Int. J. Dev. Biol. 39: 817-825 (1995)

Role of platelet-derived growth factors in mouse development

CHRISTER BETSHOLTZ*

Department of Medical Biochemistry, University of Göteborg, Göteborg, Sweden

ABSTRACT The current understanding of platelet-derived growth factor (PDGF) physiological functions *in vivo* is discussed in the context of mouse development. In particular, the review focuses on recent experiments in which genetic approaches have been applied in order to mutate the PDGF and PDGF receptor genes in the mouse. Thus, the PDGF-B and PDGF beta receptor (PDGFRb) genes were recently inactivated by homologous recombination in embryonic stem cells. Their phenotypes are highly similar, displaying cardiovascular, hematological and renal defects. The latter is particularly interesting since it consists of a specific cellular defect: the complete loss of kidney glomerular mesangial cells. As such, the phenotype not only sheds light on the developmental importance of PDGF-B-PDGFRb interactions, but also reveals information about the function of mesangial cells. Based on detailed morphological studies of mutant glomeruli and the absence of urin collection in the ruinary bladder, I propose that the mesangial cells function as interior "filter holders", the "filter" consisting of the glomerular basement membrane and associated cells types. The filter holder model would predict that glomerular filtration is critically dependent on an interior structural support of the filter, which is normally provided by the mesangial cells and the mesangial matrix. In addition to the mutants generated by gene targeting, the mouse *patch* mutation is discussed. This deletion encompassed the PDGFRa locus. The last part of the review focuses on the problems encountered when interpreting gene knockout phenotypes in the physiological functions of gene products.

KEY WORDS: *platelet-derived growth factor (PDGF), gene knockout, mesangial cells*

Introduction

Platelet-derived growth factors (PDGF) were first identified as products of platelets which stimulated the proliferation *in vitro* of connective tissue cell types such as fibroblasts (Kohler and Lipton, 1974), smooth muscle cells (Ross *et al.*, 1974) and glia cells (Westermark and Wasteson, 1976). Consequently, PDGF was assumed to play a role in wound healing. This idea has been reinforced by the demonstration that PDGF, in addition to being growth promoting, also stimulates directed migration (Grotendorst *et al.*, 1981, 1982; Seppä *et al.*, 1982; Bressler *et al.*, 1985; Siegbahn *et al.*, 1990) as well as modulated extracellular matrix production by fibroblasts and smooth muscle cells (Bauer *et al.*, 1985; Majack *et al.*, 1985, 1987). It has subsequently become clear that there exists a multitude of sources for PDGF in addition to platelets. The expression of PDGF by cells such as endothelial cells (Barrett *et al.*, 1984; Collins *et al.*, 1985, 1987), epithelial cells (Goustin *et al.*, 1985; Antoniades *et al.*, 1991; Orr-Urtreger and Lonai, 1992) and macrophages (Shimokado *et al.*, 1985; Martinet *et al.*, 1986) seems to accompany states of proliferation or functional activation as they occur in embryonic development or in tissue repair processes. Neuronal expression of PDGF, on the other hand, is increased until adulthood and stays constitutive in the uninjured adult brain (Sasahara *et al.*, 1991; Yeh *et al.*, 1991; Sasahara *et al.*, 1992). On the basis of its expression patterns *in vivo*, in mammalian embryonic and extraembryonic tissues, and in conjunction with many pathological processes in the adult, it has been proposed that PDGFs are of developmental and pathophysiological importance in addition to their putative roles as wound hormones. Detailed and recent reviews on the possible roles for PDGFs in disorders such as neoplasia and atherosclerosis have been presented elsewhere (Ross *et al.*, 1986; Raines *et al.*, 1990; Westermark and Heldin, 1991; Raines and Ross, 1993; Westermark, 1993). The primary aim here is to summarize to-date's understanding of the roles of PDGFs in mammalian development. This understanding is still rudimentary; however, through the recent analyses of embryonic PDGF ligand/receptor expression patterns, analyses of the mouse patch mutant – a natural deletion encompassing one of the PDGF receptors, and through the analyses of PDGF ligand and receptor knock-out mice generated by gene targeting, some principles are beginning to emerge. The last sections will deal with possibilities and problems with gene targeting approaches, with special reference to PDGF mutant mice.

Abbreviations used in this paper: PDGF, platelet-derived growth factor; PDGFR, platelet-derived growth factor receptor.

***Address for reprints:** Department of Medical Biochemistry, University of Göteborg, S-413 90 Göteborg, Sweden. FAX: 31416108.

0214-6282/95/$03.00

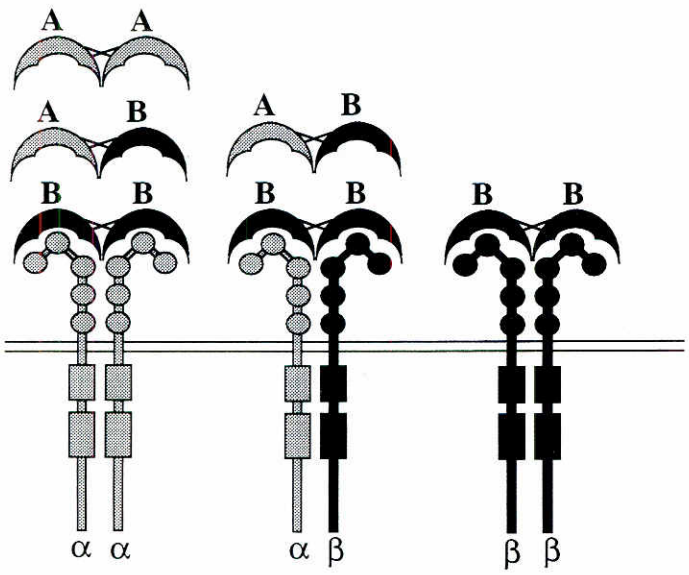


Fig. 1. Schematic illustration of the PDGF-PDGF receptor binding specificities. *Note that any PDGF isoform may bind to PDGFRα dimers whereas PDGF-BB is the only high affinity ligand for PDGFRβ dimers.*

The PDGF family of ligands and receptors

The active PDGF molecule consists of 2 polypeptide chains linked together by disulfide bonds (for reviews on the molecular structure of PDGF and PDGF receptors, see Claesson-Welsh, 1993 and Heldin *et al.,* 1993). Two types of chains, A and B, are encoded by different but related genes and may form homo- or heterodimers (PDGF-AA, PDGF-BB or PDGF-AB). These display differences in receptor binding properties. Two PDGF receptor subtypes have been identified, the α- and β-receptors (PDGFRα and PDGFRβ). These are receptor tyrosine kinases encoded by different but related genes (for a recent review on PDGF receptor signal transduction, see Cooper and Kazlauskas, 1993). The currently proposed model for PDGF ligand-receptor interaction is illustrated in Figure 1. It postulates that PDGF dimers activate PDGF receptors through the generation of active PDGF receptor dimers in which each chain of the PDGF dimer binds one receptor molecule. Here, the B-chain acts as a promiscuous ligand in that it binds both PDGFRβ or -α. PDGFRα, on the other hand, is a promiscuous receptor in that it binds both PDGF A- or B-chains. The A-chain appears to have a slightly higher affinity for PDGFRα than the B-chain, yet both affinities are compatible with physiological signaling. Although excluded from the model in Figure 1, it is possible that the A-chain also possesses a low (physiologically relevant?) affinity for PDGFRβ (Seifert *et al.,* 1993). Additional challenge to the model comes from experiments indicating that monomeric PDGF-B may exert signaling via PDGFRβ (Andersson *et al.,* 1992).

PDGF and PDGF receptor expression patterns during development

PDGFA mRNA has been shown to be present in the unfertilized frog egg (Mercola *et al.,* 1988) and both PDGFA and PDGFRα are expressed in mouse preimplantation embryos (Rappolee *et al.,* 1988) from the one-cell up to the blastocyst stage. Following implantation of such embryos, PDGFA and PDGFRα adopt a different but close (appositional) expression pattern (for details, see Morrison-Graham *et al.,* 1992; Orr-Urtreger and Lonai, 1992; Orr-Urtreger *et al.,* 1992; Palmieri, 1992; Schatteman *et al.,* 1992). In presomitic embryos, PDGFA was shown to be expressed by the primitive ectoderm as well as the visceral endoderm, whereas PDGFRα was expressed in the mesoderm. A more complex, but sandwich-like, expression pattern was seen in older mouse embryos (E10-12) in which PDGFA expression was demonstrated in the surface ectoderm, PDGFRα in the underlying dermatome, PDGFA in the myotome and PDGFRα in the loose mesenchyme. Skeletal myoblasts appear express PDGFA throughout development and they continue to do so also *in vitro* (Sejersen *et al.,* 1986). PDGFRα, on the other hand, is expressed in the prospective joints and in the developing bone, suggesting that PDGFA-PDGFRα interaction takes place at muscle-bone attachment sites. In various developing organs, PDGFA is generally expressed in epithelial structures and PDGFRα is expressed in underlying mesenchyme. This includes mammary and salivary glands, hair follicles, the lung, the adenohypophysis, olfactory regions and the eye (see also Mudhar *et al.,* 1993). Taken together, these results are suggestive of a widespread paracrine function for PDGFA; during embryogenesis, epithelium-derived PDGFA may provide migratory, proliferative or other signals to underlying or surrounding developing connective tissue.

PDGFB and PDGFRβ-expression patterns are less well-mapped in mammalian embryos. It appears as if the level of expression of this ligand-receptor pair was generally low during early development and increased later (Mercola *et al.,* 1990). Using a lacZ gene integrated under the control of the PDGFRβ promoter, PDGFRβ expression was suggested in cephalic mesenchyme at E8.5, in the heart, the somites and in limb bud mesenchyme at E10.5 and in the heart, the choroid plexus and developing vertebrae at E12.5 (Soriano, 1994). Expression of PDGFB and PDGFRβ has also been mapped in the human fetal kidney (see below).

Both PDGFA and PDGFB expression has been found in extraembryonic mammalian tissues. PDGFB expression has, for instance, been mapped to cytotrophoblasts of the placenta (Goustin *et al.,* 1985; Holmgren *et al.,* 1991) and in placental endothelial cells (Holmgren *et al.,* 1991). PDGFRβ has likewise been found in cytotrophoblasts and is also expressed in placental vascular structures (Holmgren *et al.,* 1991).

The developing central nervous system has been in focus with regard to potential sites of PDGF-PDGF receptor interaction

TABLE 1

A SUMMARY AND COMPARISON OF PDGFB AND PDGFRβ MOUSE MUTANT PHENOTYPES

PDGFB	PDGFRβ
hemorrhaging at E19	hemorrhaging at E19
anemia with erythroblastosis	anemia with erythroblastosis
trombocytopenia	thrombocytopenia
blood vessel dilation	blood vessel dilation
heart dilation and malformation	
mesangial cell deficiency	mesangial cell deficiency

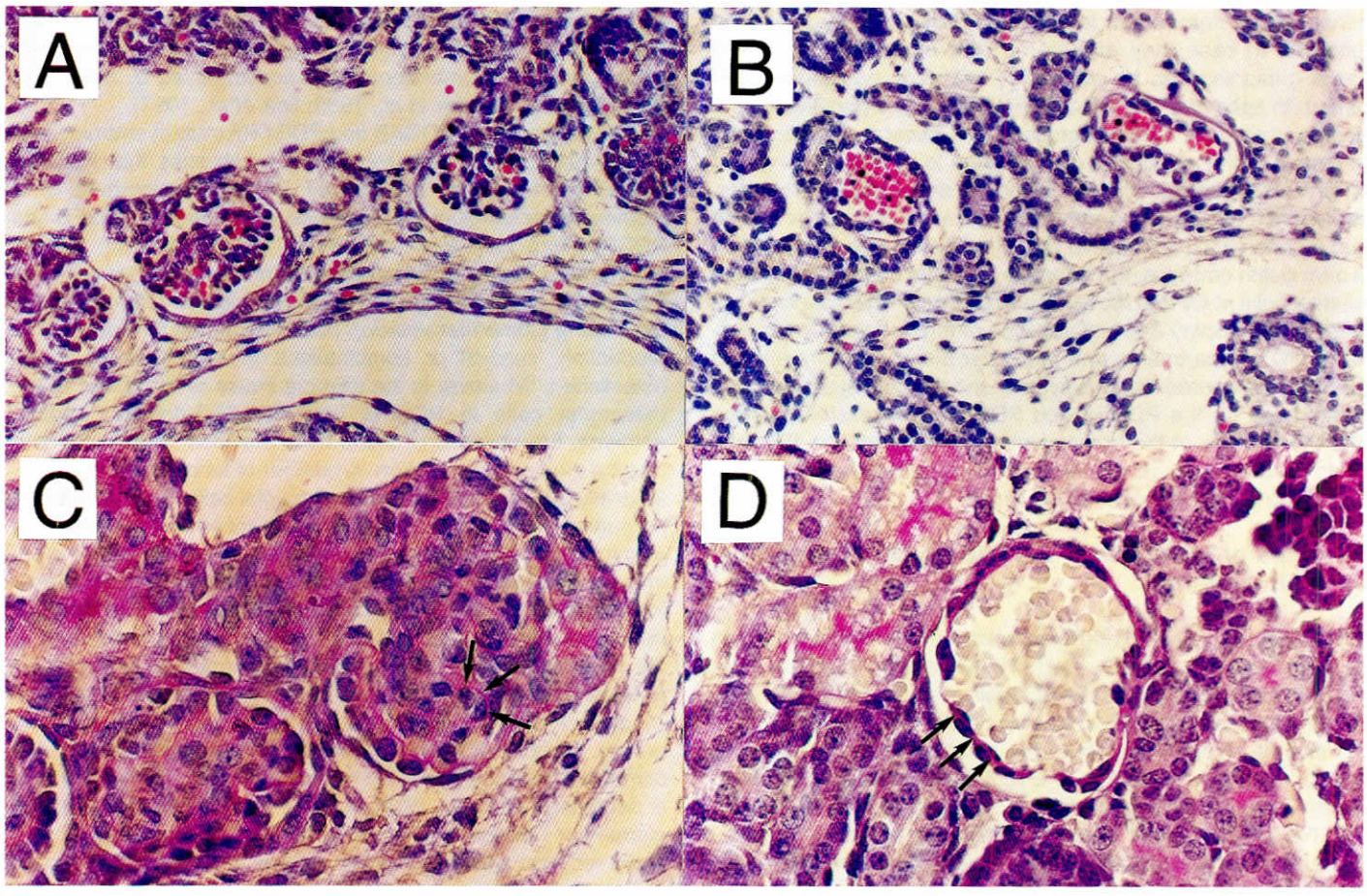


Fig. 2. Glomerulus phenotype in PDGFB mutant embryos. *Kidney sections from E17.5 PDGFB +/-* **(A,C)** *or PDGF -/- (mutant)* **(B,D)** *embryos. Note that the glomerulus is reduced in complexity from a capillary network to a single (sometimes a few) blood-filled aneurysm-like structures.* **(A,B)** *Hematoxylin eosin staining. The PAS stainings shown in* **C** *and* **D** *demonstrate that the normal folded appearance of the basement membrane* **(C,** *arrows) is absent. The capillary aneurysm-like structure in the mutant kidney* **(D)** *is delimited by a basement membrane-like structure (arrows) having cells on both surfaces.*

of physiological relevance. Neurons have been shown to express PDGFA both before birth and postnatally (Yeh *et al.,* 1991). In late prenatal, postnatal and adult brains, neuronal expression of PDGFB is also evident (Sasahara *et al.,* 1991; Sasahara *et al.,* 1992) and it has been suggested that neuronal cells may carry PDGFRβ and respond to PDGF (Smits *et al.,* 1991). Also, glial cells have been in the focus of several studies; in the optic nerve, type 1 astrocyte-derived PDGFA has been proposed to constitute the signal which drives the proliferation and determines the timing of the differentiation of O2A progenitor cells into oligodendrocytes and type 2 astrocytes (Noble *et al.,* 1988; Raff *et al.,* 1988; Richardson *et al.,* 1988; Pringle *et al.,* 1989). It has moreover been proposed that PDGFRα may be an early marker for the oligodendrocytic lineage (Pringle and Richardson, 1993; Yu *et al.,* 1994).

The mouse patch mutant

Patch is a natural mutation in the mouse that manifests as a patch-wise pigmentation deficiency in heterozygots and embryonic lethality involving a plethora of developmental abnormalities in homozygots (Grüneberg and Truslove, 1960). The patch mutation is a deletion which has been found to encompass the PDGFRα locus but potentially includes also additional genes (Smith *et al.,* 1991; Stephenson *et al.,* 1991). Thus, the phenotype of homozygous patch mutant should reflect PDGFRα-deficiency, perhaps in combination with other defects. This phenotype includes several features which correlate well with the PDGFRα expression pattern (Grüneberg and Truslove, 1960; Morrison-Graham *et al.,* 1992; Orr-Urtreger and Lonai, 1992; Orr-Urtreger *et al.,* 1992; Schatteman *et al.,* 1992; see also review by Bowen-Pope *et al.,* 1991), the pigmentation deficiency in patch heterozygots is, however, difficult to tie to the PDGFRα absence and instead suggests that the neighboring kit locus is affected. Since the structural part of the kit locus appears to be excluded from the deletion, the function of transcriptional regulatory regions may be compromised. Comparisons between the patch phenotype and PDGFA and PDGFRα gene knockout phenotypes are underway and should finally resolve these issues.

The fact that all patch embryos implant and develop normally during the early postimplantation period suggests that the PDGFA/PDGFRα coexpression seen in the preimplantation embryo

either has no function or is not rate limiting for these putative functions in case they are shared with other molecules. The same reasoning can be applied to the early postimplantation period. In spite of expression of PDGFRα in the primitive mesoderm and of PDGFA in the ectoderm and visceral endoderm, the formation of the mesoderm did not seem to be inhibited in patch embryos. Of particular interest is that as development proceeds, mesenchymal cell compartments appear to be compromised and a generalized connective tissue deficiency becomes evident in later patch embryos. For example, mutants contain fewer sclerotomal cells at E9 and those mutant embryos which survive until later stages display a virtual absence of e.g. the dermis and certain SMC compartments and moreover display malformation of the face, the central nervous system and the cardiovascular system. Based on the PDGFA and PDGFRα expression patterns and the features of the homozygous patch phenotype, it is logical to assume a crucial role for PDGFA signaling via PDGFRα in specifying embryonic connective tissue.

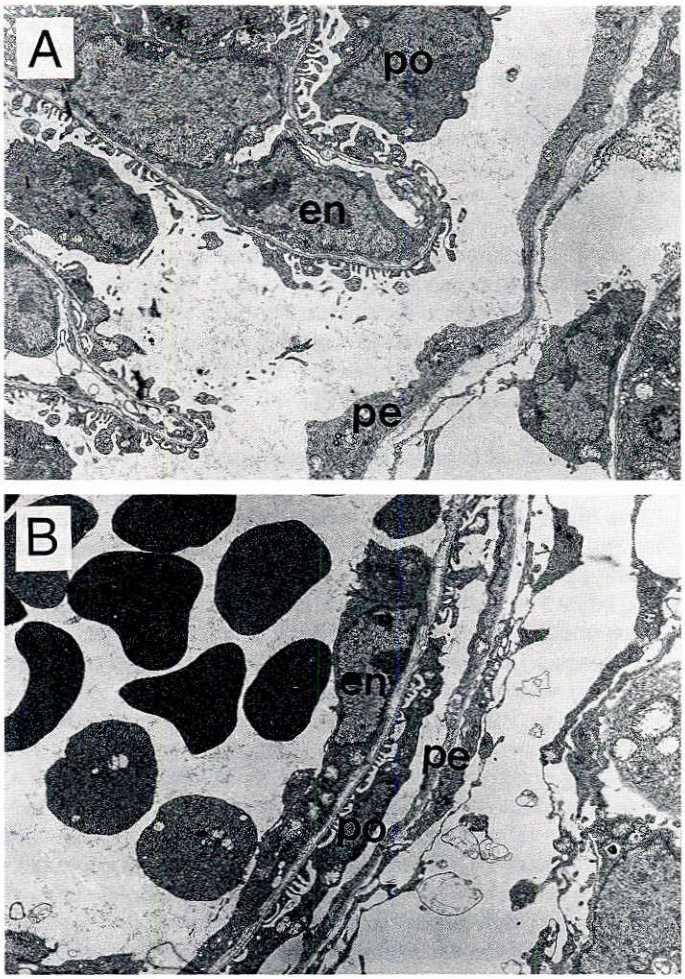
Analyses of PDGF-PDGF receptor mutants generated by gene targeting

PDGFB and PDGFRβ-deficient mice were recently generated by gene targeting (Levéen *et al.,* 1994; Soriano, 1994). A summary of their phenotypical characteristics is presented in Table 1. Both mutations are lethal to late embryos. In fact, a majority of the mutant embryos live up to the point of birth. However, following caesarean delivery at E18.5, neither mutant starts to breath regularly. Several defects common for PDGFB and PDGFRβ-mutants were observed. The most apparent one, and probably the cause of death, was a vascular or hematological defect which manifests as bleeding under the skin and in several other locations including the liver, brown fat and brain. At present, the cause of the bleedings is unclear and may relate to primary changes in the blood vessels and/or in the hematological system (see below under "The problem of defect propagation").

Both PDGFB and Rβ-deficient mice displayed a kidney glomerulus malformation in which the normal capillary tuft was replaced by a single or a few distended capillary loops (Fig. 2). Electron microscopy revealed a virtual absence of mesangial cells, whereas epithelial cells (podocytes) and endothelial cells were present, as was the basement membrane separating these two cell types. In the developing kidney, PDGFB has been shown to be expressed by the epithelial cells of the developing glomerulus (Alpers *et al.,* 1992). In the differentiated glomerulus, however, PDGFB expression is shifted from epithelial to mesangial cells (Alpers *et al.,* 1992). PDGFRβ-expression is seen in the metanephric blastema, in interstitial connective tissue and in the mesangial cells of the developing glomerulus (Alpers *et al.,* 1992, 1993). Taken together, the ligand-receptor expression patterns and the knockout phenotypes suggest that PDGFB-Rβ-interactions in the kidney drives the development of mesangial cells. More specifically, the epithelial expression of PDGFB in the early vesicular stage of the glomerulus may promote the migration of mesangial progenitors into the developing glomerulus. Alternatively, or in addition, the mesangial coexpression of PDGFB and Rβ seen later may promote the survival, differentiation or further growth of the mesangial compartment during the expansion of the glomerular tuft. It should be noted that mesangial expression of PDGFB and Rβ is downregulated in the normal adult kidney, but is highly upregulated in conjunction with various types of glomerulonephritis accompanied by mesangial proliferation (Fellström *et al.,* 1989; Iida *et al.,* 1991).

The fact that both PDGFB and PDGFRβ mutant embryos present mesangial cell deficiency firmly establishes that PDGFB-PDGFRβ interactions are crucial to the development of a glomerular mesangium. The expression patterns of PDGFB and PDGFRβ are consistent with these molecules being directly involved in mesangial cell function; however, whether this is at the level of directed migration (of mesangial precursors into the developing glomerular vesicle), proliferation, extracellular matrix production, survival or combinations thereof, remains to be established. Whatever is the ultimate cause of the failure of the mesangial cells in PDGFB and PDGFRβ-deficient mouse embryos, the absence of these cells provides some hints concerning their normal function. A number of functions have, hypothetically, been associated with mesangial cells. Their contractile properties have led to suggestions concerning regulation of glomerular blood flow, pressure and filtration. Phagocytic properties implicate functions related to basement membrane cleansing or turnover. The deposition of a firm extracellular matrix may provide mechanical resistance to the tuft, etc. The structure of the mutant glomerulus implies that the formation of a capillary network requires a mesangium. Thus, the mesangium may constitute a matrix for endothelial cell and glomerular capillary growth. An obvious structural consequence of the exchange of a complex capillary tuft with the mutant aneurysm-like structure, is a decreased area for filtration. The second consequence for glomerular filtration may relate to filtration physics. A filter generally requires structural support as well as efficient drainage of the filtrate in order to be effective. By losing its mesangium, or "filter holder", the mutant "filter" expands from the blood pressure to the margins of the Bowman's space and, consequently, the space for filtrate drainage is severely compromised (Fig. 3). The predicted result would be greatly reduced filtration. At E16.5, the first mature glomeruli are seen in the normal mouse embryo and at E18.5, glomerular filtration is ongoing as judged from the presence of urine in the urinary bladder. At the same age, PDGFB mutant embryos lacked urine in their bladders, which lends support to the prediction above.

Analyses of the PDGFA (Betsholtz *et al.,* unpublished observations) and PDGFRα (Soriano, personal communication) mutant mouse phenotypes are ongoing. Of particular interest in PDGFA-deficient mice, is a lung phenotype which presents a generalized emphysema (lack of alveolar walls) together with the absence of a specific cell type, the alveolar myofibroblast, which has many properties in common with mesangial cells (partially fibroblast-like, partially smooth muscle cell-like properties). Along with the absence of alveolar myofibroblasts is a severe reduction in alveolar elastin, which, in the normal case, colocalizes with the myofibroblasts, and has indeed been shown to be produced by these cells (Noguchi *et al.,* 1989). During mouse lung development, PDGFA has been shown to be expressed by epithelial cells and PDGFRα by the underlying mesenchyme (Orr-Urtreger and Lonai, 1992). Thus, the expression pattern is consistent with the failure of a paracrine epithelial-mesenchymal interaction involving PDGFA and PDGFRα, leading to absence of alveolar myofibroblasts in PDGFA mutant mice. In analogy



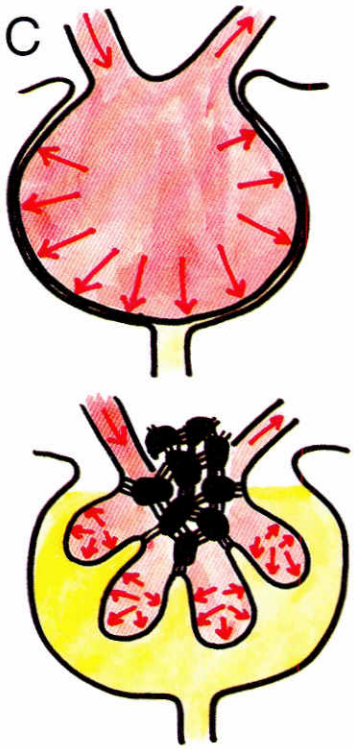


Fig. 3. Electron micrograph of part of a normal (A) and a PDGFB mutant (B) glomerulus. *Podocytes (po), endothelial cells (en) and parietal epithelial cells (pe) are indicated. Note that the filtration space between the podocytes and the parietal epithelial cells appears severely compromised in the mutant structure.* **(C)** *An illustration of the "filter holder" hypothesis to mesangial cell function. In the normal glomerulus (bottom), mesangial cells (black) and their matrix attach focally to the glomerular basement membrane forming an inside structural support that counteracts the force of the blood pressure (red arrows). This allows for collection and efficient drainage of the primary urine (yellow). In the mutant glomerulus (top) lacking mesangial cells, the blood pressure will lead to distension of the filter structure (the glomerular basement membrane together with its endothelial cells and podocytes) which consumes the collecting space and leads to the hindrance of filtrate collection and drainage.*

with the glomerulus malformation in PDGFB and PDGFRβ-deficient mice, it is reasonable to assume that the emphysematous picture results from the absence of myofibroblasts and the accompanying elastin. The scenarios are thus strikingly similar in the kidneys of PDGFB-mutants in comparison with the lungs of PDGFA-mutants. In both cases the growth factor deficiency leads to the virtual absence of a highly specialized smooth muscle-like cell type, which, together with its matrix (mesangial matrix in the glomeruli or alveolar elastin in the lung) may be instrumental in morphogenic processes leading to increased sizes of the functionally active surfaces for filtration and respiration, respectively.

Dissecting mammalian PDGF biology with the help of mutant mice: problems and possibilities

The mouse patch mutant has been extensively analyzed. In addition, mice deficient with regard to each individual member of the PDGF family of ligands and receptors have been generated and their phenotypical characterization is ongoing. Thus, the stage appears to be set for the precise elucidation of PDGF functions *in vivo*. The side-by-side comparison of the individual ligand and receptor mutant phenotypes, together with the crossing of different heterozygous mutants with the aim of generating and analyzing double deficiencies is most likely going to be extremely rewarding. Many problems have to be dealt with, however, or taken into consideration, some of which are inherent to the analysis of mutants, natural such as patch, or those generated by gene targeting.

The problem of linkage

It is obvious that for any natural deletion (and particularly if it is large) it is difficult to tie one specific known gene product, abolished by the mutation, to all aspects of the phenotype. As for the patch mutation, this would require precise knowledge of the borders of the deletion, the presence or absence of other coding sequences as well as putative transcriptional elements of importance for the correct expression of neighboring genes. The latter situation may be especially difficult to rule out, in spite of exact structural information concerning the deletion.

With embryonic stem cells as vehicles for the introduction of targeted alleles into the germline, there is a risk of entering other mutations (perhaps acquired during the phase of *in vitro* culture). These will not create too much of a problem unless they are tightly linked to the targeted locus (linkage between the phe-

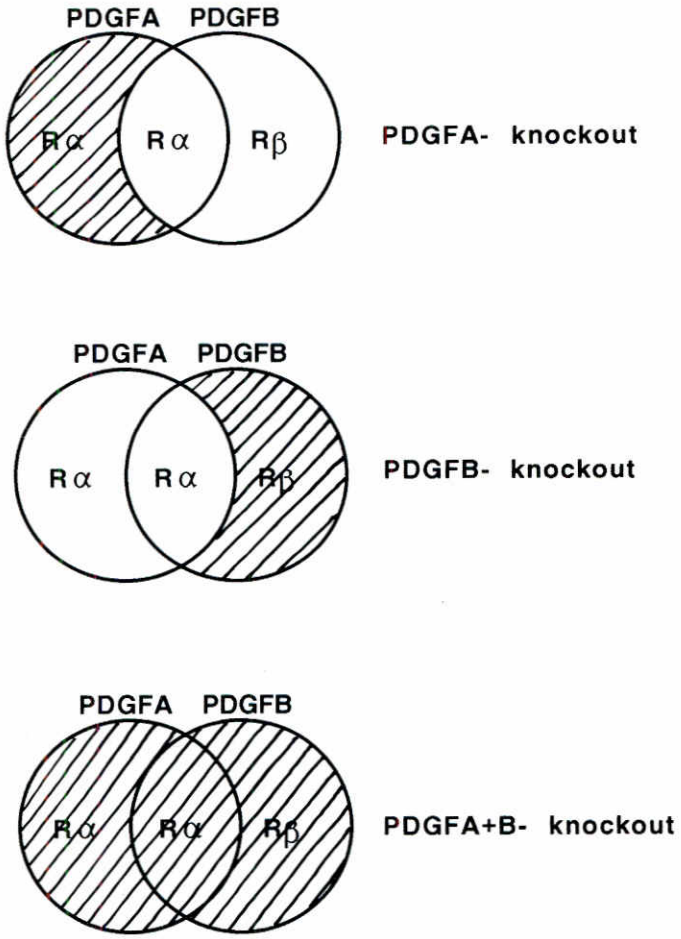


Fig. 4. The putative functional overlap between PDGFA and PDGFB and its significance for the knock-out phenotypes. *Striped areas illustrate deleted functions.*

notype and the targeted locus has to be established, to start with). Although maybe unlikely to occur, such mutations are difficult to rule out. It is known that there is a substantial risk for "random" mutation at the site of homologous recombination, thus, along with the designed mutation, additional changes may occur. It is therefore important to structurally characterize the targeted locus. However, even with perfect homologous recombination, and in the absence of unknown linked mutations, it is still difficult to formally exclude the involvement of other gene products than the one intended. This is illustrated by the dilemma of targeting vector design. A deletion of one or several crucial exons (or the entire gene) will make a "null" allele, but will neighboring genes be affected? An insertion type recombination will result in a partial duplication of the gene and therefore, putative transcriptional elements positioned within the gene of interest, but acting on a neighboring gene, are likely untouched. However, exon skipping may, in such case, produce only a partially inactivated, and thus not "null", allele. In the case of insertional mutagenesis, even the absence of detectable protein expression is not a solid proof of a null allele (a qualitative event – the loss of a gene – cannot be proven using quantitative methods such as the determination of protein or mRNA levels). Therefore, gene knock-out data are strengthened if 2 independent mutations of the same gene (e.g. different parts deleted) produce identical phenotypes. Ideally, as in the case of PDGFB and PDGFRβ discussed above, two mutually dependent proteins may be individually deleted and their phenotypes compared. Although the differences between the PDGFB and PDGFRβ mutant phenotypes (cardiac dilation; see Table 1) are tempting to be interpreted as PDGFB signaling via PDGFRα, the above mentioned potential artefacts have to be taken into consideration. The closely similar or identical phenotypes of PDGFB and PDGFRβ mutant mice (everything revealed so far except cardiac dilation, Table 1), however, should be proven beyond doubt to be caused by failure of PDGFB-PDGFRβ interactions.

The problem of overlapping functions

The PDGF ligand-receptor specificities (Fig. 1) and the biochemical indications of similarities in the signal transduction pathways between the PDGF receptor subtypes (Eriksson *et al.*, 1992) imply that there may be overlapping functions between the ligands and the receptors, respectively, *in vivo*. The highly different expression patterns of both the ligands and the receptors during e.g. embryonic development, however, imply at best a partial overlap; PDGFB is therefore not expected to compensate fully for the absence of PDGFA during embryogenesis. The uncovering of common functions may, as illustrated in Figure 4, require the double knock-out. Although this can be achieved fairly easily by crossing double heterozygotes (which we know are viable), functions shared with other genes/gene products remain a problem since they will not present unless the protein studied is rate limiting with regard to the particular function. Thus, discrepancies between the expression pattern and the corresponding phenotype are difficult to interpret. The absence of a phenotype may indicate the absence of a function, or a shared function. Alternatively, as discussed below, it may result from an indirect connection between the deficient site of expression and its obvious net result.

The problem of defect propagation

As pointed out by Bowen-Pope *et al.* in reviewing the patch phenotype (Bowen-Pope *et al.*, 1991), we tend to search for correlations between expression patterns and mutant phenotypes in such a way that we can ascribe the defects to the lack of the gene product within the affected tissue or its immediate precursor. However, the link (pathogenesis) between the lacking gene product (etiology) and the phenotype (disease) may be much more indirect. There are several aspects (in a strict sense) of the PDGFB and PDGFRβ mutant phenotypes which may fall into this category. A good illustration to the problem is the hematological phenotype presenting anemia with erythroblastosis and thrombocytopenia. Is PDGFB-PDGFRβ directly involved in the growth and differentiation of these hematopoietic lineages or of the common erythroid-megakaryocytic precursor? PDGFB has been shown to be produced by erythroid precursors (Sytkowski *et al.*, 1990) or by leukemia cells stimulated to erythroid differentiation (Alitalo *et al.*, 1987; Papayannopoulou *et al.*, 1987). In turn, PDGF may promote erythropoiesis indirectly via bone mar-

row stromal cells (Delwiche *et al.,* 1985). Thus, there are reasons to speculate that the anemia in PDGFB and PDGFRβ-deficient mice relates to the failure of erythroid precursor cells to promote stromal cells to support the erythroid precursor cells. However, the anemia may alternatively (and perhaps more likely) be secondary to the bleedings occurring in these embryos. What then causes the bleeding? Could the thrombocytopenia be involved? If so, what is the cause of the thrombocytopenia? Platelets contain PDGF and it was recently suggested that following the platelet release reaction, PDGF binds back to platelet PDGFRα which, in turn, inhibits platelet aggregation and release (Vassbotn *et al.,* 1994). If platelets are deficient with regard to PDGF, the abrogation of this putative autocrine feedback inhibitory loop may lead to platelet hyperaggregation and hence, platelet consumption, eventually leading to trombocytopenia and hemorrhage (compare to thrombotic thrombocytopenic purpura (TTP) in humans). A different scenario is that of a primary defect in blood vessels, leading to bleeding in conjunction with birth because of the blood pressure increase expected to occur at this time. This scenario lends support from the notion that PDGFB is expressed by many types of vascular endothelial cells and that PDGFB containing PDGF dimers are potent growth factors for many vascular wall cells, including smooth muscle cells. There are also certain capillary endothelial cells which express PDGFRβ (Smits *et al.,* 1989; Holmgren *et al.,* 1991). In addition to putative vascular defects, we cannot at present exclude that anemia and thrombocytopenia are secondary to deficient hormonal regulation of the precursors. Erythropoietin and thrombopoietin are produced by kidney and liver respectively and both these organs display abnormalities in the PDGFB mutants (in addition to the mesangial cell deficiency, both the kidney and the liver are reduced in size). Obviously, since hematopoiesis in mouse embryos up to the point of hemorrhage, is primarily occurring in the liver (and to some extent in the spleen and bone marrow), the reduction of the size of this organ may be partially or fully responsible for the anemia and thrombocytopenia seen.

The hematological picture may thus result from defect propagation in many steps and the same may be the case with the cardiac dilation seen in PDGFB mutant embryos (Table 1). It is clear that further studies are required to reveal the basis for the hemorrhaging seen in the PDGFB and PDGFRβ-deficient embryos and the heart phenotype of the PDGFB mutants. To this end, the detailed mapping of phenotypes and expression patterns would need to be combined with mechanistic studies in cell or organ culture or *in vivo*. For example, the transplantation of PDGF-negative hematopoietic stem cells into irradiated normal recipients should potentially resolve whether the hematological defect of the PDGFB mutant lies autonomously within the hematopoietic system or not.

The problem of embryonic lethality

Knockout animals generally provide opportunities for analyses up to the point of the first vital function of the target gene product. For the PDGFs, which seem to be obligatorily required during embryonic and early postnatal life, gene targeting does therefore not, at first glance, appear well suited for the analyses of PDGF functions in the adult. However, the creation of the null allele is just one of the many possibilities with gene targeting which allows for the analysis of the 100% or 50% reduction in the level of the gene product (the latter is frequently the case in heterozygotes).

The possibilities

Gene targeting offers numerous possibilities in addition to gene knockout. For example, the establishment of PDGF or PDGF receptor homozygous mutant embryonic stem cell lines and their subsequent use to generate wild type/mutant mosaic animals is a way to address questions concerning cell lineages and cell autonomy depending on PDGF-PDGF receptor interactions. If the mutant (or wild type) cells can be tagged in order to allow their identification in e.g. tissue sections, it may be possible to answer some of the questions raised above concerning the absence of mesangial cells. If failure of paracrine attraction of mesangial cells by epithelial cells, PDGFB-negative (but not PDGFRβ–negative) mesangial cells should be allowed to populate the mesangium. However, if mesangial deficiency is caused by failure of the putative autocrine loop established in conjunction with further growth of the developed glomerulus, PDGFB-negative cells should not be allowed to populate the mesangium.

Additional possibilities may be provided by alleles other than "null" generated by gene targeting. It has been mentioned above that insertional mutagenesis may be used to create a functionally compromised, but not fully inactive allele. Several approaches to subtle mutagenesis have been reported (Hasty *et al.,* 1991; Valancius and Smithies, 1991) including a recently reported successful attempt to generate tissue-specific gene knockout (Gu *et al.,* 1994). Among our own immediate concerns are the potential functions specifically associated with C-terminally extended forms of the PDGF chains. In PDGFB, it is known that efficient release (of PDGF-BB) required C-terminal proteolytic processing of the precursor. Cell types deficient with regard to this produce cell surface- or extracellular matrix-bound PDGF-BB (LaRochelle *et al.,* 1991; Östman *et al.,* 1991; Raines and Ross, 1992). In PDGFA, an alternative splice variant produces a C-terminally extended (Betsholtz *et al.,* 1986; Rorsman *et al.,* 1988), cell-associated (Andersson *et al.,* 1994) form of PDGF-AA. As it is tempting to speculate about specific functions associated with these PDGF forms *in vivo*, efforts aiming at the selective inactivation of these cell retention- or matrix association motifs by gene targeting are ongoing.

References

ALITALO, R., ANDERSSON, L. C., BETSHOLTZ, C., NILSSON, K., WESTERMARK, B., HELDIN, C-H. and ALITALO, K. (1987). Induction of platelet-derived growth factor gene expression during megakaryoblastic and monocytic differentiation of human leukemia cell lines. *EMBO J. 6:* 1213-1218.

ALPERS, C.E., SEIFERT, R.A., HUDKINS, K.L., JOHNSON, R.J. and BOWEN, P.D. (1992). Developmental patterns of PDGF B-chain, PDGF-receptor, and alpha-actin expression in human glomerulogenesis. *Kidney Int. 42:* 390-9.

ALPERS, C.E., SEIFERT, R.A., HUDKINS, K.L., JOHNSON, R.J. and BOWEN-POPE, D.F. (1993). PDGF-receptor localizes to mesangial, parietal epithelial, and interstitial cells in human and primate kidneys. *Kidney Int. 43:* 286-294.

ANDERSSON, M., ÖSTMAN, A., BÄCKSTRÖM, G., HELLMAN, U., GEORGE, N.C., WESTERMARK, B. and HELDIN, C.H. (1992). Assignment of interchain disulfide bonds in platelet-derived growth factor (PDGF) and evidence for agonist activity of monomeric PDGF. *J. Biol. Chem. 267:* 11260-6.

ANDERSSON, M., ÖSTMAN, A., WESTERMARK, B. and HELDIN, C.H. (1994). Characterization of the retention motif in the C-terminal part of the long splice form of platelet-derived growth factor A-chain. *J. Biol. Chem. 269:* 926-30.

ANTONIADES, H.N., GALANOPOULOS, T., NEVILLE-GOLDEN, J., KIRITSY, C.P. and LYNCH, S.E. (1991). Injury induces *in vivo* expression of platelet-derived growth factor (PDGF) and PDGF receptor mRNAs in skin epithelial cells and PDGF mRNA in connective tissue fibroblasts. *Proc. Natl. Acad. Sci. USA 88:* 565-569.

BARRETT, T.B., GAJDUSEK, C.M., SCHWARTZ, S.M., McDOUGALL, J.K. and BENDITT, E.P. (1984). Expression of the *sis* gene by endothelial cells in culture and *in vivo*. *Proc. Natl. Acad. Sci. USA 81:* 6772-6774.

BAUER, E.A., COOPER, T.W., HUANG, J.S., ALTMAN, J. and DEUEL, T.F. (1985). Stimulation of *in vitro* human skin collagenase expression by platelet-derived growth factor. *Proc. Natl. Acad. Sci. USA 82:* 4132-4136.

BETSHOLTZ, C., JOHNSSON, A., HELDIN, C-H., WESTERMARK, B., LIND, P., URDEA, M.S., EDDY, R., SHOWS, T.B., PHILPOTT, K., MELLOR, A.L., KNOTT, T.J. and SCOTT, J. (1986). cDNA sequence and chromosomal localization of human platelet-derived growth factor A-chain and its expression in tumor cell lines. *Nature 320:* 695-699.

BOWEN-POPE, D.F., VAN KOPPEN, A. and SCATTEMAN, G. (1991). Is PDGF really important? Testing the hypotheses. *Trends Genet. 7:* 113-118.

BRESSLER, J.P., GROTENDORST, G.R., LEVITOV, C. and HJELMELAND, L.M. (1985). Chemotaxis of rat brain astrocytes to platelet-derived growth factor. *Brain Res. 344:* 249-254.

CLAESSON-WELSH, L. (1993). PDGF receptors: structure and mechanism of action. In *Biology of Platelet-derived Growth Factor, 5* (Eds. B. Westermark and C. Sorg). Karger, Basel, pp. 31-43.

COLLINS, T., GINSBURG, D., BOSS, J.M., ORKIN, S.H.V. and POBER, J.S. (1985). Cultured human endothelial cells express platelet-derived growth factor B chain: cDNA cloning and structural analysis. *Nature 316:* 748-750.

COLLINS, T., POBER, J.S., HAMMACHER, A., BETSHOLTZ, C., WESTERMARK, B. and HELDIN, C-H. (1987). Cultured human endothelial cells express platelet-derived growth factor A chain. *Am. J. Pathol. 126:* 7-12.

COOPER, J.A. and KAZLAUSKAS, A. (1993). Signal transduction via the PDGF beta-receptor. In *Biology of Platelet-derived Growth Factor, 5* (Eds. B. Westermark and C. Sorg). Karger, Basel, pp. 44-73.

DELWICHE, F., RAINES, E., POWELL, J., ROSS, R. and ADAMSON, J. (1985). Platelet-derived growth factor enhances *in vitro* erythropoiesis via stimulation of mesenchymal cells. *J. Clin. Invest. 76:* 137-42.

ERIKSSON, A., SIEGBAHN, A., WESTERMARK, B., HELDIN, C-H. and CLAESSON-WELSH, L. (1992). Platelet-derived growth factor α- and β-receptors activate unique and common signal transduction pathways. *EMBO J. 11:* 543-550.

FELLSTRÖM, B., KLARESKOG, L., HELDIN, C-H., LARSSON, E., RÖNNSTRAND, L., TERRACIO, L., TUFVESON, G., WAHLBERG, J. and RUBIN, K. (1989). Platelet-derived growth factor receptors in the kidney – Upregulated expression in inflammation. *Kidney Int. 36:* 1099-1102.

GOUSTIN, A.S., BETSHOLTZ, C., PFEIFER-OHLSSON, S., PERSSON, H., RYDNERT, J., BYWATER, M., HOLMGREN, G., HELDIN, C-H., WESTERMARK, B. and OHLSSON, R. (1985). Co-expression of the *sis* and *myc* proto-oncogenes in developing human placenta suggests autocrine control of trophoblast growth. *Cell 41:* 301-312.

GROTENDORST, G., SEPPA, H.E.J., KLEIMAN, H.K. and MARTIN, G. (1981). Attachment of smooth muscle cells to collagen and their migration toward platelet-derived growth factor. *Proc. Natl. Acad. Sci. USA 78:* 3669-3672.

GROTENDORST, G.R., CHANG, T., SEPPÄ, H.E.J., KLEINMAN, H.K. and MARTIN, G.R. (1982). Platelet-derived growth factor is a chemoattractant for vascular smooth muscle cells. *J. Cell. Physiol. 113:* 261-266.

GRÜNEBERG, H. and TRUSLOVE, G.M. (1960). Two closely linked genes of the mouse. *Genet. Res. 1:* 69-90.

GU, H., MARTH, J.D., ORBAN, P.C., MOSSMANN, H. and RAJEWSKY, K. (1994). Deletion of a DNA polymerase beta gene segment in T cells using cell type specific gene targeting. *Science 265:* 103-106.

HASTY, P., RAMIREZ-SOLIS, R., KRUMLAUF, R.V and BRADLEY, A. (1991). Introduction of a subtle mutation into the Hox2.6 locus in embryonic stem cells. *Nature 350:* 243-246.

HELDIN, C.H., ÖSTMAN, A. and WESTERMARK, B. (1993). Structure of platelet-derived growth factor: implications for functional properties. *Growth Factors 8:* 245-52.

HOLMGREN, L., GLASER, A., PFEIFER-OHLSSON, S. and OHLSSON, R. (1991). Angiogenesis during human extraembryonic development involves the spatiotemporal control of PDGF ligand and receptor gene expression. *Development 113:* 749-754.

IIDA, H., SEIFERT, R., ALPERS, C. E., GRONWALD, R.G.K., PHILLIPS, P.E., PRITLZ, P., GORDON, K., GOWN, A.M., ROSS, R., BOWEN-POPE, D.F. and JOHNSON, R.J. (1991). Platelet-derived growth factor (PDGF) and PDGF receptors are induced in mesangial proliferative nephritis in the rat. *Proc. Natl. Acad. Sci. USA 88:* 6560-6564.

KOHLER, N. and LIPTON, A. (1974). Platelets as a source of fibroblast growth-promoting activity. *Exp. Cell Res. 87:* 297-301.

LAROCHELLE, W.J., MAY-SIROFF, M., ROBBINS, K.C. and AARONSON, S.A. (1991). A novel mechanism regulating growth factor association with the cell surface: identification of a PDGF retention domain. *Genes Dev. 5:* 1191-1199.

LEVÉEN, P., PEKNY, M., GEBRE-MEDHIN, S., SWOLIN, B., LARSSON, E. and BETSHOLTZ, C. (1994). Mice deficient for PDGF B show renal, cardiovascular, and hematological abnormalities. *Genes Dev. 8:* 1875-1887.

MAJACK, R.A., COOK, S.C. and BORNSTEIN, P. (1985). Platelet-derived growth factor and heparin-like glycosaminoglycans regulate thrombospondin synthesis and deposition in the matrix by smooth muscle cells. *J. Cell Biol. 101:* 1059-1070.

MAJACK, R.A., MILDBRADT, J. and DIXIT, V.M. (1987). Induction of thrombospondin messenger RNA levels occurs as an immediate primary response to platelet-derived growth factor. *J. Biol. Chem. 262:* 8821-8825.

MARTINET, Y., BITTERMAN, P.B., MORNEX, J-F., GROTENDORST, G.R., MARTIN, G.R. and CRYSTAL, R.G. (1986). Activated human monocytes express the c-*sis* proto-oncogene and release a mediator showing PDGF-like activity. *Nature 319:* 158-160.

MERCOLA, M., MELTON, D.A. and STILES, C.D. (1988). Platelet-derived growth factor A chain is maternally encoded in *Xenopus* embryos. *Science 241:* 1223-1225.

MERCOLA, M., WANG, C., KELLY, J., BROWNLEE, C., JACKSON-GRUSBY, L., STILES, C. and BOWEN-POPE, D. (1990). Selective expression of PDGF A and its receptor during early mouse embryogenesis. *Dev. Biol. 138:* 114-122.

MORRISON-GRAHAM, K., SCHATTEMAN, G.C., BORK, T., BOWEN-POPE, D.F. and WESTON, J.A. (1992). A PDGF receptor mutation in the mouse (patch) perturbs the development of a non-neuronal subset of neural crest-derived cells. *Development 115:* 133-142.

MUDHAR, H.S., POLLOCK, R.A., WANG, C., STILES, C.D.V. and RICHARDSON, W.D. (1993). PDGF and its receptors in the developing rodent retina and optic nerve. *Development 118:* 539-52.

NOBLE, M., MURRAY, K., STROOBANT, P., WATERFIELD, M.D. and RIDDLE, P. (1988). Platelet-derived growth factor promotes division and motility and inhibits premature differentiation of the oligodendrocyte/type-2 astrocyte progenitor cell. *Nature 333:* 560-562.

NOGUCHI, A., REDDY, R., KURSAR, J.D., PARKS, W.C. and MECHAM, R.P. (1989). Smooth muscle isoactin and elastin in fetal bovine lung. *Exp. Lung Res. 4:* 537-552.

ORR-URTREGER, A. and LONAI, P. (1992). Platelet-derived growth factor-A and its receptor are expressed in separate but adjacent cell layers of the mouse embryo. *Development 115:* 1045-1058.

ORR-URTREGER, A., BEDFORD, M.T., DO, M-S., EISENBACH, L. and LONAI, P. (1992). Developmental expression of the alpha-receptor for platelet-derived growth factor, which is deleted in the embryonic lethal Patch mutation. *Development 115:* 289-303.

ÖSTMAN, A., ANDERSSON, M., BETSHOLTZ, C., WESTERMARK, B. and HELDIN, C-H. (1991). Identification of a cell retention signal in the B-chain of PDGF and in the long splice version of the A-chain. *Cell Regul. 2:* 503-512.

PALMIERI, S., PAYNE, J., STILES, C.D., BIGGERS, J.D. and MERCOLA, M. (1992). Expression of mouse PDGF-A and PDGF alpha receptor genes during pre- and post-implantation development: evidence for a developmental shift from an autocrine to a paracrine mode of action. *Mech. Dev. 39:* 181-191.

PAPAYANNOPOULOU, T., RAINES, E., COLLINS, S., NAKAMOTO, B., TWEEDDALE, M. and ROSS, R. (1987). Constitutive and inducible secretion of

platelet-derived growth factor analogs by human leukemic cell lines coexpressing erythroid and megakaryocytic markers. *J. Clin. Invest. 79:* 859-66.

PRINGLE, N., COLLARINI, E.J., MOSLEY, M.J., HELDIN, C.H., WESTERMARK, B. and RICHARDSON, W.D. (1989). PDGF A chain homodimers drive proliferation of bipotential (O-2A) glial progenitor cells in the developing rat optic nerve. *EMBO J. 8:* 1049-56.

PRINGLE, N.P. and RICHARDSON, W.D. (1993). A singularity of PDGF alpha-receptor expression in the dorsoventral axis of the neural tube may define the origin of the oligodendrocyte lineage. *Development 117:* 525-33.

RAFF, M.C., LILLIEN, L.E., RICHARDSON, W.D., BURNE, J.F. and NOBLE, M.D. (1988). Platelet-derived growth factor from astrocytes drives the clock that times oligodendrocyte development in culture. *Nature 333:* 562-565.

RAINES, E.W. and ROSS, R. (1992). Compartmentalization of PDGF on extracellular binding sites dependent on exon-6-encoded sequences. *J. Cell Biol. 116:* 533-543.

RAINES, E. and ROSS, R. (1993). Platelet-derived growth factor *in vivo*. In *Biology of Platelet-derived Growth Factor, 5* (Eds. B. Westermark and C. Sorg). Karger, Basel, pp. 74-114.

RAINES, E.W., BOWEN-POPE, D.F. and ROSS, R. (1990). Platelet-derived growth factor. In *Handbook of Experimental Pharmacology. Peptide Growth Factors and Their Receptors, Part I* (Eds. M.B. Sporn and A.B. Roberts). Springer-Verlag, Heidelberg, pp. 173-262.

RAPPOLEE, D.A., BRENNER, C.A., SCHULTZ, R., MARK, D. and WERB, Z. (1988). Developmental expression of PDGF, TGF-α, and TGF-β genes in preimplantation mouse embryos. *Science 241:* 1823-1825.

RICHARDSON, W.D., PRINGLE, N., MOSLEY, M.J., WESTERMARK, B. and DUBOIS, D.M. (1988). A role for platelet-derived growth factor in normal gliogenesis in the central nervous system. *Cell 53:* 309-19.

RORSMAN, F., BYWATER, M., KNOTT, T.J., SCOTT, J. and BETSHOLTZ, C. (1988). Structural characterization of the human platelet-derived growth factor A-chain cDNA and gene: alternative exon usage predicts two different precursor proteins. *Mol. Cell. Biol. 8:* 571-577.

ROSS, R., GLOMSET, J.A., KARIYA, B. and HARKER, L. (1974). A platelet-dependent serum factor that stimulates the proliferation of arterial smooth muscle cells *in vitro*. *Proc. Natl. Acad. Sci. USA 71:* 1207-1210.

ROSS, R., RAINES, E.W. and BOWEN-POPE, D.F. (1986). The biology of platelet-derived growth factor. *Cell 46:* 155-169.

SASAHARA, A., KOTT, J.N., SASAHARA, M., RAINES, E.W., ROSS, R. and WESTRUM, L.E. (1992). Platelet-derived growth factor B-chain-like immunoreactivity in the developing and adult rat brain. *Dev. Brain Res. 68:* 41-53.

SASAHARA, M., FRIES, J.W.U., RAINES, E.W., GOWN, A.M., WESTRUM, L.E., FROSCH, M.P., BONTHRON, D.T., ROSS, R. and COLLINS, T. (1991). PDGF B-chain in neurons of the central nervous system, posterior pituitary and in a transgenic model. *Cell 64:* 217-227.

SCHATTEMAN, G.C., MORRISON-GRAHAM, K., VAN KOPPEN, A., WESTON, J.A. and BOWEN-POPE, D.F. (1992). Regulation and role of PDGF receptor alpha-subunit expression during embryogenesis. *Development 115:* 123-131.

SEIFERT, R.A., VAN, K.A. and BOWEN, P.D. (1993). PDGF-AB requires PDGF receptor alpha-subunits for high-affinity, but not for low-affinity, binding and signal transduction.*J. Biol. Chem. 268:* 4473-80.

SEJERSEN, T., BETSHOLTZ, C., SJÖLUND, M., HELDIN, C-H., WESTERMARK, B. and THYBERG, J. (1986). Rat skeletal myoblasts and arterial smooth muscle cells express the gene for the A chain but not the gene for the B chain *(c-sis)* of platelet-derived growth factor (PDGF) and produce a PDGF-like protein. *Proc. Natl. Acad. Sci. USA 83:* 6844-6848.

SEPPÄ, H., GROTENDORST, G., SEPPÄ, S., SCHIFFMANN, E. and MARTIN, G.R. (1982). Platelet-derived growth factor is chemotactic for fibroblasts. *J. Cell Biol. 92:* 584-588.

SHIMOKADO, K., RAINES, E.W., MADTES, D.K., BARRETT, T.B., BENDITT, E.P. and ROSS, R. (1985). A significant part of macrophage-derived growth factor consists of at least two forms of PDGF. *Cell 43:* 277-286.

SIEGBAHN, A., HAMMACHER, A., WESTERMARK, B. and HELDIN, C-H. (1990). Differential effects of the various isoforms of platelet-derived growth factor on chemotaxis of fibroblasts, monocytes and granulocytes. *J. Clin. Invest. 85:* 916-920.

SMITH, E.A., SELDIN, M.F., MARTINEZ, L., WATSON, M.L., CHOUDHURY, G.G., LALLEY, P.A., PIERCE, J., AARONSON, S., BARKER, J., NAYLOR, S.L. and SAKAGUCHI, A.Y. (1991). Mouse platelet-derived growth factor receptor alpha gene is deleted in W19H and patch mutations on chromosome 5. *Proc. Natl. Acad. Sci. USA 88:*4811-5.

SMITS, A., HERMANSSON, M., NISTÉR, M., KARNUSHINA, I., HELDIN, C-H., WESTERMARK, B. and FUNA, K. (1989). Rat brain capillary endothelial cells express functional PDGF B-type receptors. *Growth Factors 2:* 1-8.

SMITS, A., KATO, M., WESTERMARK, B., NISTÉR, M., HELDIN, C-H. and FUNA, K. (1991). Neurotrophic activity of platelet-derived growth factor (PDGF): rat neuronal cells possess functional PDGF β-type receptors and respond to PDGF. *Proc. Natl. Acad. Sci. USA 88:*8159-8163.

SORIANO, P. (1994). Abnormal kidney development and hematological disorders in PDGF beta-receptor mutant mice. *Genes Dev. 8:* 1888-1896.

STEPHENSON, D.A., MERCOLA, M., ANDERSON, E., WANG, C., STILES, C.D., BOWEN-POPE, D.F. and CHAPMAN, V.M. (1991). Platelet-derived growth factor receptor a-subunit gene (Pdgfra) is deleted in the patch (Ph) mutation. *Proc. Natl. Acad. Sci. USA 88:* 6-10.

SYTKOWSKI, A.J., O'HARA, C., VANASSE, G., ARMSTRONG, M.J., KRECZKO, S. and DAINIAK, N. (1990). Characterization of biologically active, platelet-derived growth factor-like molecules produced by murine erythroid cells *in vitro* and *in vivo*. *J. Clin. Invest. 85:* 40-6.

VALANCIUS, V. and SMITHIES, O. (1991). Testing an "in-out" targeting procedure for making subtle genomic modifications in mouse embryonic stem cells. *Mol. Cell. Biol. 11:* 1402.

VASSBOTN, F., HAVEN, O.K., HELDIN, C-H. and HOLMSEN, H. (1994). Negative-feedback regulation of human platelets via autocrine activation of the platelet-derived growth factor α-receptor. *J. Biol. Chem.* 269: 13874-13879.

WESTERMARK, B. (1993). PDGF and its receptors in human tumor cells. In *The Biology of PDGF, 5* (Eds. B. Westermark and C. Sorg). Karger, Basel, pp. 146-162.

WESTERMARK, B. and HELDIN, C-H. (1991). Platelet-derived growth factor in autocrine transformation. *Cancer Res. 51:* 5087-5092.

WESTERMARK, B. and WASTESON, Å. (1976). A platelet factor stimulating human normal glial cells. *Exp. Cell Res. 98:* 170-174.

YEH, H-J., RUIT, K.G., WANG, Y-X., PARKS, W.C., SNIDER, W.D. and DEUEL, T.F. (1991). PDGF A-chain gene is expressed by mammalian neurons during development and in maturity. *Cell 64:* 209-216.

YU, W-P., COLLARINI, E.J., PRINGLE, N. and RICHARDSON, W.D. (1994). Embryonic expression of myelin genes: evidence for a focal source of oligodendrocyte precursors in the ventricular zone of the neural tube. *Neuron 12:* 1353-1362.