Roles of PDGF in animal development

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Summary

Recent advances in genetic manipulation have greatly expanded our understanding of cellular responses to platelet-derived growth factors (PDGFs) during animal development. In addition to driving mesenchymal proliferation, PDGFs have been shown to direct the migration, differentiation and function of a variety of specialized mesenchymal and migratory cell types, both during development and in the adult animal. Furthermore, the availability of genomic sequence data has facilitated the identification of novel PDGF and PDGF receptor (PDGFR) family members in *C. elegans*, *Drosophila*, *Xenopus*, zebrafish and mouse. Early data from these different systems suggest that some functions of PDGFs have been evolutionarily conserved.

Introduction

Platelet-derived growth factor (PDGF) was first identified in a search for serum factors that stimulate the proliferation of arterial smooth muscle cells (Ross et al., 1974). Since then, mammalian PDGFs have been extensively characterized in culture-based assays, where they have been shown to drive cellular responses including proliferation, survival, migration, and the deposition of extracellular matrix (ECM) and tissue remodeling factors. Knockout studies have demonstrated that many of these cellular responses to PDGFs are essential during mouse development. The genes that encode two ligands, *Pdgfa* and *Pdgfb*, and both receptors, PDGF receptor alpha and PDGF receptor beta (*Pdgfra*, *Pdgfrb*), have been knocked out in the mouse. These studies have demonstrated that PDGFB and PDGFRβ are essential for the development of support cells in the vasculature, whereas PDGFA and PDGFRα are more broadly required during embryogenesis, with essential roles in numerous contexts, including central nervous system, neural crest and organ development (Levéen et al., 1994; Soriano, 1994; Boström et al., 1996; Soriano, 1997; Fruttiger et al., 1999; Karlsson et al., 1999; Gnessi et al., 2000; Karlsson et al., 2000). Because of the severe and pleiotropic phenotypes of *Pdgfa* and *Pdgfra* knockout mouse embryos, many primary functions of PDGFs remained elusive until being addressed in experiments using conditional gene ablation and gain-of-function transgenics. *Pdgfr* signaling mutants have also been generated in which specific tyrosine residues in the receptor cytoplasmic domains have been mutated to phenylalanines. These mutations disrupt the interactions of PDGFRs with individual cytoplasmic signaling proteins and, in some cases, abrogate a subset of receptor functions (Heuchel et al., 1999; Tallquist et al., 2000; Klinghoffer et al., 2002) (M. Tallquist and P.S., unpublished). Together, such in vivo studies have demonstrated that the PDGFs perform distinct cellular roles at successive stages of mouse embryogenesis. In many contexts, PDGFs are mitogenic during early developmental stages, driving the proliferation of undifferentiated mesenchyme and some progenitor populations (reviewed by Betsholtz et al., 2001). During later maturation stages, PDGF signaling has been implicated in tissue remodeling and cellular differentiation, and in inductive events involved in patterning and morphogenesis. In mouse and *Drosophila*, PDGFs also direct cell migration, both at short and long distances from signal sources.

This review discusses the known roles of PDGFs in development, with emphasis on cellular responses to PDGFs and how they contribute to neural/oligodendrocyte development, vascular and hematopoietic development, neural crest cell development, organogenesis, somitogenesis and skeletal patterning. Although most published studies of PDGF functions in vivo have been performed in mouse, early studies of PDGF- and PDGFR-related proteins in other model organisms suggest that some known PDGF roles (e.g. in glial/neural development) are conserved from fly to man, whereas others (e.g. in the neural crest) are specific to, but conserved among, vertebrates (Table 1).

The PDGF signaling family

In both mouse and human, the PDGF signaling network consists of four ligands, PDGFA-D, and two receptors, PDGFRα and PDGFRβ (Fig. 1). All PDGFs function as secreted, disulfide-linked homodimers, but only PDGFA and B can form functional heterodimers. PDGFRs also function as homo- and heterodimers, and in vitro assays have demonstrated that the ligands differ in their affinities for the αα, αβ and ββ receptors, as indicated in Fig. 2 (Heldin and Westermark, 1999; Li et al., 2000; Bergsten et al., 2001; LaRochelle et al., 2001). All known PDGFs have characteristic ‘PDGF domains’, which include eight conserved cysteines that are involved in inter- and intramolecular bonds. However, differences in structure and proteolytic processing segregate the ligands into two subfamilies. PDGFA and B comprise one subfamily: the amino terminal prodomains of these proteins are cleaved intracellularly so that they are secreted in their active forms (Heldin and Westermark, 1999). In addition, PDGFB and one splice form of PDGFA have negatively charged motifs near

Table 1. Similarities in the developmental expression of PDGF/PDGFR family members in different model organisms*

Tissue/cell type	Mouse (see text for references)	Zebrafish (Liu et al., 2002a; Liu et al., 2002b)	*Xenopus* (Ho et al., 1994)	*Drosophila* (Duchek et al., 2001; Cho et al., 2002; Munier et al., 2002)	*C. elegans* (Popovici et al., 2002)
Hematopoietic and vascular cells	PDGFB – vascular endothelium PDGFC – vascular smooth muscle cells PDGFD – fibroblastic adventitia PDGFRβ – vascular mural cells			PVR – hemocytes PVFs – along hemocyte migration routes	
Lung	PDGFA – lung epithelium PDGFC – trachea, lung epithelium and mesenchyme, bronchial smooth muscle PDGFRα – lung mesenchyme			PVR, PVF1 – trachea	
Gonads	PDGFA – testis cords PDGFB – endothelial cells, late embryogenesis testis cords and Leydig cells PDGFC – mesonephros/testis border PDGFRα,β – interstitial cells PDGFRβ – perimyoid cells			PVR – all follicle cells in egg chambers, border cells PVF1 – oocyte	
Nervous system	PDGFA – neurons, astrocytes PDGFC – ventral horn of spinal cord, floor plate, olfactory placode and epithelium PDGFRα – astrocytes, oligodendrocyte precursors, subset of developing neurons PDGFB, PDGFRβ – postnatal neurons	PDGFRα – placodes, optic cup, cranial ganglia, anterior and posterior lateral line ganglia		PVR – midline glia PVF2,3 – ventral nerve cord	VER-1 – amphid and phasmid sheath cells VER-2 – neural cells in head ganglia (ADL) VER-3 – dorsal ganglia head neuron (ALA)
Neural crest and neural crest migration route	PDGFA,C – surface ectoderm PDGFRα – subset of non-neuronal cardiac and cranial neural crest cells	PDGFA – pharyngeal arches PDGFRα – trunk and cranial neural crest	PDGFA – neural ectoderm, otic vesicle, pharyngeal endoderm PDGFRα – cephalic neural crest		
Somites	PDGFA,C – myotome PDGFRα – throughout early somite, then restricted to sclerotome, dermatome	PDGFRα – newly formed somites, sclerotome			
Gut	PDGFA,C – intestinal epithelium PDGFC – smooth muscle layers PDGFRα – mesenchyme, including villus clusters			PVF2,3 – foregut, hindgut	VER-1 – muscular, first and last intestinal cells VER-3 – pharynx saucer muscle cell, anal sphincter muscle
Kidney	PDGFA – early nephron epithelium, Henle's loop PDGFB – glomerular capillary endothelium, nephron epithelium (?) PDGFC – metanephric mesenchyme, early nephron epithelium PDGFD – fibrous capsule around kidney PDGFRα – mesenchyme PDGFRβ – metanephric mesenchyme, glomerular mesangial/endothelial precursors	PDGFA – pronephros		PVF1,2,3 – Malpighian tubules	

**Drosophila* and *C. elegans* each have a single PDGF- or VEGF (vascular endothelial growth factor)-like signaling network. PVF1, 2, 3 and PVR are the *Drosophila* PDGF/VEGF-like factors and their receptor, respectively. VER-1, 2, 3 are *C. elegans* VEGFR-like proteins. (To date, no definitive VER ligands have been identified or characterized.)

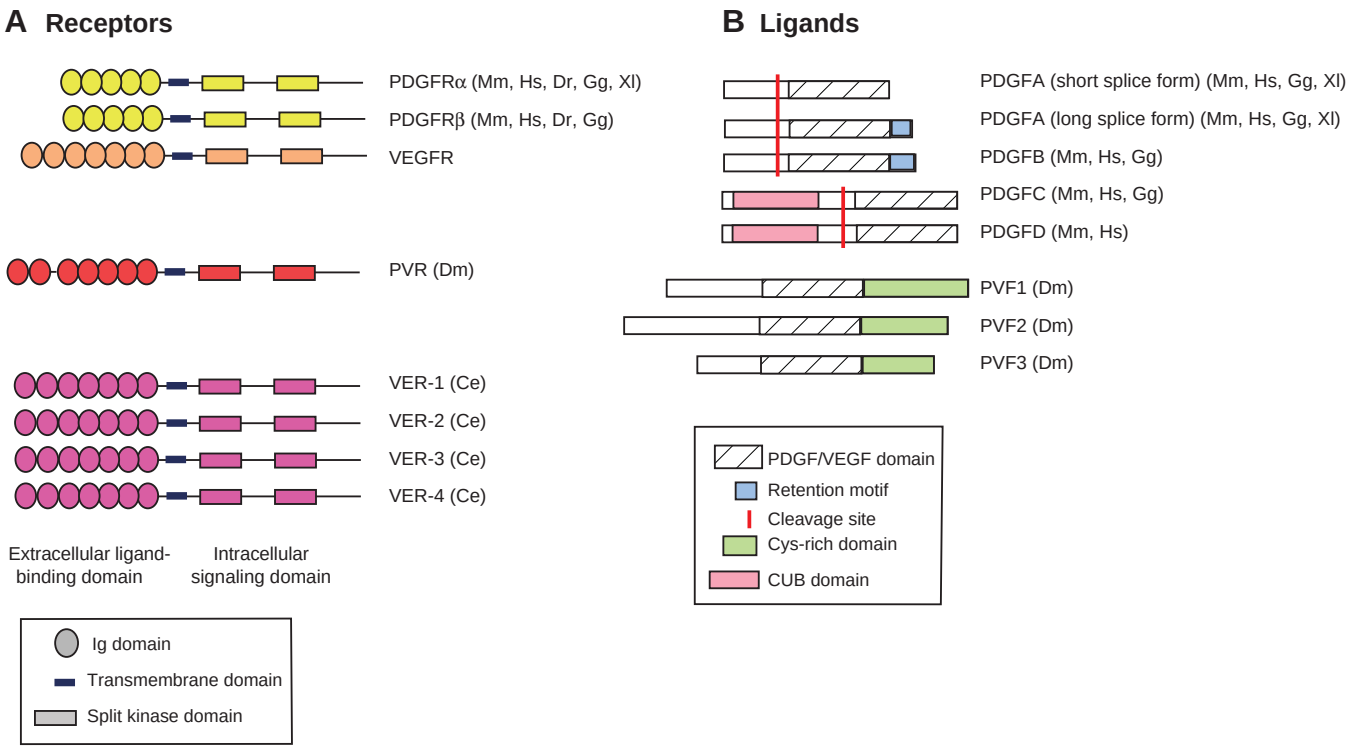

Fig. 1. The PDGF/VEGF signaling superfamily. PDGFRs (A), PDGFs (B) and related proteins (A,B) identified in different organisms. Vertebrate VEGFs and VEGFRs are not included in this figure, but a prototypical mammalian VEGFR is shown for comparison (A). Mouse, human, *Drosophila* and *C. elegans* proteins are discussed in the text (see text for references). In some teleosts, including zebrafish and pufferfish, the *Pdgfrb* genomic locus appears to have been duplicated, giving these species three PDGF receptors, PDGFRα, PDGFRβ1.1, and PDGFRβ1.2 (Williams et al., 2002). There are three known splice variants of chick PDGFA, which correspond to the short and long forms identified in mouse, as well as an additional short form (Ataliotis, 2000; Hamada et al., 2000; Horiuchi et al., 2001). Only one receptor (PDGFRα) and two splice variants of PDGFA have been identified in *Xenopus* (Mercola et al., 1988; Bejcek et al., 1990; Jones et al., 1993). *C. elegans* and *Drosophila melanogaster* each have a single PDGF/VEGF-like signaling network. *C. elegans* has four VEGFR-like proteins, VER-1 to VER-4, but no definitive VER ligand has been identified or characterized. *Drosophila* has three PDGF/VEGF ligands, PVF-1,2 and 3, and one receptor, PVR. Two splice forms of PVF-1 have been identified that differ at their N termini. Three splice variants of PVR have also been identified: two differ in the region between the second and third immunoglobulin (Ig) domains, and the third is truncated after the second Ig domain. Mm, *Mus musculus* (mouse); Hs, *Homo sapiens* (human); Dr, *Danio rerio* (zebrafish); Gg, *Gallus gallus* (chick); Xl, *Xenopus laevis* (frog); Dm, *Drosophila melanogaster* (fruit fly); Ce, *Caenorhabditis elegans* (nematode).

their carboxy termini that are cleaved extracellularly. These 'retention motifs' may interact with ECM components and/or retain these ligands near their producing cells until being cleaved (Heldin and Westermark, 1999). Unlike PDGFA and PDGFB, the C and D ligands are activated post-secretion by cleavage of their N-terminal CUB domains, which are repeat regions first identified in complement subcomponents C1r/C1s, sea urchin uEGF and human BMP-1 (Bork, 1991; Li et al., 2000; Bergsten et al., 2001). The extracellular proteases that activate PDGFC and D in vivo have not been identified, although plasmin can cleave and activate them in cell culture (Li et al., 2000; Bergsten et al., 2001).

PDGFRs are receptor tyrosine kinases. Each receptor has five immunoglobulin repeats in the extracellular ligand-binding domain and a split tyrosine kinase domain in the cytoplasmic region. Upon ligand binding, PDGFRs dimerize, activating the tyrosine kinase domains, which then autophosphorylate several tyrosine residues in the receptor cytoplasmic domains. This creates docking sites for signaling proteins and adaptors that initiate signal transduction upon PDGFR binding (Fig. 3). Both receptors can activate many of the same major signal transduction pathways, including the Ras-MAPK (mitogen activated protein kinase), phosphatidylinositol 3-kinase (PI3K) and phospholipase Cγ pathways. However, the array of interacting proteins differs slightly between PDGFRα and PDGFRβ, resulting in some differences in their functional capabilities in vivo (Rosenkranz and Kazlauskas, 1999; Klinghoffer et al., 2001). For more extensive discussions of signal transduction downstream of PDGFRs, see Heldin, and Heldin and Westermark (Heldin, 1998; Heldin and Westermark, 1999).

Recently, proteins related to mammalian PDGFs have been identified in several organisms (Fig. 1). In vertebrates, the PDGF signaling family is closely related to the vascular endothelial growth factor (VEGF) family, which functions in vasculogenesis and hematopoiesis. Although no PDGFs or PDGFRs have been identified in *Caenorhabditis elegans*, four VEGFR homologs (VER-1 to VER-4) and one putative ligand have been identified on the basis of sequence homology (Popovici et al., 1999; Popovici et al., 2002). In *Drosophila melanogaster*, three PDGF/VEGF ligands (PVF1,2,3) and a single receptor (PVR) have been identified (Duchek et al.,

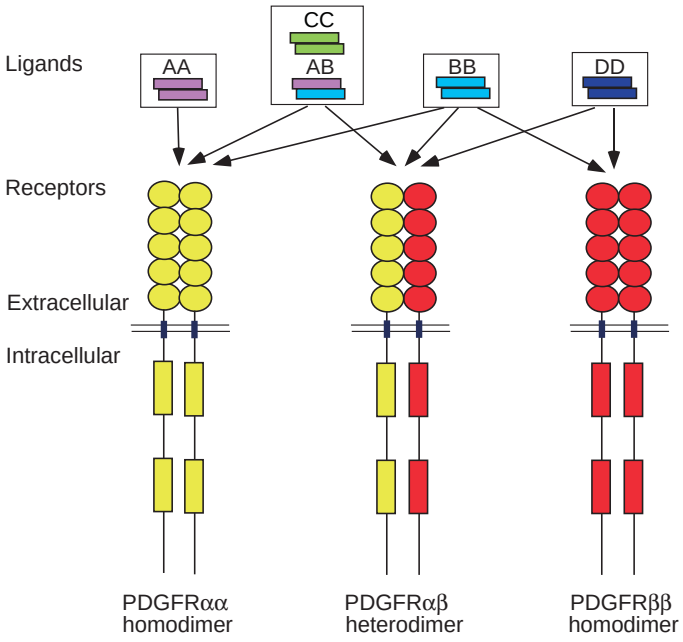

Fig. 2. Mammalian PDGF/PDGFR binding interactions. The ability of different homo- and heterodimeric PDGF ligands to bind the different receptor dimers in vitro is shown. Arrows between ligands and receptors indicate the ability of a given ligand dimer to bind to and activate the designated receptor. PDGF dimers are represented by the letters of their constituent subunits, for example, AA is a PDGFA homodimer, and AB is a heterodimer of PDGFA and PDGFB.

2001; Heino et al., 2001; Pietrokovski and Shilo, 2001; Cho et al., 2002). Alternative splicing of *Pvr* generates isoforms that differ in their expression patterns and ligand binding affinities (P. Rørth, personal communication) (Cho et al., 2002). The fact that these invertebrates each have a single VEGF- or PDGF-like signaling network suggests that the two vertebrate networks might have evolved through the duplication and diversification of a single ancestral signaling family, or through the evolutionary convergence of signaling families that have distinct origins.

PDGF roles in the development of neural and vascular support cells

Oligodendrocyte and neural development in the CNS

In the mammalian central nervous system (CNS), oligodendrocytes deposit an insulating layer of myelin around neuronal projections; these myelin sheaths are essential in facilitating neurotransmission. Oligodendrocytes differentiate postnatally from PDGFRα-expressing progenitor (O2A) cells, which arise in the mouse around embryonic day 12.5 (E12.5) in the periventricular zone of the neuroepithelium. From E12.5-E15.5, O2A cells proliferate and migrate to fill the neural tube/spinal cord (Calver et al., 1998). During this time, *Pdgfa* is expressed by neurons and astrocytes throughout the spinal cord, and PDGFC is expressed in the floor plate and ventral horn regions (Fig. 4A) (Fruttiger et al., 1999; Aase et al., 2002). Although the role of PDGFC in the embryonic spinal cord is not yet known, the expansion of the O2A population (E12.5-E15.5) is significantly impaired in spinal cords of *Pdgfa* null and *Pdgfra* signaling mutant mouse embryos; consequently, mutant pups exhibit CNS hypomyelination and tremor phenotypes (Fruttiger et al., 1999; Klinghoffer et al., 2002).

PDGFA/PDGFRα signaling is not required for the initial specification of O2A cells, or for their survival during the proliferative period (E13.5-E15.5) (Fruttiger et al., 1999; Klinghoffer et al., 2002). However, data from cell culture and transgenic experiments suggest that PDGF drives, and is limiting for, O2A proliferation in vivo (vanHeyningen et al., 2001). In primary O2A cell cultures, PDGF stimulates proliferation at rates that correlate with PDGF concentration (vanHeyningen et al., 2001). Additionally, transgenic overexpression of PDGFA in embryonic spinal cord neurons rescues the *Pdgfa*-null oligodendrocyte phenotype and increases the O2A proliferation rate between E13 and E17 in a dose-dependent manner (Calver et al., 1998; Fruttiger et al., 1999; vanHeyningen et al., 2001).

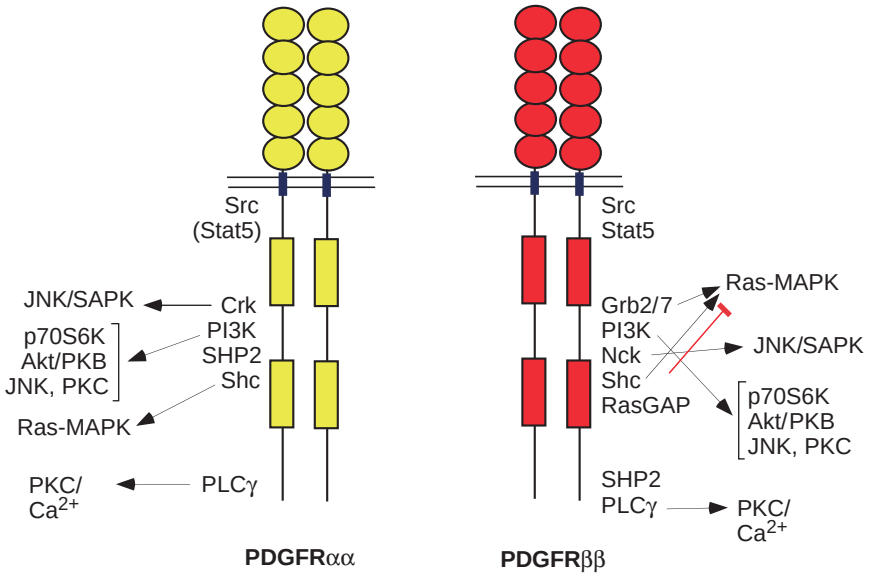

Fig. 3. Signal transduction downstream of activated PDGFRs. Upon ligand binding, PDGFRs dimerize, activate intracellular tyrosine kinase domains, and autophosphorylate several tyrosine residues to generate docking sites for signaling and adaptor proteins. Proteins that interact with activated (ligand-bound) mammalian PDGFRα and PDGFRβ homodimers are shown. Also indicated are the signaling pathways activated by the receptor-binding proteins (reviewed by Heldin and Westermark, 1999). Stat5, Signal transducer and activator of transcription 5; PI3K, phosphatidylinositol 3-kinase; SHP2, SH2-containing protein tyrosine phosphatase; PLCγ, phospholipase Cγ; JNK, Jun N-terminal kinase; SAPK, stress activated protein kinase; p70S6K, p70 S6 kinase; PKB, protein kinase B; PKC, protein kinase C; MAPK, mitogen activated protein kinase; RasGAP, Ras GTPase-activating protein.

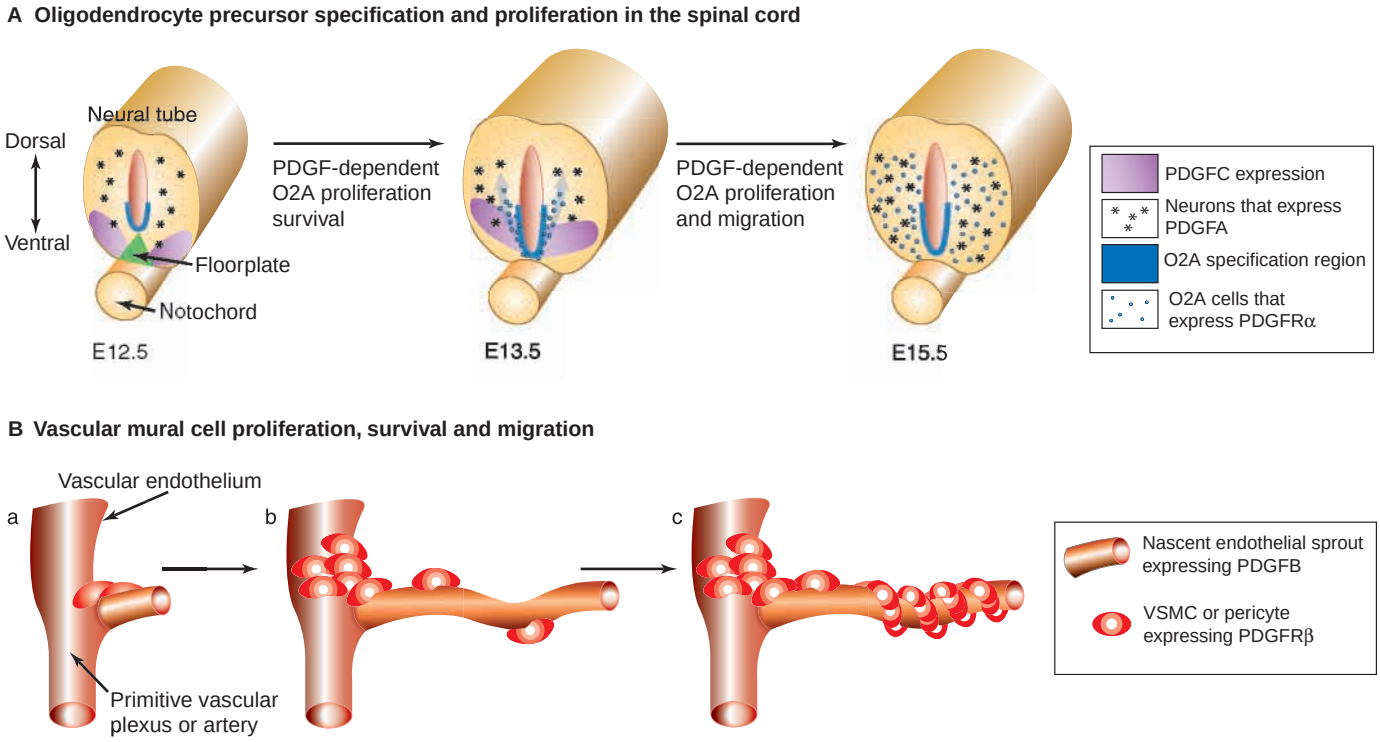

Fig. 4. Roles of PDGF in the developing nervous and circulatory systems. (A) Oligodendrocyte precursor cell development in the mouse spinal cord. Oligodendrocyte precursor (O2A) cells, which express PDGFRα, arise in the ventral periventricular zone of the mouse embryonic neural tube around E12.5. From E12.5-E15.5, O2A cells proliferate and migrate laterally and dorsally to fill the spinal cord. During this time, PDGFA is expressed by neurons and astrocytes in the spinal cord, and PDGFC is expressed by cells in the floor plate and ventral horn regions. There is evidence that PDGFA/PDGFRα signaling drives O2A cell proliferation in the spinal cord (E12.5-E15.5). PDGFRα signaling may also influence the migration of O2A cells. The general migration route taken by O2A progenitors is depicted by gray arrows. (B) Vascular mural cell proliferation and migration in the mouse. During angiogenesis, primitive vascular networks (or plexa) are remodeled through branching, sprouting, and pruning of the vascular endothelium. As new vessels form, they recruit and are coated by mural cells, contractile cells that support and stabilize new vessels. The two major classes of vascular mural cells are pericytes, which form single cell layers around capillaries, and vascular smooth muscle cells (VSMCs), which coat veins and arteries. During angiogenic remodeling, PDGFB is expressed in nascent vascular endothelial sprouts (a) and drives the proliferation of PDGFRβ-expressing pericytes and VSMCs near arterial walls and primitive vascular plexa (b). PDGFB also directs the migration and/or survival of these mural cells along endothelial sprouts (b,c). Upon reaching their destination, VSMCs and pericytes encircle and associate tightly with the endothelium (c); survival and anti-proliferative factors produced by mural cells stabilize nascent vessels.

In *Pdgfa*-null mice, graded dysmyelination has been observed in the brain and optic nerve: the most severely hypomyelinated regions are those farthest from the origins of oligodendrocyte progenitors (Fruttiger et al., 1999). Similarly, in *Pdgfra* signaling mutants, O2A cells fail to reach the dorsal region of the spinal cord, and the initial migration of these cells away from the ventricular zone is delayed (Klinghoffer et al., 2002). These observations suggest that the migration of oligodendrocytes or their progenitors is impaired in the absence of normal PDGF signaling. PDGF is capable of driving chemotactic migrations of oligodendrocytes and neural stem cells in primary cultures (Forsberg-Nilsson et al., 1998; Simpson and Armstrong, 1999), but further studies are needed to determine whether this is a primary physiological function of PDGF signaling, or whether the in vivo observations reflect proliferation defects prior to migration.

Mutant analysis has not revealed roles for PDGF in mouse neurogenesis, although PDGFRα is expressed in some neuronal populations in the developing CNS, and PDGFB and PDGFRβ are expressed in postnatal neurons (Vignais et al., 1995; Nait-Oumesmar et al., 1997; Fruttiger et al., 1999; Enge et al., 2003). However, preliminary evidence suggests that PDGF-related signals are involved in neural and glial development in *C. elegans* and *Drosophila*. *C. elegans* VER proteins are expressed by specific neurons and sheath cells (analogous to glial cells), but the functions of VER signaling are not yet known (Table 1) (Popovici et al., 2002). In *Drosophila*, *Pvr* is expressed by the ventral midline glia, and *Pvf2* and *Pvf3* are expressed in the ventral nerve cord (Cho et al., 2002). Overexpression of *Pvf1* in postmitotic neurons induces neuronal pathfinding and synaptogenic defects (Kraut et al., 2001). Functional studies in these organisms may help elucidate conserved roles of PDGFs in neural development.

PDGF roles in vascular mural cell development

During angiogenesis, primitive vascular networks are remodeled through endothelial sprouting, branching and pruning, and contractile mesenchymal cells, known as mural cells, are recruited to coat nascent vessels. The two major classes of mural cells are vascular smooth muscle cells

(VSMCs) and pericytes; these cells provide survival and antiproliferative factors that stabilize nascent vascular endothelial sprouts (Lindahl et al., 1997a; Benjamin et al., 1998; Hellström et al., 2001). The development of vascular mural cells requires PDGFB/PDGFRβ signaling (Levéen et al., 1994; Soriano, 1994; Lindahl et al., 1997a; Hellström et al., 1999). In *Pdgfb* and *Pdgfrb* knockout mice, which die perinatally with extensive hemorrhaging, numerous vessels lack or are incompletely covered by mural cells (Levéen et al., 1994; Soriano, 1994). Consequently, endothelial sprouts hyperproliferate and give rise to dilated, ectopic capillaries that are unstable, hyperpermeable and vulnerable to degeneration or regression (Lindahl et al., 1997a; Hellström et al., 2001; Enge et al., 2002).

In the mouse embryo, PDGFB secreted by vascular endothelial cells is a chemotactic, and perhaps survival, signal for *Pdgfrb*-expressing VSMC and pericyte progenitors as they leave the arterial walls and primitive plexa to migrate along newly formed endothelial sprouts (Fig. 4B) (Lindahl et al., 1997a; Hellström et al., 1999). There is also evidence that PDGFB signaling drives the proliferation of VSMC and pericyte progenitors. In wild-type mice, *Pdgfb* is expressed at sites of pericyte proliferation, and there is a twofold decrease in VSMC proliferation around developing arteries in *Pdgfb*-null mice (Hellström et al., 1999). Furthermore, there are reduced numbers of spinal cord pericytes in embryos homozygous for *Pdgfrb* signaling mutant alleles, which could be attributed to deficient pericyte proliferation or survival (M. Tallquist and P.S., unpublished). In vitro, PDGFs (AB, BB, CC) can directly induce angiogenic sprouting and branching of vascular endothelium (Cao et al., 2002), but this role has not been demonstrated in vivo.

The analysis of chimeric mice that comprise both wild-type and *Pdgfrb*-null cells has demonstrated that in a competitive in vivo context, there is a selection for *Pdgfrb*-positive cells in all VSMCs and pericytes examined (Crosby et al., 1998; Lindahl et al., 1998). Although this suggests a requirement for PDGFRβ in these cells, observations in knockout embryos indicate that some VSMC and pericyte lineages are not affected, or are only mildly affected, by the disruption of PDGFB/PDGFRβ signaling (Lindahl et al., 1997a; Fruttiger et al., 1999; Hellström et al., 1999). For example, in *Pdgfb*-null animals, pericytes are present, albeit in low numbers, in skeletal muscle, skin and adrenal gland; Itoh cells (pericyte-like cells in the liver) and VSMCs around developing arteries appear to develop normally in the absence of PDGFB/PDGFRβ signaling (Soriano, 1994; Lindahl et al., 1997a; Hellström et al., 1999). These results may reflect tissue-specific functions of other factors in mural cell development. Although their functions in the vasculature are not known, PDGFC and D are expressed in VSMCs and in connective tissue surrounding arteries, respectively, and both are expressed by endothelial cells (Uutela et al., 2001).

PDGF/VEGF signaling in embryonic hematopoeitic cell migration

In *Drosophila*, PVR signaling is essential for the embryonic migration of hemocytes, the precursors of the fly blood cell lineage (Cho et al., 2002). These cells arise as bilaterally symmetric clusters of mesoderm in the head region of stage 8 *Drosophila* embryos, and subsequently undergo stereotyped anterior, ventral and posterior migrations (Fig. 5A). The posterior migration requires PVR signaling: a mutation in *Pvr* blocks hemocyte migration into the tail and results in their clustering in the head region (Cho et al., 2002). All three *Pvfs* are expressed along the hemocyte migration route and must simultaneously be knocked down to mimic the receptor mutant phenotype (Cho et al., 2002). However, results from ectopic expression experiments indicate that the ligands differ in their capacity to influence hemocyte behavior in vivo. For example, hemocyte migration is re-directed to sites of high ectopic *Pvf2*, but not *Pvf1*, expression (Cho et al., 2002). Similarly, overexpression of *Pvf2*, but not of *Pvf1*, drives hemocyte hyperproliferation in *Drosophila* larvae (Munier et al., 2002). PVF2 (and/or PVF3) may therefore be the relevant ligand(s) for hemocyte development in vivo.

Hematopoietic progenitor cells also undergo stereotyped migrations during vertebrate development (Traver and Zon, 2002). Although *Pdgfb* and *Pdgfrb* knockout mouse embryos exhibit hematopoietic defects, liver transplant experiments have demonstrated that PDGFB/PDGFRβ signaling is not directly required for the embryonic specification, migration or differentiation of hematopoietic stem cells (Levéen et al., 1994; Soriano, 1994; Kaminski et al., 2001). In mice, these processes may rely instead on VEGF signaling, as VEGFR2 is essential for development of the hematopoeitic lineage (Shalaby et al., 1995). The split from the PVR in an open circulatory system to PDGFRs and VEGFRs in a closed circulatory system may reflect an evolutionary need to discriminate signaling to different cell types in the blood and vasculature.

PDGF functions in neural crest: proliferation, migration and tissue remodeling

In the mouse, PDGFRα is required cell autonomously for the development of a subset of non-neuronal neural crest cell derivatives in the cardiac and cranial regions (Morrison-Graham et al., 1992; Soriano, 1997; Tallquist and Soriano, 2003). Deletion of *Pdgfra* in the murine neural crest cell lineage leads to defects in palatal closure and fusion, nasal and cardiac septation, and the development of several bone and cartilage structures (Morrison-Graham et al., 1992; Soriano, 1997; Tallquist and Soriano, 2003). Furthermore, thymus size is often reduced in the absence of PDGFRα signaling (Morrison-Graham et al., 1992; Tallquist and Soriano, 2003). Homozygous *Patch* mutant embryos (which carry a large genomic deletion that encompasses the *Pdgfra* gene) and NCC-*Pdgfra* embryos (which have *Pdgfra* conditionally deleted in neural crest cells) exhibit several cardiac septation and remodeling defects similar to those observed in neural crest ablation studies (Kirby et al., 1983; Kirby and Waldo, 1990; Morrison-Graham et al., 1992; Tallquist and Soriano, 2003). This implies that these *Pdgfra* mutants are deficient for functional cardiac neural crest cells, though it is unclear whether the deficiency is in the number of cells that reach target tissues, or in neural crest cell differentiation or function at target sites. *Pdgfa* and *Pdgfc* are highly expressed in neural crest target tissues in the mouse, including the epithelial lining of the branchial arches and branchial pouches (Orr-Urtreger and Lonai, 1992; Ding et al., 2000; Aase et al., 2002). These ligands may act as long-range migration cues or post-migratory signals for neural crest cells in the cranial region. In *Xenopus* and zebrafish embryos, *Pdgfa* and *Pdgfra* are similarly

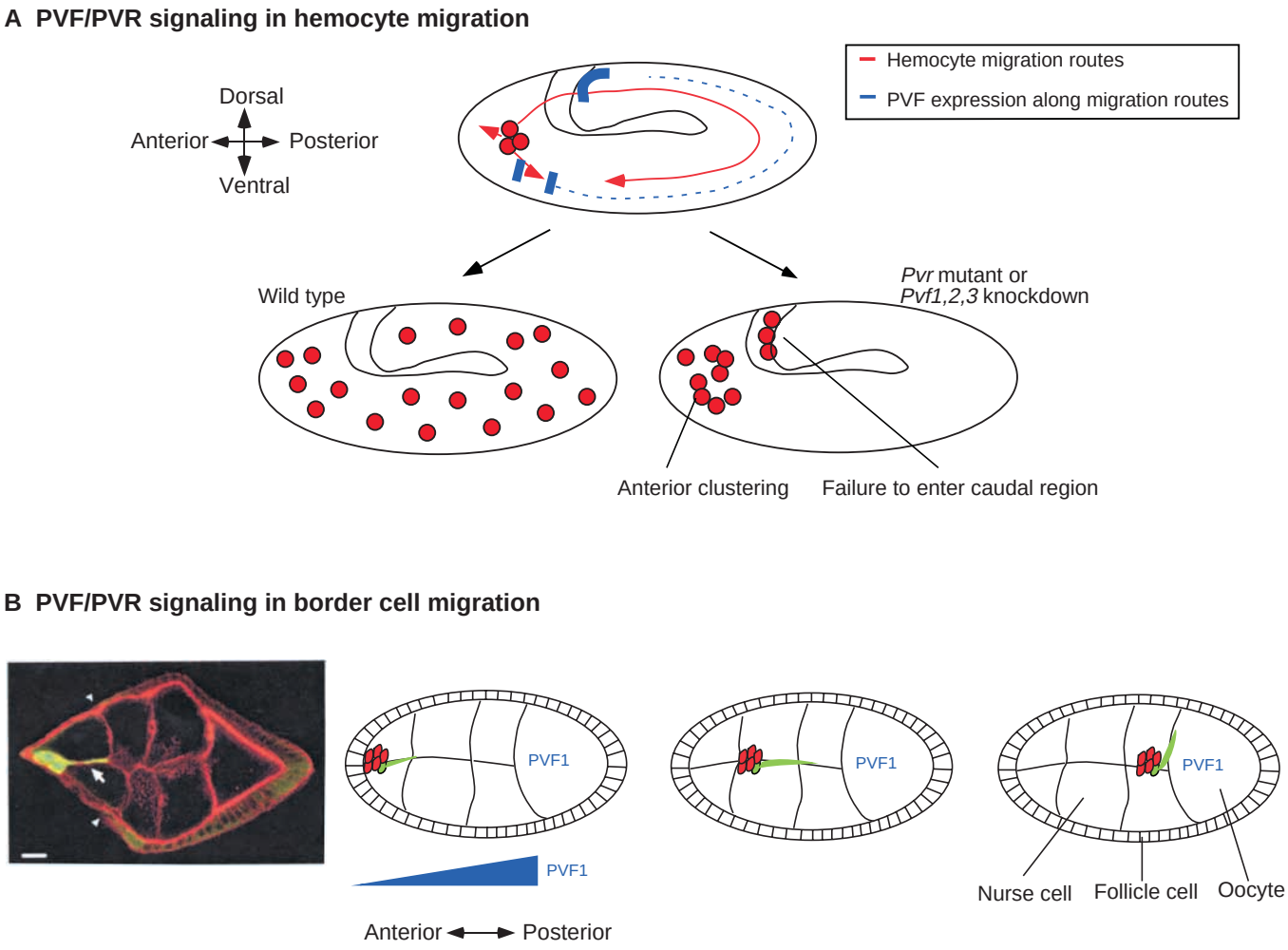

Fig. 5. PVF/PVR signals direct cell migration during *Drosophila* development. (A) Hemocyte migration. Hemocytes (red) originate as bilateral clusters in the anterior region of *Drosophila* embryos. These cells migrate anteriorly, posteriorly and ventrally to populate the wild-type embryo (red arrows indicate migration routes). PVF ligands are expressed along the embryonic hemocyte migration routes and guide or enable hemocyte migration: in *Pvr* mutant or *Pvf1,2,3* knockdown embryos, hemocytes cluster in the head region and fail to enter the caudal region of the embryo. (B) Border cell cluster migration in response to PVF1. In the left-most panel, a stage 9 *Drosophila* egg chamber expressing *lacZ* in border cells is stained with anti-β-galactosidase (green; border cells) and phalloidin (red; actin), and shows a long cellular extension (LCE; white arrow), which protrudes from one cell of the border cell cluster toward the oocyte. PVF1 is expressed by oocytes in *Drosophila* egg chambers, and PVR is expressed by all follicle cells, including the border cell cluster (red, green). In response to a graded PVF1 signal (blue), one border cell of the cluster protrudes a long cellular extension (LCE, green) toward the source of PVF1. This LCE guides the migration of the border cell cluster from the anterior cortex of the egg chamber to its final posterior location next to the oocyte. The panel on the left is copied with permission from Fulga and Rørth (Fulga and Rørth, 2002). Scale bar: 20 μm.

expressed in the pharyngeal region and neural crest, respectively, indicating that PDGF roles in neural crest are likely to be conserved among vertebrates (Table 1) (Ho et al., 1994; Liu et al., 2002a; Liu et al., 2002b).

PDGFRα signaling has been implicated in the migration and survival of cranial neural crest cells. In explant experiments, PDGFA enhances neural crest cell motility (without affecting proliferation) and stimulates cultured neural crest cells to secrete matrix metalloproteinase 2 (MMP2) and its activator, MT-MMP (Robbins et al., 1999; Li et al., 2001). MMP2 influences neural crest cell migration and may play a role in tissue remodeling (Robbins et al., 1999). In *Pdgfra*-null embryos, increased apoptosis and differences in ECM deposition have been observed along the neural crest migratory pathway (Morrison-Graham et al., 1992; Soriano, 1997). However, NCC-*Pdgfra* mutant embryos do not exhibit defects in neural crest survival, migration or proliferation, and phenotypes observed in these embryos suggest that PDGFRα is required for postmigratory neural crest functions (Tallquist and Soriano, 2003). The discrepancy between the *Pdgfra*-null and conditional-null data could reflect cell non-autonomous requirements for PDGF signaling in neural crest cell migration and/or survival, or could be due to differences in the precise location or stage at which the neural crest defects were analyzed in the different studies.

PDGF roles in organogenesis

PDGFs play distinct roles at successive stages of mammalian

Table 2. PDGF roles in mammalian organogenesis.

	Early role	Later role	References
Kidney	Mesenchymal proliferation* (4) Proliferation/survival of mesangial cell precursors (1) Interstitial cell proliferation and/or survival* (3)	Migration of mesangial cells into glomerular space (2) Glomerular angiogenesis* (5)	(1) Levéen et al., 1994; Soriano, 1994; Lindahl et al., 1998 (2) Lindahl et al., 1998; Arar et al., 2000; Ricono et al., 2003 (3) Li et al., 2000 (4) Levéen et al., 1994 (5) Cao et al., 2002
Prenatal testis	Proliferation in testis and at coelomic surface (6)	Branching of coelomic vessel (6,7) Fetal Leydig cell differentiation (6) PMC differentiation or function* (7,8)	(6) Brennan et al., 2003 (7) Uzumcu et al., 2002 (8) Gnessi et al., 1993; Chiarenza et al., 2000
Postnatal testis	Interstitial cell proliferation (9)	Maturation of seminiferous tubules (9) Adult Leydig cell differentiation (9) Migration, survival, and/or function of adult Leydig cells or their precursors* (9)	(9) Gnessi et al., 2000
Skin/hair	Proliferation of dermal layer (10)	Proliferation of *Pdgfra*-expressing mesenchymal cells (10) Adhesion or matrix deposition between tissue layers* (12) Organization of pre-papillae (10) Formation of the dermal sheath lining (10) Formation of the hair canal at the dermis/epidermis interface (11) Hair cycle re-entry (10) Melanocyte development (12)	(10) Karlsson et al., 1999 (11) Takakura et al., 1996 (12) Soriano, 1997
Intestine	Proliferation of mesenchyme between epithelium and muscle layers	Proliferation of *Pdgfra*-expressing mesenchyme Organization of villus clusters Differentiation of epithelial and mesenchymal villus cell types Migration and/or function of *Pdgfra*-positive mesenchymal cells within forming villi*	Karlsson et al., 2000
Lung	Mesenchymal proliferation* (14)	Proliferation of *Pdgfra*-expressing mesenchyme Migration and/or survival of *Pdgfra*-expressing mesenchymal cells* (13,15) Induction of septation, differentiation of alveolar myofibroblasts, secretion of matrix and remodeling factors* (13)	(13) Boström et al., 1996; Lindahl et al., 1997b (14) Li and Hoyle, 2001 (15) Sun et al., 2000
Somites/ skeleton	Somitic or presomitic mesoderm proliferation (16) Reciprocal signaling between sclerotome and myotome (17)	Chondrogenesis (17,18) Neural arch positioning (19) Vertebral arch closure (16,19,20) Development of craniofacial bones and cartilage (16,17,20)	(16) Soriano et al., 1997 (17) Tallquist and Soriano, 2003 (18) Ataliotis, 2000 (19) Payne et al., 1997 (20) Grüneberg and Truslove, 1960

*Putative and/or hypothesized functions of PDGFs; these roles have been suggested by expression, cell culture, and/or preliminary functional data.

organogenesis (Table 2, Fig. 6). During early stages, PDGFs drive mesenchymal proliferation. For example, PDGFRα signaling is essential for interstitial cell proliferation in the early embryonic testis and kidney, and for mesenchymal proliferation in early intestine, skin and lung development (Karlsson et al., 1999; Karlsson et al., 2000; Li et al., 2000; Sun et al., 2000; Li and Hoyle, 2001; Brennan et al., 2003). In each of these contexts, PDGFRα is broadly expressed in the mesenchyme, and the mitogenic PDGF function is elicited by paracrine signals from local epithelium. There is no evidence that PDGFs act as long-range proliferative signals during development, and although ligands and receptors are co-expressed in some cell types, the role(s) and regulation of autocrine signaling in organogenesis are not yet understood.

After an early period of organ growth, *Pdgfr* expression generally becomes restricted to a small subpopulation of mesenchymal cells that stops proliferating and undergoes directed migrations. At these stages of organ maturation, PDGFs are no longer mitogenic but instead drive the migration, differentiation and/or function of *Pdgfr*-expressing cells (Fig. 6). Some of these later roles for PDGF are discussed below; others are summarized in Table 2. The roles of *Pdgfr*-expressing cells are not clear in several contexts, but they are often positioned such that they may participate in inducing or supporting morphogenetic processes.

PDGF functions in the developing kidney

PDGFB/PDGFRβ signaling is required for the development of capillary tufts in glomeruli, which are filtration units in the kidney. In *Pdgfb* and *Pdgfrb* knockout mice, these tufts either

do not form or consist of enlarged, unbranched capillary loops that lack mesangial cells (pericyte-like cells surrounding glomerular capillaries) (Levéen et al., 1994; Soriano, 1994). There is evidence that *Pdgfrb*-positive mesenchymal progenitors give rise to both endothelial and mesangial cells of glomerular capillary tufts, which are thought to form by de

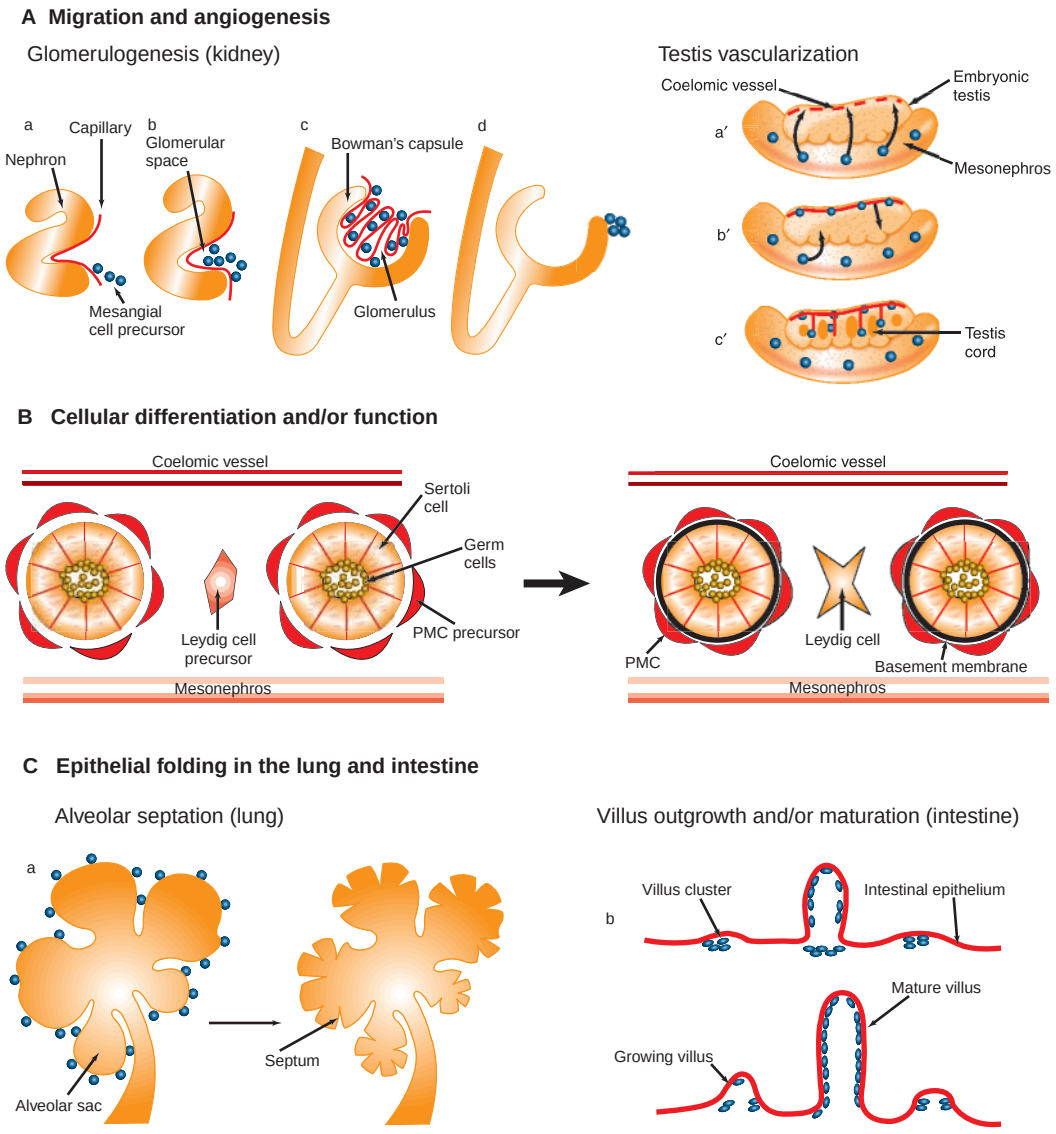

Fig. 6. PDGF roles in mammalian organogenesis. In several early developmental contexts, epithelial PDGF drives proliferation of undifferentiated mesenchyme expressing PDGFRα. However, during organ maturation, PDGF signals direct several other cellular functions. (See text for more details.) (A) Cell migration and angiogenesis. In the developing kidney, PDGFB/PDGFRβ signaling is essential for the migration of *Pdgfrb*-expressing endothelial/mesangial cell precursors (blue) from (a) the cleft of the S-shaped nephron into (b,c) the glomerular space. These cells give rise to the glomerular capillary bed (red) and its mesangial cells (pericyte-like cells that coat glomerular capillaries; c). (d) In *Pdgfb*- and *Pdgfrb*-null embryos, few *Pdgfrb*-expressing precursors migrate to the nephron cleft, and these cells fail to enter the glomerular space; capillary beds fail to form in these embryos and capillary aneurysms are observed in the glomerular space. The embryonic testis vasculature develops through the formation of the coelomic vessel (a′,b′) and the branching of this vessel between testis cords (c′). Endothelial cells (blue) migrate from the mesonephros into the testis, where they contribute to the coelomic vessel and its branches (red). PDGFRα is required for both endothelial cell migration and branching of the coelomic vessel. In both the kidney and testis, PDGF signals may directly induce angiogenic branching of the primitive vasculature, although this has not been clearly shown in vivo. (B) Cellular differentiation and/or function. PDGF signals are essential for the differentiation and/or function of interstitial cell types in the developing testis. PDGFRα is required for the differentiation of both fetal and adult Leydig cells. The ligand(s) required for fetal Leydig cell development are not yet known, but PDGFA is required for the development of adult Leydig cells, which replace fetal Leydig cells during postnatal testis maturation. There is evidence from in vitro studies that PDGF signals may induce perimyoid cell (PMC) differentiation and/or function. These cells originate in the interstitium and differentiate into contractile, smooth muscle-like cells that associate tightly with testis cords. Together, PMCs and Sertoli cells produce the basement membrane around cords. The source(s) of PDGFs that direct interstitial cell differentiation/function is not clear, although PDGFs are secreted from the coelomic vessel, the mesonephros and testis cords. (C) Epithelial folding. *Pdgfra*-expressing mesenchymal cells cluster at sites of future epithelial folding during lung, skin and intestine morphogenesis. Although the *Pdgfra*-expressing cells are essential for these morphogenetic processes, their roles and the role(s) of PDGF signaling in folding morphogenesis are not yet known. Late in embryogenesis, *Pdgfra*-positive mesenchymal cells (blue) in the lung migrate to sites of alveolar septation (a), a postnatal process in which the air sac epithelium invaginates and is lined with specialized matrix. Septation does not occur in the absence of the *Pdgfra*-positive cells, whose development requires PDGFA during embryogenesis. During intestine maturation, *Pdgfra*-positive mesenchymal cells (blue) cluster at sites of future villus formation (b). The intestinal epithelium subsequently folds into the lumen of the intestine and *Pdgfra*-positive cells migrate from the clusters to line the forming villus. *Pdgfra* expression is maintained in these cells during villus maturation, and, in PDGFA-null mice, villus structure and epithelial differentiation are abnormal.

novo vasculogenesis and angiogenesis. *Pdgfrb*-expressing cells originate in the metanephric mesenchyme and migrate, first to the nephron cleft (before glomerulogenesis) and then into the glomerular space where vasculogenesis and angiogenesis take place (see Fig. 6A) (Hyink et al., 1996; Woolf and Loughna, 1998; Ricono et al., 2003). In *Pdgfb*- and *Pdgfrb*-null embryos, there is a reduction, and in some cases a loss, of the *Pdgfrb*-positive mesenchymal population at the nephron cleft. This suggests that although these cells are capable of reaching the cleft, their migration or premigratory proliferation is impaired (Levéen et al., 1994; Lindahl et al., 1998). PDGFB/PDGFRβ signaling is not required for proliferation or survival of the putative progenitor cells at the nephron cleft, but is absolutely required for their migration into the glomerular space (Fig. 6A) (Lindahl et al., 1998; Arar et al., 2000; Ricono et al., 2003). Given the angiogenic potential of PDGFs demonstrated in vitro (Cao et al., 2002), PDGFs may also induce branching of the glomerular capillary endothelium.

PDGF functions in mouse and *Drosophila* gonad development

PDGF signaling is required for the development of the interstitium and vasculature in the mouse testis (Fig. 6A,B). Perhaps due to these roles, *Pdgfra*-null embryos have enlarged testis cords, a phenotype also seen in wild-type embryonic testes treated with PDGFR inhibitors (Uzumcu et al., 2002; Brennan et al., 2003). Early in embryonic testis development, *Pdgfa*, *Pdgfb* and *Pdgfra* are expressed in scattered cells within the XY gonad, and *Pdgfc* and *Pdgfra* are expressed at the coelomic surface and the mesonephros/gonad boundary (Brennan et al., 2003). At this time, PDGFRα signaling is required for the proliferation and/or differentiation of interstitial cells, including fetal Leydig cells (Fig. 6B) (Brennan et al., 2003), which are testosterone-producing interstitial cells that differentiate shortly after testis cord formation. Culture-based studies indicate that PDGF signaling may also be involved in the development or function of perimyoid cells (PMCs) later in embryonic testis development. PMCs are smooth muscle-like interstitial cells that associate tightly with testis cords and, together with Sertoli cells, secrete components of the basement membrane that surrounds testis cords (Hadley et al., 1985; Skinner et al., 1985). At the time of PMC differentiation, *Pdgfa* expression becomes restricted to testis cords and *Pdgfrs* are expressed by all interstitial cells (Uzumcu et al., 2002; Brennan et al., 2003). PDGF stimulation of cultured PMCs induces the expression of smooth muscle-specific genes, enhanced ECM production, cellular contraction and stress fiber formation (Gnessi et al., 1993; Chiarenza et al., 2000). These in vitro findings may reflect in vivo roles of PDGFs in PMC differentiation or function. In the postnatal testis, PDGFs continue to be essential for interstitial development: PDGFA (which does not appear to be essential in the embryonic testis) is required postnatally for adult Leydig cell development, interstitial cell proliferation and completion of spermatogenesis (Gnessi et al., 2000). The molecular mechanisms underlying these roles await further investigation.

PDGF signaling is also essential for the development of the testis vasculature in the mouse (Brennan et al., 2003). The embryonic testis becomes vascularized through the angiogenic branching of the coelomic vessel, which forms at the testis surface and extends branches between the testis cords (Brennan et al., 2002). Endothelial cells migrate from the mesonephros into the testis and contribute to the coelomic vessel branches. This migration is thought to be driven by an attractive signal from Sertoli cells (Merchant-Larios and Moreno-Mendoza, 1998; Nishino et al., 2001). PDGFRα signaling is required for both coelomic vessel branching and endothelial migration (Fig. 6A) (Brennan et al., 2003). PDGF signals may directly induce angiogenic branching of the coelomic vessel. However, co-culture experiments have demonstrated that endothelial migration requires PDGFRα signaling specifically in the testis (Brennan et al., 2003), suggesting that PDGFs are not themselves long-range chemoattractants for endothelial cells, but are required upstream of the activation or secretion of a different signaling protein.

In contrast to this indirect role of PDGF in cell migration during mammalian testis development, PVF1 directly induces migration during the development of the *Drosophila* egg chamber. In this context, PVF1 is secreted by the oocyte and acts as a long range chemoattractant for border cells, a cluster of somatic cells that express PVR (Fig. 5B) (Duchek et al., 2001; McDonald et al., 2003). In response to a graded PVF1 signal, one border cell of the cluster sends out a long cellular extension (LCE) towards the oocyte. The LCE adheres to nurse cells along the migration route and guides the migration of the border cell cluster from the anterior cortex of the egg chamber to its final localization adjacent to the oocyte (Fulga and Rørth, 2002). PVR signaling is partially redundant with EGFR (epidermal growth factor receptor) signaling in directing border cell migration (Duchek et al., 2001). However, ligand specificity has been demonstrated in misexpression experiments in which border cell migration is redirected to sites of high PVF1, but not PVF2 or Gurken (an EGFR ligand), expression (McDonald et al., 2003). PVF1 is also required for the normal distribution of *Drosophila* E-Cadherin in migrating border cells (McDonald et al., 2003). These cellular responses to a PVF1 gradient in the developing egg chamber may represent common mechanisms by which PDGFs induce and guide cell migration.

Lung and intestine development: signaling centers at sites of epithelial folding?

Epithelia undergo patterned folding or invagination during the maturation stages of mammalian lung, intestine and skin development. In each of these organs, PDGF signaling is required for the development of mesenchymal cells that express *Pdgfra* and localize at sites of future folding (Fig. 6C). The roles of these cells, and specifically of PDGF signaling to these cells, are unknown, but phenotypic data suggest that they may play an active role in inducing or regulating morphogenesis or differentiation.

In the mammalian lung, PDGFA/PDGFRα signaling is required for alveolar septation, a postnatal process whereby the air sac epithelium invaginates to form septa and is lined by specialized matrix and mesenchymal cells (Boström et al., 1996; Klinghoffer et al., 2002). This process depends on a *Pdgfra*-positive mesenchymal population, the embryonic development of which requires PDGFA. *Pdgfra*-positive cells originate subjacent to distal branches of embryonic lung epithelium; these cells associate tightly with growing tubules and may modulate embryonic lung growth and/or branching (Sun et al., 2000). Late in gestation, *Pdgfra*-positive cells

undergo PDGFA-dependent migrations, dispersing as single cells to line prospective terminal sacs throughout the lung (Lindahl et al., 1997b). These cells localize near sites of future septation at the onset of alveogenesis (Boström et al., 1996; Lindahl et al., 1997b). *Pdgfra*-positive mesenchymal cells in the lung may be precursors to alveolar myofibroblasts (Lindahl et al., 1997b), or might provide signals that induce septum formation, matrix deposition or differentiation (e.g. of alveolar smooth muscle cells) during alveogenesis. Further studies are needed to distinguish between these models, and to determine whether postnatal PDGFRα-mediated signals are required for alveogenesis.

In the developing skin and intestine, postmitotic *Pdgfra*-positive mesenchymal cells similarly migrate to form clusters that underlie sites of future epithelial folding (Karlsson et al., 1999; Karlsson et al., 2000). In the intestine, these cells are called villus cluster cells and pericryptal fibroblasts. Although their functions in morphogenesis are not known, phenotypic data suggest that these clusters provide instructive or permissive signals for villus outgrowth (Karlsson et al., 2000). For example, *Pdgfa*-null animals have fewer villus clusters, possibly due to an earlier mitogenic role of PDGFA, and fewer villi extend into the intestinal lumen (Karlsson et al., 2000). Furthermore, in *Pdgfa*- and *Pdgfra*-null mice, villi are often pleated with aberrant distributions of differentiated cell types. These phenotypes could be secondary to the loss of earlier roles of PDGFs in villus cluster cells. However, mesenchymal cells from the clusters migrate into growing villi during villus outgrowth and maintain *Pdgfra* expression throughout intestinal development (Karlsson et al., 2000). *Pdgfa* is not expressed in the villus epithelium (Karlsson et al., 2000), but conditional inactivation of *Pdgfra* and/or *Pdgfc* in the intestine may reveal PDGF roles in villus maturation.

PDGF signaling in somite and skeletal patterning

Phenotypic analysis of *Pdgfra*-null mouse embryos suggests that, as in organogenesis, PDGFs play successive roles in somite and skeletal development. PDGF signals have been implicated in driving somitic or presomitic mesoderm proliferation: *Pdgfra* is expressed throughout epithelial somites, and the somites of *Pdgfra*-null mouse embryos are smaller than those of wild-type littermates (Soriano, 1997). Later in development, *Pdgfra* expression becomes restricted, with high expression in sclerotome and lower expression in dermatome. *Pdgfa* and *Pdgfc* are both expressed in the myotome (Orr-Urtreger and Lonai, 1992; Ding et al., 2000; Aase et al., 2002). In *Pdgfra*-null embryos, myotome compartments in rostral somites are abnormally shaped and are frequently fused with the myotome of adjacent somites. These abnormalities are paralleled by later skeletal phenotypes, which are, in many cases, characterized by the aberrant growth or fusion of skeletal elements (Soriano, 1997). Together, expression and phenotypic data suggest that PDGFRα signaling is involved in feedback signaling between the myotome and the sclerotome, although the specific cellular responses to PDGF signaling during somitogenesis are unknown (Soriano, 1997; Tallquist et al., 2000).

Experimental data suggest that PDGFs induce chondrocyte differentiation during the outgrowth of limbs and of somite-derived skeletal elements (such as the ribs). In somite micromass cultures, PDGF-AA and -BB are potent effectors of chondrogenesis, driving chondrocyte maturation without inducing proliferation (Tallquist et al., 2000). Similarly, studies in the chick have demonstrated that PDGF-AA induces cartilage formation in vivo and in limb bud micromass cultures (Ataliotis, 2000).

PDGFs and human disease

Pdgf and *Pdgfr* mutant mice exhibit several phenotypes that are reminiscent of human diseases. These mice can serve as experimental models in which to study the etiology, progression and treatment of these diseases, and the contributions of different genes and/or pathways to disease development. For example, *Pdgfra* mutant mice model neural crest deficiency disorders, such as cardiac and nasal septation defects, cleft face and cleft palate (Grüneberg and Truslove, 1960; Morrison-Graham et al., 1992; Soriano, 1997; Tallquist and Soriano, 2003). In addition, knockout and transgenic studies have highlighted two distinct modes by which aberrant PDGF function can lead to the lung disease emphysema. *Pdgfa*-null mice and *Pdgfra* signaling mutants that survive past birth exhibit early postnatal emphysema as a result of failed alveolar septation (Boström et al., 1996; Lindahl et al., 1997b; Sun et al., 2000; Klinghoffer et al., 2002). By contrast, transgenic overexpression of *Pdgfb* in the lung gives rise to emphysema in adult mice; these animals have thickened septa, as well as enlarged saccules, severe inflammation and fibrosis (Hoyle et al., 1999).

The relevance of these and other mouse mutant phenotypes to human disease and development has been highlighted by findings of abnormal PDGF/PDGFR expression or activity in human patients. A common phenotype in *Pdgfra*-null mouse embryos is a failure in neural tube closure; genetic studies have demonstrated that *Pdgfra* and *Pax1* interact genetically in the development of spina bifida occulta (Grüneberg and Truslove, 1960; Helwig et al., 1995; Soriano, 1997; Payne et al., 1997; Joosten et al., 1998). In humans, there are common *Pdgfra* promoter polymorphisms that underlie variation in transcriptional activity, and specific haplotype combinations correlate with a predisposition to neural tube defects (Joosten et al., 2001). Promoter haplotypes with higher *Pdgfra* transcriptional activity are over-represented in patients with sporadic spina bifida; conversely, homozygosity for a common promoter haplotype with low transcriptional activity was not observed in any cases of sporadic spina bifida, suggesting that this variant protects or selects against the development of this condition (Joosten et al., 2001).

Although *Pdgfb* and *Pdgfrb* knockout mice die perinatally, PDGFB roles in adult vascular, kidney and retina pathogenesis have been elucidated through studies using conditional knockout mice, transgenic mice and mice harboring mutations that alter PDGF activity without causing early lethality. In the vasculature, *Pdgfrb* activation caused by loss of the low density lipoprotein receptor-related protein (LRP1) in VSMCs leads to aneurysms and the development of atherosclerotic lesions. These vascular defects are associated with the disruption of the elastic lamina around vessels, and with hyperproliferation of VSMCs (Boucher et al., 2003). In addition, mice that lack *Pdgfb* or *Pdgfrb*, as well as some *Pdgfrb* signaling mutants, exhibit cardiac hypertrophy (Levéen et al., 1994; Soriano, 1994; Klinghoffer et al., 2001). Whether this reflects a primary defect in the cardiomyocytes or a physiological response to

other vascular defects remains unclear. The form of cardiac hypertrophy in *Pdgfrb* signaling mutants resembles that commonly associated with hypertension (Klinghoffer et al., 2001). However, hyperproliferation of cardiac fibroblasts was observed in transgenic mice overexpressing *Pdgfc*, which also have cardiac hypertrophy (Li et al., 2000). The disease states in the *Pdgfb* and *Pdgfrb* mutant mice may be etiologically distinct from the hypertrophy in the *Pdgfc* overexpression model.

The kidney and the retina are both particularly sensitive to mutations in the *Pdgfrb* signaling domain: some signaling mutant mice (which survive to adulthood) exhibit the hallmarks of glomerulosclerosis and proliferative retinopathy (Klinghoffer et al., 2001) (M. Tallquist and P.S., unpublished). In humans, PDGFs have been shown to be upregulated in glomerulosclerosis, as well as in diabetic and allograft-related nephropathies (Langham et al., 2003; Eitner et al., 2003), and PDGF inhibitors can inhibit pathogenic mesenchymal proliferation in the kidney (Savikko et al., 2003). PDGFA and PDGFB are also upregulated in human patients with diabetic proliferative retinopathy (Freyberger et al., 2000), and PDGF inhibition using a dominant-negative PDGFRα can inhibit the progression of proliferative vitreoretinopathy in an ex vivo model (Ikuno and Kazlauskas, 2002). In the mouse retina, proliferative disease can result from either excessive PDGF in astrocytes or deficient PDGF in the vascular endothelium, both of which result in a shortage of pericytes (Fruttiger et al., 1996; Andrews et al., 1999; Klinghoffer et al., 2001; Enge et al., 2002; Forsberg-Nilsson et al., 2003). These responses to the up- and downregulation of PDGF indicate that PDGF-directed retinopathy therapies will probably need to be targeted in a cell type- (or PDGFR-) specific manner.

PDGFs have also been implicated in the etiology of human cancer. Many years ago, the transforming gene in Simian Sarcoma Virus, *sis*, was found to encode PDGFB (Doolittle et al., 1983; Waterfield et al., 1983). Since then, PDGF hyperactivity has been observed in invasive gastric carcinomas and gliomas, and in several other types of human cancer (Nakamura et al., 1997; Hermanson et al., 1992; Hermanson et al., 1996). In human gastric cancers, PDGF has been found to be an effective prognostic marker: high levels of PDGFA correlate with high grade carcinomas and reduced patient survival (Katano et al., 1998). A recent study also identified *Pdgfra*-activating mutations in a subset of human gastrointestinal stromal tumors, for which *Pdgfra* may prove to be a useful molecular marker and therapeutic target (Heinrich et al., 2003). Further studies of PDGF function in both normal and diseased gastrointestinal tracts may shed light on how gastric tumors originate and progress.

Both PDGFs and PDGFRs are upregulated in human gliomas and astrocytomas, and *Pdgfra* expression levels are higher in more advanced forms of gliomas than in less malignant glial tumors (Hermanson et al., 1992; Hermanson et al., 1996). Transgenic overexpression of PDGFB in neural progenitors or glial cells induces the formation of oligodendrogliomas and oligoastrocytomas in the mouse (Dai et al., 2001). In these mouse models, it was recently shown that *Ink4a-Arf*, a tumor suppressor gene that is commonly mutated in high-grade human gliomas, cooperates with PDGF in the development and malignant progression of gliomas (Dai et al., 2001). This is one example of a disease model, generated by genetic manipulation of PDGF in the mouse, which could prove useful in elucidating both the cellular roles of PDGFs in tumorigenesis, and the way(s) in which PDGFs or PDGFRs interact with other oncogenes and tumor suppressor genes in the progression of human cancer.

Closing remarks

Over the next few years, our knowledge of PDGF-dependent developmental processes should be vastly expanded through functional studies of recently identified PDGF family members in various organisms, and through experiments in which multiple ligands or receptors are simultaneously disrupted. Because requirements for PDGF signaling in early development can obscure later roles, it will also be necessary to use conditional mutagenesis to elucidate the serial roles of PDGFs within specific developmental contexts. Although expression data suggest that some PDGF roles are conserved among different organisms, this needs to be investigated through functional studies. The different model systems each have their strengths that will be invaluable in elucidating how the PDGFs and receptors are regulated, the cellular mechanisms by which they exert their actions, and how they interact with other signaling systems during development. In addition to elucidating normal developmental functions of PDGFs, genetic manipulations of PDGF function have identified disease states that are induced and/or influenced by aberrant PDGF activity. These model systems are invaluable tools for investigating disease etiology, therapeutic approaches and the interactions of PDGFs with other genes that contribute to multigenic diseases. To address these problems effectively, we need to understand the specific cellular functions that are driven by PDGF signaling in normal development and physiology.

As the roles of recently identified factors become known, we will be able to address the question of how receptor mutant phenotypes relate to those of the ligands. In both flies and mammals, questions remain as to the genetic interactions between PDGF ligands and their receptors. In the mouse, the *Pdgfrb* and *Pdgfb* knockout phenotypes appear virtually the same (Levéen et al., 1994; Soriano, 1994), suggesting that PDGFD may have non-essential or subtle roles during embryogenesis. However, the *Pdgfa*- and *Pdgfra*-null phenotypes differ dramatically in their severity: *Pdgfra*-null embryos do not survive past E15, with most dying by E11.5, whereas many *Pdgfa*-null mice survive past birth (M. Hellström, C. Betsholtz and P.S., unpublished) (Boström et al., 1996; Tallquist and Soriano, 2003). This discrepancy is likely to be due to PDGFRα-mediated functions in response to PDGFB and PDGFC. Preliminary evidence indeed suggests that *Pdgfc*-null embryos exhibit cleft palate and spina bifida, and die perinatally. In addition, *Pdgfa/Pdgfc* double-null embryos recapitulate phenotypes associated with *Pdgfra* deficiency (H. Ding and A. Nagy, personal communication).

The early lethality of *Pdgfra*-knockout mice indicates that the PDGFs play essential roles in early embryonic development that are not yet fully understood. Studies in *Xenopus* have implicated PDGF signaling in gastrulation, neural tube closure, and mesoderm adhesion or migration during early embryonic development (Ataliotis et al., 1995; Symes and Mercola, 1996). Only a subset of the phenotypes observed in *Xenopus* experiments are recapitulated in

Pdgfra/Pdgfrb double-null mouse embryos, which exhibit failed anterior neural fold closure but no gastrulation defects (P.S., unpublished results). This may reflect the functional divergence of PDGFs between amniotic and anamniotic species. Recent studies have demonstrated that PDGFRα and PDGFRβ are essential for extraembryonic development in the mouse. PDGFRα is expressed in the parietal endoderm (E8-E10) and in cells lining the chorioallantoic plate (E9-E13). In wild-type embryos, PDGFRα-expressing cells migrate from the periphery to populate the chorioallantoic plate. This migration fails to occur in *Pdgfra*-null embryos, and the chorioallantoic plate vasculature does not develop normally (Hamilton et al., 2003), perhaps contributing to their early lethality. PDGFB/PDGFRβ signaling is required in extraembryonic tissues at later stages (E13-E17) for the maturation of the vasculature in the labyrinthine layer of the placenta, where fetal/maternal gas and nutrient exchange takes place. The labyrinthine layers of *Pdgfb*- and *Pdgfrb*-null embryos have deficient numbers of pericytes and trophoblasts, abnormally large vessels and a reduction in vascular surface area (Ohlsson et al., 1999). It is not known how these extraembryonic vascular defects impact embryonic development.

Another unresolved question is how PDGFs and PDGFRs are regulated at the levels of transcription, splicing, and ligand maturation or cleavage. Establishing what factors contribute to these different aspects of PDGF regulation will help associate PDGF signaling with other genetic pathways. For example, mouse PAX1 has been shown to regulate *Pdgfra* transcription and to interact genetically with *Pdgfra* in mouse neural tube development (Helwig et al., 1995; Joosten et al., 1998). Alternative splicing of *Pdgfa* is conserved among vertebrates, and in vitro studies suggest that this modulates the range of PDGFA activity by dictating the usage of the retention motif (Mercola et al., 1988; Heldin, 1998; Heldin and Westermark, 1999; Horiuchi et al., 2001). However, the in vivo function(s) and developmental utilization of PDGFA variants and PDGF retention motifs have not yet been determined, nor is it known which proteases cleave the retention motifs of PDGFA and B, or the CUB domains of PDGFC and D, in vivo. These proteases define the diffusibility and sites of action of PDGFs, and so studies of their localization and regulation will greatly further our understanding of PDGF functional regulation in vivo. It is not clear whether the activity of invertebrate ligands is similarly regulated by cleavage of retention motifs or CUB-like domains. However, *Drosophila Pvf1* is subject to alternative splicing, and the two known variants differ at their amino termini (Cho et al., 2002). Splice-form-specific analyses of PDGFs, PVFs and PVR are needed to clarify how alternative splicing modulates PDGF signaling in vivo.

To understand why cellular responses to PDGFs differ at distinct times or locations in development, we need to determine what other signals influence PDGF-mediated responses, and to identify transcriptional targets of PDGF signaling in different cellular contexts. A cell's response to PDGF is probably influenced by its expression of PDGF receptors and ligands as well as by its differentiation state and local signaling environment. Different PDGF ligands may drive distinct responses in some developmental contexts. However, the response of a given cell to the same ligand may change over time because of crosstalk with other signaling pathways. For example, a recent study demonstrated that antagonistic crosstalk occurs between LRP1 and PDGFRβ signaling in the vasculature (Boucher et al., 2003). In addition, PDGF signals might drive different transcriptional responses in naive versus committed or differentiated cells due to developmental changes in chromatin structure or the activity of transcriptional regulatory factors. The identification of transcriptional targets of distinct PDGFs in different contexts should shed light on the mechanisms by which cellular responses to PDGFs are specified.

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