells in the embryonic and extraembryonic areas, respectively. PDGF-A was mainly expressed in flattened cells covering the embryonic mesoderm, whereas Pdgfra expression characterized the more columnar cells of the extraembryonic area. At 6.5 days p.c., before this expression pattern became established, Pdgfra was expressed both in the parietal and visceral endoderm (Orr-Urtreger et al., 1992). Hence at a certain stage of development Pdgfra expression had to be replaced by PDGF-A expression in the visceral endoderm of the embryonic pole. This could be connected to the ingress of receptor-bearing mesoderm cells between the visceral (embryonic) endoderm and the primitive ectoderm.

Another developmentally regulated product of the visceral endoderm has been reported by Dziadek and Adamson (1978). They found that embryonic, but not extraembryonic type, visceral endoderm cells selectively synthesize α-fetoprotein. The visceral and parietal endoderm are differentiated derivatives of the primitive endoderm of the blastocyst (Gardner and Rossant, 1979; Gardner, 1983). The morphological change between embryonic and extraembryonic visceral endoderm cell types could be an additional step in primitive endoderm differentiation. These differentiation steps are thought to be connected to interactions between the visceral endoderm and the egg cylinder (Dziadek, 1978; Hogan et al., 1981; Gardner, 1983) and they may involve, among other factors, PDGF-A and Pdgfra.

Developmentally regulated changes of PDGF and PDGF receptor expression were also observed in connection with somite differentiation. Mesenchymal aggregates, representing early somites, expressed mainly the receptor (Orr-Urtreger et al., 1992). Following differentiation however, the myotome became restricted to ligand transcription, whereas the dermatome expressed the receptor (Figs 2 and 9B). A third developmental switch of PDGF-A expression was observed during lens development, where the surface ectoderm, which expresses PDGF-A in all of its localizations, switched to Pdgfra expression, whence it invaginated to form the lens vesicle (Figs 8 and 9C).

PDGF-A expression in the floor plate of the neural tube may also be worth noting here, because of this structure’s effect on neuronal cell type and axon guidance, as well as on morphogenetic interactions between somites and their derivatives and other axial structures (Yamada et al., 1991; Bovolenta and Dodd, 1991).

**Epithelio-mesenchymal interactions and the localized expression of Pdgfra and PDGF-A**

Classical transplantation and transfilter reconstruction experiments demonstrate the role of epithelio-mesenchymal interactions in the development of numerous organs (for a review see Saxen et al., 1980). The specificity of these interactions largely depends on the anatomical and genetic origin of the mesenchyme (Deuchar, 1975; Kollar and Fisher, 1980). Although this emphasizes the importance of the mesenchyme-to-epithelium direction, it is assumed that a circuit of interactions is at the basis of organogenetic induction.

We studied the relative expression of PDGF-A and Pdgfra in numerous organs (see Table I). In most, with the exception of the eye, the receptor was expressed in the mesenchyme, whereas the ligand in the epithelium. It follows that the direction of interaction may be a consistent element of developmental regulation of PDGF-A and Pdgfra. Following this argument, it remains to be determined whether there are receptor ligand pairs mediating interactions in the opposite, mesenchyme-to-epithelium direction.

It should be taken into consideration that the actual developmental activity of the PDGF system, which contains two receptors (α and β) and two ligands (A and B), is probably more complex than appears from the expression of one ligand-receptor pair (Heldin and Westmark, 1989). Its complexity is expected to be highest during the period of organogenesis, when both the β receptor and PDGF-B are transcriptionally active, whereas in the early embryo the α and A isoforms are expressed selectively (Mercola et al., 1990).

We conclude that the appositional regulation of Pdgfra and PDGF-A coincides in developmental time and space with a number of inductive events, which are known to occur across cell sheets. In addition we observed that the relative expression of Pdgfra and PDGF-A may change, ‘switch’, in connection with morphological and functional changes in the cells which transcribe them, probably as a result of developmental regulation.

**Modes of spatial regulation of growth factors and their receptors**

The appositional expression pattern of PDGF-A and Pdgfra described here, is not unique or exclusive for these molecules or to murine development. Similar transcriptional localization was recently reported by Holmgren and his colleagues (1991) for the B and β isoforms of PDGF. They demonstrated that during advanced placental development, PDGF-B and its β receptor were transcribed at separate, but adjacent sites. PDGF-R β was expressed in fibroblast-like and smooth muscle cells surrounding the blood vessel, whereas PDGF-B transcripts were displayed in cells situated more centrally, in the blood vessel endothelium. In the
earliest capillaries PDGF-B and PDGF-R β expression could not be distinguished and this was interpreted as an early autocrine phase. PDGF-A expression, which they have also investigated, was detected in smooth muscle cells of the blood vessel intima, whereas its receptor, Pdgfra, was transcribed in the mesenchymal stroma of the placenta. These data (Holmgren et al., 1991) suggest that human PDGF-B and PDGF-R β, similar to PDGF-A and Pdgfra, display appositional expression patterns at certain localizations. They also confirm our findings regarding the expression of the A and α isoforms, and collectively indicate that appositional regulation may be characteristic for this receptor tyrosine kinase subfamily and their ligands.

Recently the developmental expression of another related receptor-ligand pair c-kit/W and its ligand SCF/Steel, has been reported. The kit receptor, which belongs to the PDGF-R sub-group of receptor tyrosine kinases (Ullrich and Schlessinger, 1990), is expressed at multiple sites, including those defective in its W mutations (Orr-Urtreger et al., 1990). Studies comparing the expression of this receptor and its ligand simultaneously, revealed that in several tissues and organs, including visceral endoderm, primitive ectoderm, adult gonads, olfactory bulb, cerebral cortex, hippocampus and cerebellum, c-kit and SCF, similar to PDGF-A and Pdgfra, are transcribed in separate but adjacent sheets of cells (Motro et al., 1991; Keshet et al., 1991).

Another member of the PDGF-R subfamily is the c-fms receptor. Its ligand, CSF-1, is a macrophage-granulocyte growth factor (Sherr et al., 1985). Strong accumulation of CSF-1 was reported in the uterine epithelium, whereas c-fms was expressed in the decidua and later in the spongiosphoblast, suggesting that they may contribute to materno-foetal interactions through a separately localized, complementary transcriptional regulation (Regenstreif and Rossant, 1989).

The localized expression of fibroblast growth factors (FGF) and their receptors can also be compared. FGF receptors belong to a separate subgroup of receptor tyrosine kinases. Developmental expression of FGF-4 (Niswander and Martin, 1992) and FGF-5 (Haub and Goldfarb, 1991; Hebert et al., 1991) was detected in certain areas of the primitive ectoderm and primitive streak. Later they were mostly expressed in the nascent mesoderm. The localized expression of the two FGF receptors, flg and bek (bek has high affinity for FGF-4; A. Yayon, personal communication) was also investigated at this stage of development. Their transcription was localized to the primitive ectoderm of the early embryo, and on its appearance concentrated to the mesoderm and its derivatives (Orr-Urtreger et al., 1991), similar to FGF-4 and FGF-5. It follows, that at least during early development, for which comparable data are available, the two FGF receptors as well as the two fibroblast growth factors were expressed in the same cell sheets. These data taken together suggest that the developmental localization of certain FGF and FGF-R could be significantly different from the appositional expression pattern seen in the PDGF-R subgroup and their ligands.

Appositional regulation restricts the sites where a receptor and its ligand are transcribed. It is remarkable that this exacting regulation should be reinforced by various additional mechanisms operating at different molecular levels. They include alternative splicing (Flanagan et al., 1991), post-translational modification (LaRochelle et al., 1991) and the mature protein’s affinity to proteoglycans (Klagsbrun and Baird, 1991; Massagué, 1991). These mechanisms localize polypeptide growth factors to the cell membrane, or to intracellular matrix components close to their source of secretion. One reason for these multiple mechanisms may be to ensure the accurate localization of inductive interactions and to fend off the consequences of misregulated oncogenes.

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References


Appositional localization of PDGF-A and its receptor

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