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Platelet-derived growth factor: mechanism of action and possible in vivo function

Review

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Introduction

Platelet-derived growth factor (PDGF) was discovered as a constituent of platelet α-granules with growth-promoting activity for smooth muscle cells and fibroblasts; subsequent studies have shown that PDGF is synthesized by a number of different normal and transformed cell types (reviewed in Ross *et al.,* 1986; Westermark *et al.,* 1989). PDGF is generally thought to have a pivotal role in the regulation of normal cell proliferation, and it has been suggested that it also is a mediator of pathological cell growth, e.g., in tumor development as well as in the generation of atherosclerosis, tissue fibrosis, and other nonmalignant proliferative disorders. In normal as well as pathological conditions, the effect of PDGF is most likely exerted locally in an autocrine or paracrine manner. The capacity of PDGF to act as an autocrine growth factor was unraveled by the finding that it is similar to the product of the *sis* oncogene of simian sarcoma virus (SSV) (Doolittle *et al.,* 1983; Waterfield *et al.,* 1983); the transforming properties of this retrovirus were shown to be exerted by a PDGF-like growth factor acting in an autocrine manner (reviewed in Westermark *et al.,* 1987).

We summarize in this review some recent advances in our understanding of the mechanism of action of PDGF and its possible in vivo function.

Different isoforms of PDGF

Structural analysis of purified PDGF revealed that it is a dimeric 30-kDa component composed of disulphide-bonded A and B polypeptide chains. Both chains are synthesized as precursor molecules that undergo proteolytic processing after synthesis and dimerization; in the mature parts of slightly more than 100 amino acids, the two chains are ~60% similar in amino acid sequence with a perfect conservation of the eight cysteine residues (Betsholtz *et al.,* 1986b). All three possible dimers, AA, AB, and BB, have been identified and purified from platelets and transformed cells (Stroobant and Waterfield, 1984; Heldin *et al.,* 1986; Hammacher *et al.,* 1988; Bowen-Pope *et al.,* 1989; Hart *et al.,* 1990). The three isoforms differ in their functional properties (see further below) as well as in their secretory behaviors; whereas both the A-chain and B-chain precursors contain signal sequences and PDGF-AA and PDGF-AB are rapidly secreted from the producer cell, a 24-kDa form of PDGF-BB remains to a large extent associated with the producer cell and relatively small amounts of 30-kDa PDGF-BB are secreted (Robbins *et al.,* 1985; Östman *et al.,* 1988). The mechanism behind the generation and retention of the 24-kDa PDGF-BB and its functional significance remain to be elucidated.

The A-chain occurs as two variants as a result of differential splicing of the transcript; the three most C-terminal amino acids in the shorter, more common, variant are replaced in the longer variant by 18 different amino acids containing a long stretch of basic amino acids (Bonthron *et al.,* 1988; Rorsman *et al.,* 1988). Interestingly, the B-chain precursor also has a similar sequence of basic residues in its C-terminal. Furthermore, an endothelial cell growth factor with the same spacing of cysteine residues as that found in PDGF, denoted vascular permeability factor (Keck *et al.,* 1989) or vascular endothelial growth factor (Leung *et al.,* 1989), was also found to occur in two variants, most likely as a result of differential splicing of the gene. Significantly, the longer variant of this factor displayed a basic stretch of amino acids at an analogous position as that in the long version of the PDGF A-chain (Betsholtz *et al.,* 1990). These findings raise the interesting possibility

that the conserved basic sequence has a function, maybe in targeting the factor to a specific location. This possibility is further illustrated by the finding that, in artificial mutants of the A- and B-chains lacking the signal sequences, the basic sequences mediate association of the products with the nucleus (Lee *et al.,* 1987; Maher *et al.,* 1989).

Two different PDGF receptor types

The recent availability of all three PDGF isoforms has led to the identification of two different PDGF receptor types (Hart *et al.,* 1988; Heldin *et al.,* 1988). The α-receptor (also called A-type receptor) binds all PDGF isoforms, whereas the β-receptor (also called B-type receptor) binds PDGF-BB with high affinity and PDGF-AB with lower affinity, but does not bind PDGF-AA with any appreciable affinity. Human fibroblasts have both α- and β-receptors, but there are also examples of normal cells having only α-receptors, e.g., O-2A progenitor cells of the rat optic nerve (Hart *et al.,* 1989), and only β-receptors, e.g., rat brain capillary endothelial cells (Smits *et al.,* 1989). Both receptor types transduce potent mitogenic signals, but in human fibroblasts only the β-receptor mediates chemotaxis and actin reorganization (Nistér *et al.,* 1988a; Hammacher *et al.,* 1989; Siegbahn *et al.,* 1990). However, the signal transduction from the receptors may to some extent be cell-type specific, because a hematopoietic cell line transfected with α-receptor cDNA responded chemotactically to PDGF (Matsui *et al.,* 1989b).

The PDGF α-receptor is synthesized as a precursor of 140 kDa, which undergoes maturation to a 170-kDa cell-surface-expressed species (Claesson-Welsh *et al.,* 1989b). The β-receptor is slightly larger, the precursor having a size of 160 kDa and the fully glycosylated receptor a size of 180 kDa (Hart *et al.,* 1987; Keating and Williams, 1987). The receptors are turned over fairly rapidly, and ligand binding increases the rate of internalization and degradation even further.

Cloning of cDNAs for the two PDGF receptors revealed that the predicted protein sequences are very similar (Yarden *et al.,* 1986; Claesson-Welsh *et al.,* 1988, 1989a; Gronwald *et al.,* 1988; Matsui *et al.,* 1989a). The extracellular parts of the two receptors each contain five immunoglobulin-like domains and have an overall amino acid sequence similarity of 30%. There are 8 and 11 putative glycosylation sites in the α- and β-receptors, respectively, only a few of which are conserved. The intracellular portions of the receptors contain protein tyrosine kinase domains, which in each receptor contain inserted sequences of ~100 amino acids without homology to kinase domains. The amino acid sequence similarity between the receptors is high in the kinase domain (80% identity) as well as in the sequence between the transmembrane domain and the first sequence of the kinase domain (83% identity), whereas it is lower in the kinase insert (35% identity) and in the C-terminal tail (27% identity). The structural organizations of the PDGF receptors are similar to those of the CSF-1 receptor (Coussens *et al.,* 1986) and the c-*kit* product (Yarden *et al.,* 1987; Qui *et al.,* 1988), a receptor for an as-yet-unknown ligand.

Studies of mutants of the PDGF β-receptor have given some initial insight into the role of the different domains of the receptor in its function. Thus, a receptor mutant in which the kinase activity was extinguished by mutation of the ATP binding lysine residue was found to be unable to mediate most of the cellular signals, including inositol lipid breakdown, calcium release, elevation of intracellular pH, gene expression, actin reorganization, chemotaxis, or DNA synthesis (Escobedo *et al.,* 1988; Westermark *et al.,* 1990). The kinase negative receptor mutant was able to bind ligand with normal affinity and to mediate internalization and degradation of ligand as well as receptor, but with lower rate compared with the wild-type receptor (Sorkin *et al.,* unpublished observations). Another important question relates to the function of the unique kinase insert domain of the receptor. Mutants in which the insert was deleted were able to transduce some of the early signals of PDGF, such as phosphatidylinositol turnover and c-*fos* induction, but did not mediate a mitogenic effect. Notably, the kinase activities of the mutated receptors were decreased and the substrate specificities altered, which suggests that the inability to mediate a mitogenic signal of mutated receptors was due to inabilities to phosphorylate and activate a crucial substrate(s) in the mitogenic pathway (Escobedo and Williams, 1988; Severinsson *et al.,* 1990).

Activation by dimerization of PDGF receptors

The extracellular ligand-binding domains of the PDGF receptors are linked to the intracellular effector domains by a single stretch of amino acids. It is not easy to envision how a signal can be transmitted across the cell membrane through a single receptor. The possibility that

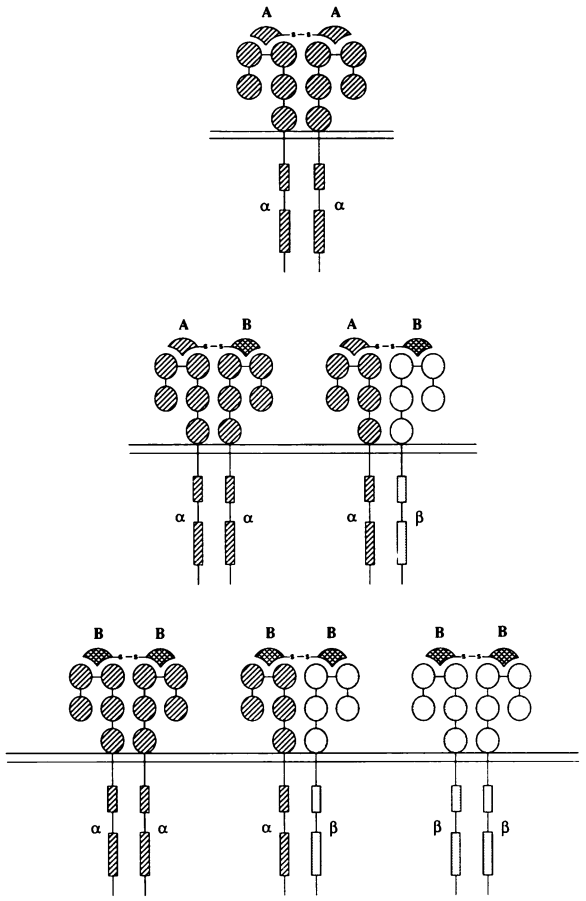

***Figure 1.* Schematic illustration of the abilities of the various isoforms of PDGF to bind to and dimerize PDGF α- and β-receptors.** The receptors are drawn to indicate that they are each composed of five extracellular Ig-like domains and an intracellular split tyrosine kinase domain. The model is based on the notion that PDGF is a bivalent ligand and that the A subunit binds to α-receptors only, whereas the B subunit binds to both α- and β-receptors. For discussion, see the text.

ligand binding induces receptor dimerization and oligomerization has been proposed in the case of the epidermal growth factor (EGF) receptor (reviewed by Schlessinger, 1988). Analogously, incubation of purified PDGF β-receptors with PDGF-BB was found to lead to dimerization of receptors, as revealed by experiments utilizing covalent cross-linkers (Bishayee *et al.*, 1989; Heldin *et al.*, 1989). Cross-linking occurred in a dose-dependent manner and decreased at higher concentrations of PDGF-BB; higher oligomeric forms of receptors were not seen. This suggests that each subunit in the dimeric ligand binds one receptor molecule, such that the ligand forms a bridge between the receptors. Furthermore, dimerization was found to be closely associated with activation of the protein tyrosine kinase of the receptor, measured as autophosphorylation as well as phosphorylation of exogenous substrates (Heldin *et al.*, 1989). Further evidence in support of ligand-induced PDGF receptor dimerization has been obtained by coprecipitation experiments (Seifert *et al.*, 1989).

The mechanism whereby dimerization of receptors leads to their activation remains to be elucidated. One possibility is that cross-phosphorylation between the receptors in the dimer is involved. The two major autophosphorylation sites in the PDGF β-receptor were recently localized to Tyr-751 in the kinase insert domain and Tyr-857 in the second segment of the kinase domain (Kazlauskas and Cooper, 1989). The consequences of the phosphorylation of each of these sites for the catalytic properties of the receptor are, however, not known.

The structural similarity between the two PDGF receptor types suggests that α-receptors also undergo dimerization after binding of any of their ligands. Furthermore, it is possible that heterodimeric receptor complexes can be formed. This could explain the finding that, although PDGF-AB binds with a 10-fold lower affinity than PDGF-BB to PDGF β-receptors (Severinsson *et al.*, 1989), it is as potent a mitogen as PDGF-BB for human foreskin fibroblasts, in which activation of α-receptors only gives a limited mitogenic response. It is thus possible that PDGF-AB can bind to and activate the β-receptor efficiently only in the presence of α-receptors, by binding simultaneously to one α- and one β-receptor. Indirect evidence in support of this possibility was recently obtained by investigations of the ability of different PDGF isoforms to induce actin reorganization in human fibroblasts (Hammacher *et al.*, 1989). PDGF-AB and PDGF-BB induces actin reorganization but not PDGF-AA, suggesting that the response is mediated by the β-receptor but not the α-receptor. Furthermore, the response of PDGF-AB, but not that of PDGF-BB, was inhibited by blocking of the α-receptors by an excess of PDGF-AA or by down-regulation of the α-receptor by pretreatment with PDGF-AA at 37°C. In fact, in the absence of α-receptors, PDGF-AB acted as a β-receptor antagonist, presumably because it bound to β-receptors in a monovalent manner without activating them (Hammacher *et al.*, 1989). The abilities of the various isoforms of PDGF to mediate homo- and heterodimerization of PDGF α- and β-receptors are depicted in Figure 1.

Substrates for the PDGF receptor kinase

As mentioned above, an intact tyrosine kinase of the β-receptor is essential for signal transduction. This is probably true also for the α-receptor, although data are not yet available for this receptor. It is thus likely that the phosphorylation of specific substrates on tyrosine residues by the receptor kinases is a crucial event in the transmission of the signals that lead to cell division, chemotaxis, and other effects of PDGF. Some substrates that are interesting candidates for involvement in the mitogenic pathway have recently been identified. Their identification has been simplified by the finding that they remain associated with the activated PDGF receptor after phosphorylation; they can be coimmunoprecipitated with the receptor.

One of the identified substrates is phospholipase C-γ (PLC-γ), which catalyzes the degradation of phosphatidylinositol-4,5-bisphosphate (PI-4,5-P_2), and thus generates two different second messengers, inositol-1,4,5-trisphosphate, which releases intracellularly stored Ca^{2+}, and diacylglycerol, which activates protein kinase C. PLC-γ has been shown to be a substrate for the PDGF receptor both in vivo and in vitro (Kumjian *et al.,* 1989; Meisenhelder *et al.,* 1989; Wahl *et al.,* 1989). The phosphorylation is most likely direct, i.e., no intermediary steps are involved, and PLC-γ can be coimmunoprecipitated with the receptor. However, it has not yet been possible to determine whether the phosphorylation of PLC-γ is associated with an increase in its catalytic activity. If this is the case, it could in part explain the stimulatory effect of PDGF on phosphatidylinositol turnover.

PDGF has also been found to stimulate the activity of phosphatidylinositol 3′ kinase (PI-3′ kinase), an enzyme that phosphorylates the D-3 position of phosphatidylinositol (Auger *et al.,* 1989; Coughlin *et al.,* 1989). This gives rise to PI-3-P, PI-3,4-P_2, and PIP_3, precursor molecules for novel inositol phosphates with still-unknown effects on signal transduction. The PI-3′ kinase is also activated by the $pp60^{c\text{-}src}$/polyoma middle T complex (Courtneidge and Heber, 1987; Kaplan *et al.,* 1987). The fact that the PI-3′ kinase activity can be precipitated both by PDGF receptor antibodies (Coughlin *et al.,* 1989) and by antiphosphotyrosine antibodies (Auger *et al.,* 1989) after PDGF stimulation suggests that the enzyme is a substrate for the receptor kinase, although this has not been shown directly.

A cellular GTPase-activating protein (GAP) has also been shown to be phosphorylated on tyrosine residues and to associate with PDGF receptors after stimulation with PDGF-AA as well as PDGF-BB (Molloy *et al.,* 1989). Maximum association with the β-receptor requires autophosphorylation of both of the identified sites (Kazlauskas *et al.,* 1990). GAP specifically interacts with Ras proteins that have been implicated in signal transduction pathways of several growth factors (Mulcahy *et al.,* 1985). It is an interesting possibility that tyrosine phosphorylation of GAP affects its association with Ras proteins and thus modulates the signals that arise from the GAP-Ras complex.

Another potentially interesting candidate substrate for the PDGF receptor kinase is the serine/threonine kinase Raf-1. Raf-1 was found to be phosphorylated in PDGF-stimulated intact cells and to be coimmunoprecipitated with the receptor (Morrison *et al.,* 1988, 1989). Tyrosine phosphorylation of Raf-1 was found to lead to a four- to sixfold increase in its serine/threonine kinase activity. It is of interest that protein kinase C, which is structurally related to Raf-1, is also activated in PDGF-stimulated cells, but by an indirect mechanism involving stimulation of phosphatidylinositol turnover (see above).

The data described above suggest that the PDGF receptors, after ligand binding and activation, become associated with kinase substrates that remain associated with the receptor after phosphorylation on tyrosine residues (Figure 2).

PDGF-inducible genes

It is clear from the previous section that PDGF stimulation generates a number of signals at the inner leaflet of the cell membrane, including phosphatidylinositol breakdown products and a phosphorylation cascade. Virtually nothing is known about how these signals are transmitted through the cytoplasm to the nucleus, but, several minutes to several hours after PDGF stimulation, a number of different genes are induced (Cochran *et al.,* 1983; Linzer and Nathans, 1983; Almendral *et al.,* 1988). A systematic investigation led to the identification of ~80 early-growth-response genes, most or all of which are induced by PDGF (Almendral *et al.,* 1988). Some of these genes code for proteins that probably are not involved directly in the triggering of cell proliferation, like matrix protein and cytoskeletal proteins; others code for growth factors or growth inhibitors that may modulate the growth response; yet others code for transcription factors, like c-*fos,* c-*jun,* c-*myc,* and c-*myb,* which may be involved in the regulation of the machinery for DNA synthesis and other events that are linked to cell division.

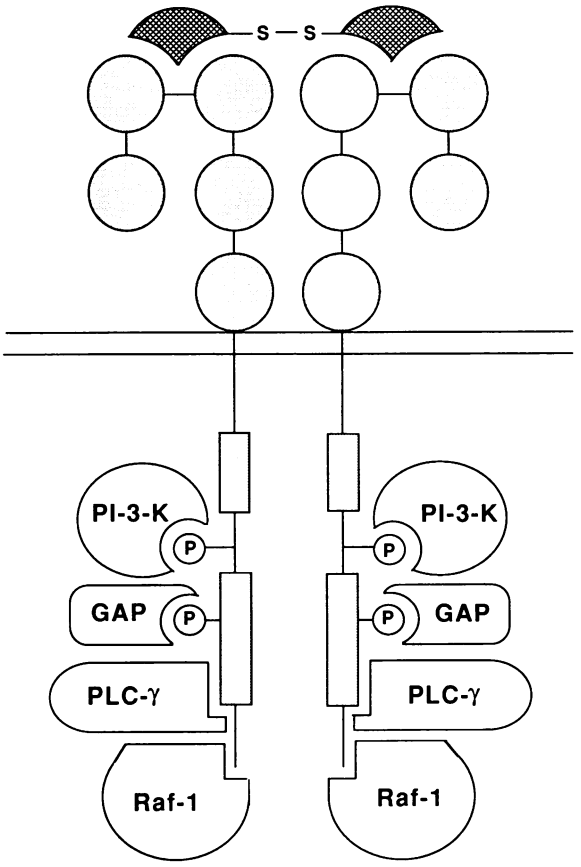

Figure 2. **Schematic illustration of a ligand-activated, dimeric PDGF receptor complex, associated with kinase substrates.** Note that the exact domains in the receptors that interact with the individual substrates are not known. For discussion, see the text.

Possible in vivo role of PDGF

The in vivo function of PDGF remains speculative. PDGF is likely to be involved in regulation of cell growth and differentiation during embryonal development, because it has been found to be expressed in mouse (Rappolee *et al.,* 1988) and *Xenopus* (Mercola *et al.,* 1988) embryos and in human placenta (Goustin *et al.,* 1985). mRNA for the A-chain but not the B-chain was found to be expressed early in mouse embryogenesis (day 6.5–8.5) together with mRNA for the α-receptor, which was expressed earlier and more abundantly than the β-receptor mRNA (Mercola *et al.,* 1990). Interestingly, the expression of α-receptor mRNA was found to be stimulated by the morphogen retinoic acid in F9 mouse teratocarcinoma cells (Mercola *et al.,* 1990). Furthermore, a function for PDGF has been demonstrated in the developing rat optic nerve, where PDGF secreted by type-1 astrocytes controls the differentiation of O-2A progenitor cells into oligodendrocytes and type-2 astrocytes (Noble *et al.,* 1988; Raff *et al.,* 1988; Richardson *et al.,* 1988). Interestingly, the O-2A cells were found to have PDGF α-receptors but no β-receptors (Hart *et al.,* 1989), and the type-1 astrocytes were found to secrete PDGF-AA but not the other isoforms of PDGF. This is thus an example of specificity within the PDGF system; other cells in the environment that have only β-receptors, like capillary endothelial cells (Smits *et al.,* 1989), will not be stimulated.

Very little is known about the mechanisms that regulate the production of PDGF A- and B-chains in vivo. In vitro studies have indicated that the two genes are differentially controlled (Betsholtz *et al.,* 1986b) and that their expression in cultured cells is modulated by external stimuli. For example, transforming growth factor (TGF)-β induces the B-chain in AKR-2B cells (Leof *et al.,* 1986), the A-chain in human foreskin fibroblasts (Paulsson *et al.,* 1988) and in a human leukemia cell line (Mäkelä *et al.,* 1987), and both A- and B-chains in endothelial cells (Daniel *et al.,* 1987). Furthermore, IL-1 has been found to induce the A-chain in smooth muscle cells; in fact, the mitogenic activity of IL-1 on these cells is mediated via production of PDGF-AA (Raines *et al.,* 1989). Interestingly, PDGF itself was found to induce the synthesis of PDGF A-chain in human fibroblasts, suggesting a positive autocrine feedback mechanism in the prereplicative phase (Paulsson *et al.,* 1987). Similar examples of autoinduction have been found for EGF/TGF-α (Coffey *et al.,* 1987) and TGF-β (Van Obberghen-Schilling *et al.,* 1988).

The facts that PDGF is released by platelets and by cells involved in the inflammatory reaction and that it stimulates proliferation, chemotaxis, and matrix production suggest a role in the adult individual in tissue repair processes. Initial experiments using in vivo models for wound healing are in support of this hypothesis. Thus Sprugel *et al.* (1987) found that PDGF administrated in a collagen gel in Gortex tubes inserted in the skin of rats promoted the formation of granulation tissue, and Pierce *et al.* (1989) found that local application of PDGF-BB increased the strength of the healing wound.

PDGF may also be involved in adverse reactions, such as malignancies (see below) and other conditions involving an excess of cell proliferation. Thus, in chronic inflammatory conditions, the stimulatory effect of PDGF on connective tissue cells may lead to tissue fibrosis (Martinet *et al.,* 1987). Furthermore, the stimulatory effects of PDGF on arterial smooth

muscle cells, may at sites of injury of the endothelial cell layer cause an unscheduled proliferation and migration of smooth muscle cells into the intima of the vessel wall and thereby promote atherosclerosis (Ross, 1986).

Of importance for the understanding of the effects of PDGF in vivo is the recent observation that the PDGF β-receptor is not present on cells in certain normal tissues, despite that the corresponding cell types have receptors when grown in tissue culture (Terracio *et al.,* 1988). Receptors were found to be induced, however, in conjunction with inflammation in vivo (Rubin *et al.,* 1988). Analogously, rat liver fat-storing cells have been found to respond to PDGF (Pinzani *et al.,* 1989), and the response is enhanced by conditioned medium from Kupffer cells by induction of PDGF receptors (Friedman and Arthur, 1989). Furthermore, elevation of the intracellular cyclic adenosine monophosphate (cAMP) concentration in rat Schwann cells leads to induction of PDGF receptors and PDGF responsiveness (Weinmaster and Lemke, 1990). These findings suggest that the response to PDGF in vivo depends not only on the availability of ligand, but also on the expression of the corresponding receptor, which may be regulated by humoral factors, released, e.g., from inflammatory cells. Other cell types, such as porcine endometrial cells (Terracio *et al.,* 1988) and human arachnoideal cells (Wang *et al.,* unpublished observations), constitutively express PDGF β-receptors in vivo. Data on α-receptors in vivo are not yet available; however, recent studies have revealed that this receptor can be modulated in vitro: it is down-regulated after exposure of 3T3 cells to TGF-β (Gronwald *et al.,* 1989).

PDGF and cell transformation

It is interesting that PDGF, one subunit of which itself is the product of the protooncogene c-*sis,* induces a signal pathway that involves at least two protooncogene products at the cell membrane, Raf and Ras, and a number of protooncogene products in the nucleus. It is conceivable that constitutive activation of these products, e.g., after retroviral transduction or other mutational events, leads to transformation of the cells by subverting the mitogenic pathway of PDGF. These observations on PDGF and its mechanism of action are consistent with the general hypothesis that oncogene products act by subverting the mitogenic pathway of growth factors at different levels, thereby giving the cell a constitutive growth stimulus (reviewed in Heldin *et al.,* 1987). Whereas this could explain the loss of growth control characteristic of transformed cells, other genetic alterations are probably needed to account for the fully malignant phenotype. Thus, normal human fibroblasts infected with SSV appeared morphologically transformed, but were not immortalized and senesced just like uninfected cells (Johnsson *et al.,* 1986). The finding that the B-chain of PDGF is virtually identical to part of $p28^{sis}$, the transforming protein of SSV, stimulated experiments to determine the effect of overexpression of either of the two chains of PDGF in cells with PDGF receptors. Genomic (Gazit *et al.,* 1984) or cDNA (Clarke *et al.,* 1984; Josephs *et al.,* 1984) sequences of the PDGF B-chain (c-*sis*) under the control of viral promoters were shown to cause efficient cell transformation. Similar experiments with the A-chain of PDGF revealed that it too has transforming properties, albeit less efficient compared with the B-chain (Beckmann *et al.,* 1988; Bywater *et al.,* 1988), most likely because it stimulates α-receptors only, whereas PDGF-BB stimulates both α- and β-receptors.

The findings that PDGF has a potent transforming effect in gene transfer experiments raise the question whether unscheduled production of PDGF contributes to the growth of spontaneous tumors. This possibility is supported by the finding that PDGF is frequently produced by cell lines established from human malignancies. As an example, 24 and 17 human glioblastoma cell lines of 24 investigated produced A- and B-chains, respectively (Nistér *et al.,* 1988b). Because most of these cell lines also expressed one or both PDGF receptor types, autocrine stimulation of cell growth may have occurred. Investigations of sections of glioblastoma tumors by in situ hybridization technique revealed that PDGF production is common also in vivo (Hermansson *et al.,* 1988). In addition to being produced by the tumor cells, the B-chain of PDGF was also produced in the hyperplastic endothelium, which also was found to have β-receptors. Thus, it is possible that PDGF is of importance in autocrine stimulation of growth of normal as well as malignant cells of human glioblastoma.

There are also several examples of PDGF-producing cell types that lack PDGF receptors, e.g., mammary carcinoma cell lines (Rozengurt *et al.,* 1985; Bronzert *et al.,* 1987; Perez *et al.,* 1987). Whereas an autocrine effect of PDGF in these cases is highly unlikely, it is possible that PDGF production could contribute to some ex-

tent to the formation of the tumor stroma by a paracrine mechanism.

Autocrine stimulation by PDGF: intracellular or extracellular event?

Studies on *sis*-transformed cells have provided ample evidence that PDGF-BB elicits an autocrine growth response (Johnsson *et al.,* 1985). It is a matter of controversy, however, whether the growth stimulatory signal is generated at the plasma membrane or within an intracellular compartment; evidence has been presented in favor of both views. The extensive downregulation of PDGF β-receptors in *sis*-transformed cells has been taken as an indication of a rapid turnover of activated cell-surface receptors (Garrett *et al.,* 1984). But the argument has also been raised that intracellular activation of the receptor leads to a shunt to the degradative pathway and therefore to a very low expression of cell-surface receptors (Keating and Williams, 1988). The presence of mitogenically active intracellular forms of the *sis* product is consistent with this idea (Lokeshwar *et al.,* 1990). Several authors have shown conclusively that the PDGF β-receptor protein tyrosine kinase is indeed activated inside the cell, most likely already in the endoplasmic reticulum (Hanninck and Donoghue, 1988; Huang and Huang, 1988; Keating and Williams, 1988; Fleming *et al.,* 1989). The intracellular activation hypothesis also fits with the recent finding that a recombinant v-*sis* protein, made to remain in the lumen of the endoplasmic reticulum by a C-terminal KDEL retention signal, has transforming activity (Bejcek *et al.,* 1989). However, one cannot exclude the possibility that transformation is caused by ligands that escape retention.

A strong argument in favor of an extracellular autocrine growth stimulation in *sis*-transformed cells was provided by the finding that PDGF antibodies attenuate acute transformation of human fibroblasts by SSV and inhibit serum-free growth of SSV-transformed cells (Johnsson *et al.,* 1985). Further evidence has been obtained using suramin, a polysulfonate known to block the binding of PDGF (Williams *et al.,* 1984; Betsholtz *et al.,* 1986a) and the v-*sis* product (Garrett *et al.,* 1984) to PDGF receptors. Addition of suramin to *sis*-transformed cells leads to an upregulation of PDGF receptors (Garrett *et al.,* 1984; Johnsson *et al.,* 1986) and a complete reversal of the transformed phenotype (Betsholtz *et al.,* 1986a). Although it has been argued that suramin is taken up by the cells and exerts its receptor-blocking activity inside the cell (Huang and Huang, 1988), there is conclusive evidence that the protein tyrosine kinase activity of intracellular receptors in *sis*-transformed cells is indeed unaffected by suramin (Keating and Williams, 1988; Fleming *et al.,* 1989); only kinase activity associated with cell surface receptors is inhibited (Fleming *et al.,* 1989). Another strong argument in favor of the extracellular mechanism was raised by Hanninck and Donoghue (1988). These authors found that alteration of the intracellular transport system by monensin did not inhibit autophosphorylation of an immature PDGF receptor in cells expressing *sis,* but extinguished c-*fos* expression.

In our opinion, most experimental data are compatible with the hypotheses that the *sis* product and the cognate receptor interact inside the cell and that this leads to activation of the receptor kinase. This is a sterile interaction, however. To generate a mitogenic signal, the ligand-receptor complex has to be translocated to the plasma membrane, probably to interact with the proper receptor kinase substrates. This model is schematically depicted in Figure 3.

Future perspectives

In spite of significant advances in recent years, several of the most important structural and functional aspects of PDGF and its receptors remain elusive. The gross structure of PDGF has been defined, but the localization of the intra- and interchain disulfide bonds as well as the three-dimensional structure of PDGF await elucidation. A related question concerns the structure of the intracellular, truncated 24-kDa form of PDGF-BB. The three-dimensional structure of the PDGF receptors in their free and ligand-binding conformation is another important area for future research. The discovery of a family of enzymes as substrates for the protein tyrosine kinase activities of the PDGF receptors is a major breakthrough in the area concerning the signal transduction pathway of PDGF. But what is their precise role in mitogenesis, and what is the nature of the signals that carry the message further to the nucleus and change the transcriptional activity?

The *sis* gene is one of the best-characterized oncogenes with regard to its function in cell transformation. The only remaining issue concerns the subcellular location of the transforming factor: is the autocrine growth signal generated inside the cell or at the cell surface? Although we favor the latter view, the possibility of an intracellular growth activation has not been definitely ruled out.

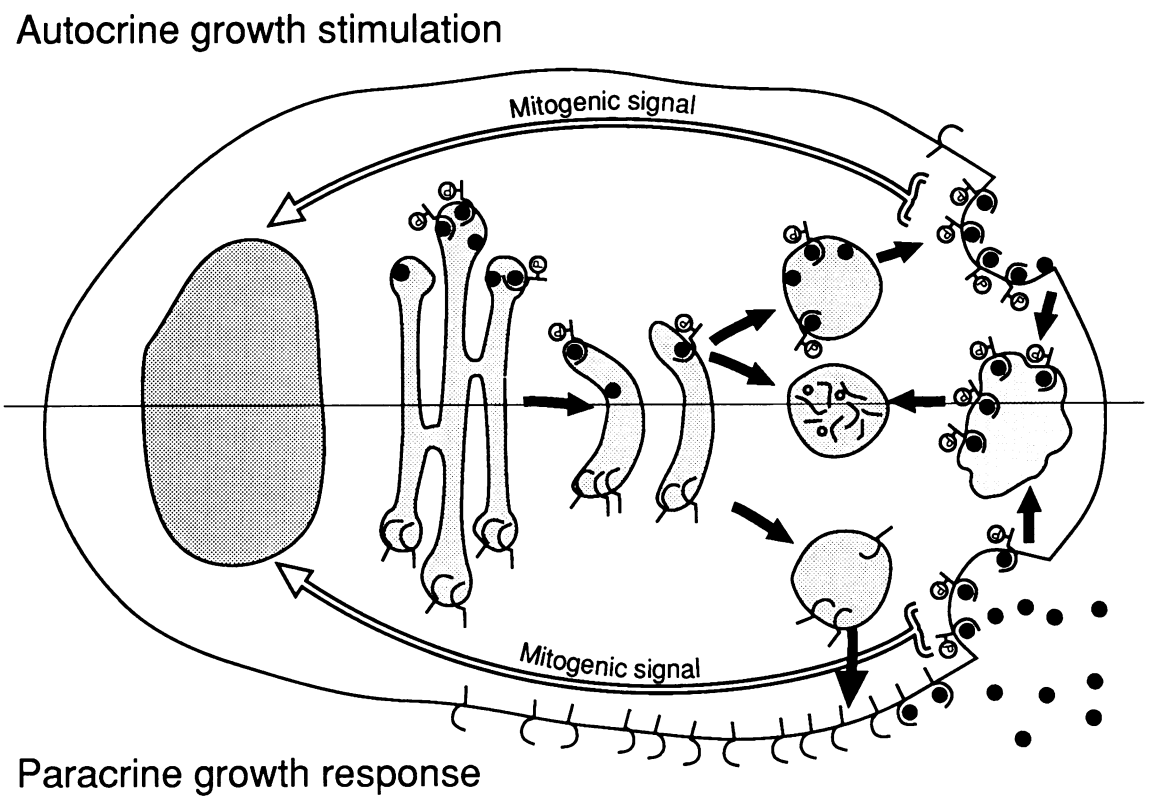

Figure 3. **Schematic illustration of autocrine growth stimulation in a cell producing PDGF, in comparison with the effect of exogenous PDGF on a responsive cell.** The symbols indicate: ●, PDGF; ⌇, PDGF receptor; ⌇ⓟ, activated and autophosphorylated PDGF receptor. According to this model, the newly synthesized receptors bind ligand and become activated already in the endoplasmic reticulum or in the Golgi apparatus, but the ligand-receptor complex needs to be transported to the cell membrane before a mitogenic signal is initiated. For discussion, see the text.

An important question concerns the role of PDGF in the development of human malignancies. No structural alterations in the genes encoding PDGF or its receptors have been found in human tumor cells. Although many examples have been found of tumor cells that produce one or several of the isoforms of PDGF and the cognate receptor, formal proof of an autocrine stimulation has not been obtained. At present, we are therefore left with the question whether PDGF is a driving force in tumor development or merely an innocent bystander. A change of direction toward the study of tumors at very early stages of development will probably be helpful.

The number of potential target cells for PDGF is continually increasing as is the number of cells producing PDGF. Our understanding of the role of PDGF in normal development and physiology is, however, only fragmentary. Detailed studies on the distribution of PDGF and its receptors in vivo as well as manipulation of their genes in experimental animals are therefore warranted.

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