# Signal Transduction by the Platelet-Derived Growth Factor Receptor

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**When platelet-derived growth factor (PDGF) binds to its receptor on a quiescent fibroblast or smooth muscle cell, it stimulates a remarkably diverse group of biochemical responses, including changes in ion fluxes, activation of several kinases, alterations in cell shape, increased transcription of a number of genes, and stimulation of enzymes that regulate phospholipid metabolism. These and other reactions culminate, hours later, in DNA replication and cell division. How does the receptor for PDGF recognize and bind its specific ligand and then transduce this signal across the cell membrane via a single membrane-spanning region? Which of the immediate cellular responses are directly involved in the biochemical pathways that lead to DNA synthesis? How does the PDGF receptor trigger a diverse group of responses? Recent studies of the PDGF receptor have provided insight into these issues.**

SERUM DERIVED FROM CLOTTED WHOLE BLOOD IS BETTER AT stimulating proliferation of vascular smooth muscle cells and fibroblasts than is plasma from which platelets have been removed. It was this observation that led to the discovery of platelet-derived growth factor (PDGF) as a growth-promoting substance that is released from platelets when they adhere to the surface of an injured blood vessel (*1, 2*). The earliest studies of PDGF emphasized its role in stimulating the proliferation of vascular smooth muscle cells, a process that can cause pathological narrowing of the lumen of a blood vessel at the site of an atherosclerotic plaque. In recent years it has become apparent that tissues other than platelets are also sources of PDGF. For example, endothelial cells that line the inner surface of blood vessels produce PDGF under the tight control of a number of factors that regulate transcription of PDGF genes (*3, 4*). Macrophages may be an important source of PDGF in the normal response of tissue to wounding and in inflammatory conditions (*5*). Other roles for PDGF in normal developmental biology have been proposed as a result of the finding of PDGF in the placenta (*6*), the early mouse embryo (*7*), *Xenopus* embryos (*8*), and astrocytes of developing optic nerves (*9*).

The human platelet form of PDGF is predominantly a disulfide-linked dimer of two homologous peptides termed A and B (*10*). Homodimeric AA and BB forms of PDGF have been found in several cultured cell lines and appear to participate in a number of processes in vivo, although the specific biological roles of the AB, AA, and BB dimeric forms have not been clarified. The homodimeric form of PDGF encoded by the v-*sis* oncogene is closely related to the BB homodimer in its amino acid sequence (*11, 12*). When expressed in cells that have PDGF receptors, this oncogene-encoded protein causes cell transformation by activating the receptors inside the cell before the receptors reach the cell surface in their biosynthetic pathway (*13*). From recent studies of the expression of the A chain, B chain, and receptor for PDGF in diseased blood vessels it seems likely that PDGF plays a role in atherosclerosis (*14*). The PDGF system may be involved in other fibroproliferative pathological processes, including pulmonary fibrosis, glomerulonephritis, myelofibrosis, keloid formation, and carcinogenesis. However, the definitive role of PDGF in these processes will probably not be understood until a specific antagonist of the action of this factor is available or until animals are produced that are deficient in PDGF or its receptor.

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## Structure of the PDGF Receptor

The PDGF receptor was first identified as a 180- to 190-kD membrane glycoprotein by the covalent cross-linking of $^{125}$I-labeled PDGF (AB heterodimeric form) to intact cells (*15*) or to membrane preparations (*16*). The receptor can be found on vascular smooth muscle cells, fibroblasts, and glial cells, but is not present on endothelial cells or on most hematopoietic cells. Like the insulin, epidermal growth factor (EGF), colony-stimulating factor–1 (CSF-1), and insulin-like growth factor–1 (IGF-1) receptors, the PDGF receptor has intrinsic tyrosine kinase activity (*17–19*), and its amino acid sequence contains an easily recognizable tyrosine kinase domain (*20*). When quiescent fibroblasts are stimulated by PDGF, the predominant tyrosine-phosphorylated species is the receptor itself (*20*). It was this property of the PDGF-activated kinase that allowed purification of the PDGF receptor by affinity chromatography with antibodies that recognize phosphotyrosine (*21*). The receptor was phosphorylated by stimulating mouse fibroblasts with human platelet PDGF that was predominantly of the AB form. Thus the receptor was purified on the basis of the sensitivity of its kinase to the AB form of PDGF. Oligonucleotide probes designed from partial amino acid sequences were used to isolate the cDNAs for the mouse (*20*) and closely related human (*22–24*) PDGF receptors.

Several features of the amino acid sequence of the PDGF receptor (Fig. 1) have been used to categorize related receptor molecules into a class that includes the receptor for CSF-1 (*25*) and the c-*kit* proto-oncogene, which is a putative receptor for an unknown ligand (*26*). The most striking of these structural features is the organization of the extracellular region into five immunoglobulin-like domains (D1 to D5) (Fig. 1). The other distinctive characteristic of this class of receptors is that there is a large region that interrupts the coding sequence of the tyrosine kinase domain (*20*). Thus the kinase domain, which is clearly recognizable by its amino acid homology to

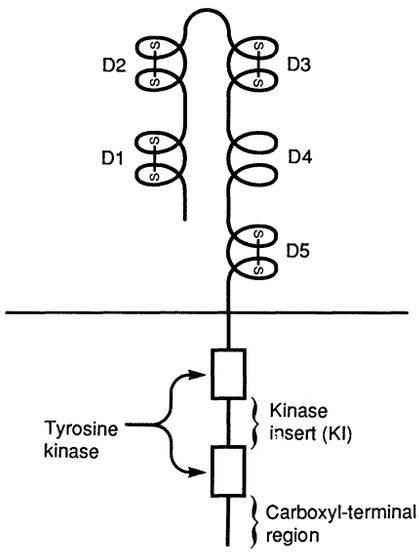



**Fig. 1.** Representation of PDGF receptor structural domains. The extracellular portion of the molecule consists of five immunoglobulin-like domains, D1 to D5. The cytoplasmic region contains sequences homologous to other tyrosine kinases (boxes). The kinase sequences are interrupted by the kinase insert (KI) domain. The c-*kit* protein, CSF-1 receptor, and a recently discovered PDGF receptor-like molecule (*70*) have an identical organization of domains in that they have five immunoglobulin domains and a kinase insert region.

the other tyrosine kinases such as the oncogene tyrosine kinases, is considerably larger than the more "conventional" tyrosine kinase domains (*27*). We have termed the sequence that interrupts the kinase domain the "kinase insert" (KI) region of the receptor (*20*). The KI regions of the PDGF receptor, c-*kit* protein (*26*), and CSF-1 (*25*) receptor are different in specific sequences and length, but are found at precisely the same location within their respective kinase domains. It is possible that the KI sequences represent a structural "excursion" from the sequences that actually form the active site of the kinase. All of the tyrosine kinase molecules that have large KI domains also have five external immunoglobulin-like domains, an indication that the receptors in this group may have a common ancestry.

The other features of the PDGF receptor determined from the primary amino acid sequence are the presence of a single membrane-spanning segment and a "juxtamembrane" region that connects the first kinase domain with the transmembrane domain (*20*). Little is known about the juxtamembrane region except that its length of approximately 47 amino acids is highly conserved among the receptor tyrosine kinases. The carboxyl-terminal domain of the PDGF receptor is distinctive in sequence but has no easily predictable structure, and its function is also unknown.

Gronwald *et al.* (*23*) and Heldin *et al.* (*28*) showed that radioiodinated AA and BB forms of PDGF bind to cells with at least two different specificity patterns, an indication that more than one type of PDGF receptor exists. Although interpretations of the data on PDGF receptor subtypes have differed, the findings are all consistent with the existence of more than one type of PDGF receptor. The relative amounts of the subtypes seem to differ among different types of cells. The mitogenic and tyrosine kinase activation of 3T3 cells and smooth muscle cells appear to be due predominantly to the form of the receptor that preferentially binds the BB form of PDGF (*29*). Although the AA form of PDGF binds and activates this receptor at high concentrations (*29*), it binds with higher affinity to another receptor subtype (*24, 28*) whose amino acid sequence and biological role have not yet been determined. Since most of the information regarding signal transduction has come from studies of the receptor that preferentially binds BB PDGF, this article deals exclusively with this type of receptor, and for simplicity, the term "PDGF receptor" is used to denote the BB receptor subtype.

## The PDGF-Binding Domain of the Receptor

When the primary structure of the mouse PDGF receptor was determined by cDNA sequencing (*20*), the regularity of the spacing of cysteine residues in the extracellular domain was an obvious structural feature that distinguished the PDGF receptor from the insulin and EGF receptors, which have well-defined cysteine-rich clusters. After close analysis of the extracellular domain of the PDGF receptor, five β-sheet–rich repeats of around 100 amino acids, each resembling an immunoglobulin variable or constant region, were identified. A representation of a typical immunoglobulin G molecule is shown in Fig. 2. The constant region domains, labeled $C_H$ and $C_L$, are structurally similar to each other, as are the variable domains, $V_L$ and $V_H$, which form a binding site for antigen. Disulfide bonds are important structural components of each domain and also hold together the heavy and light chains (Fig. 2). The domains associate in pairs through noncovalent interactions. The structures of immunoglobulin variable and constant domains have been studied extensively by crystallographic techniques (*30*). Each domain can be described as a sandwich of two β-sheets that are, in turn, formed by an antiparallel arrangement of β-strands (represented by the letters A to D in Fig. 3). The two sheets of each domain are pinned together by a single disulfide bond, indicated by S-S in Fig. 3. A variable domain differs from a constant domain in that it possesses one or two extra β-strands, labeled C′ and C″ in Fig. 3. The amino acids that form the contact sites with antigen are typically located in the loops between β-strands and are termed complemen-

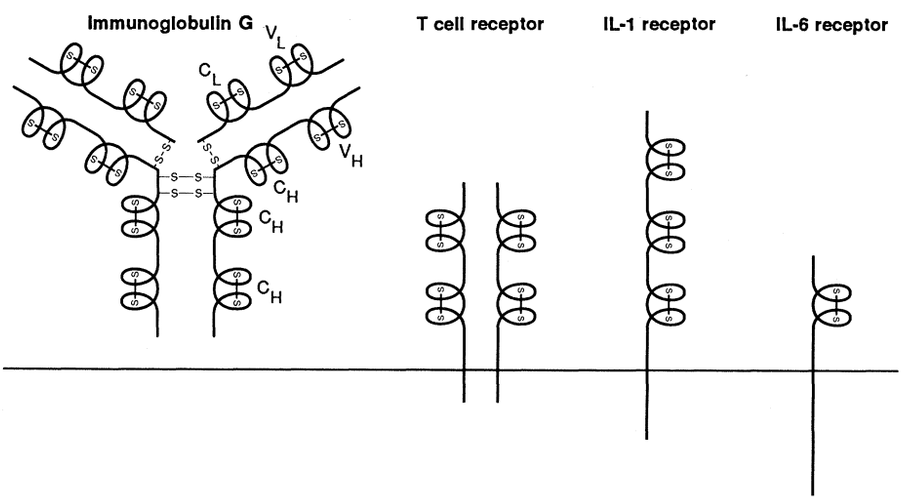



**Fig. 2.** Schematic representation of an immunoglobulin G (IgG) molecule and some receptor molecules that have immunoglobulin-like domains. The light chain constant and variable ($C_L$ and $V_L$) domains and heavy chain constant and variable ($C_H$ and $V_H$) domains are shown. Disulfide bonds are represented by S-S.

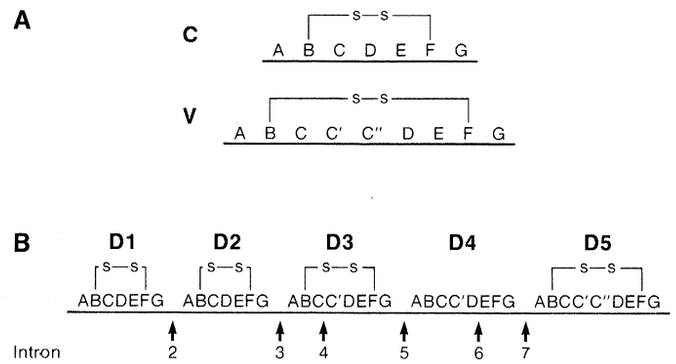



**Fig. 3.** Folding patterns of (**A**) immunoglobulin constant (C) and variable (V) domains and (**B**) postulated folding pattern of PDGF receptor immunoglobulin-like domains. Each of the letters A to G represents a β-strand. Disulfide bonds are represented by S-S. The complementarity determining regions of the variable domain lie between strands B and C, between C′ and C″, and between F and G. The positions of introns of the PDGF receptor gene are shown by arrows (*72*).

tarity determining regions. Although there is considerable variability in specific sequences among immunoglobulin domains, the topology of the β-strands is highly conserved (*31*).

The proposal that the extracellular region of the PDGF receptor consists of five immunoglobulin-like domains is based on a number of observations. First, the amino acid sequence contains five regular repeats, each of the appropriate size for an immunoglobulin domain. Each repeat, except the fourth, has characteristically spaced cysteine residues that are a diagnostic feature of an immunoglobulin domain. The receptor sequence has other important features of an immunoglobulin domain—for example, the presence of characteristically positioned tryptophan and tyrosine residues within the immunoglobulin-like domains (*31*). Direct sequence comparison of segments of the receptor with corresponding segments of true immunoglobulin domains shows a statistically significant similarity between PDGF receptor domains and immunoglobulin domains (Table 1). The argument that the receptor domains assume the folding pattern of immunoglobulin domains can be strengthened by examining the predicted secondary structure of the receptor. The β-strand topology predicted by Bazan and Fletterick (*32*), using the Garnier-Robson algorithm (*33*), a turn predictor program (*34*), and a Fourier analysis of hydrophobic sequence pattern, is shown in Fig. 3B. The first two domains of the receptor have only six potential β-strands and would therefore resemble an immunoglobulin constant domain. The third and fourth domains are long enough to form an extra strand. The fifth domain more closely resembles a variable heavy chain domain in length. The CSF-1 receptor and c-*kit* protooncogene have an identical pattern of domain organization, suggesting that this domain structure (Fig. 3) is important for both ligand recognition and signal transduction. A final point in support of the proposed domain structure of the PDGF receptor relates to the exon structure of the receptor gene. A common characteristic of molecules in the immunoglobulin gene superfamily is that most of the immunoglobulin-like domains are encoded by a single exon that defines the boundaries of the domain (*31*). For a few of these molecules, especially those in the cell adhesion molecule group, a single additional intron is found within the domain sequences (*31*). As depicted in Fig. 3, the intron-exon junctions of each of the PDGF receptor immunoglobulin domain are at the domain boundaries except for the single introns that fall within the coding sequences of the third and fourth domains (*35*).

The presence of immunoglobulin-like domains in the extracellular regions of transmembrane proteins seems to be a feature shared by a number of molecules that have recognition functions. The PDGF receptor, CSF-1 receptor, and c-*kit* protein belong to a structurally related family that probably includes additional members. Several other growth-promoting receptors, including the T cell receptor (*36*), the interleukin-1 receptor (*37*), and the interleukin-6 receptor (*38*), have immunoglobulin-like domains (Fig. 2). A number of cell adhesion molecules also have immunoglobulin-like domains. In particular, the neural cell adhesion molecule (*39*) is clearly similar to the PDGF receptor in immunoglobulin domain organization receptor (Table 1). On the basis of this comparison, one can speculate that growth factor receptors may be involved in direct cell-cell interactions and may function as adhesion molecules.

## Signal Transduction Across the Membrane

The mature PDGF receptor sequence has only one hydrophobic sequence that is long enough to span the plasma membrane. The amino acid sequence for the membrane-spanning region is predicted to form a rigid α-helix that has rather unremarkable surface features except perhaps some potential hydrogen bonding structures involving the serine, threonine, and tryptophan residues. Many transmembrane regions of other receptors contain several serines and threonines that have no known function. We examined the sequences of transmembrane segments for the known growth factor receptors and have found no recognizable pattern in the positioning of these residues. The membrane-spanning regions of the PDGF receptor, CSF-1 receptor, and c-*kit* protein have no significant sequence similarity, except for the presence of hydrophobic residues. However, the transmembrane regions of the human and mouse PDGF receptors (*22–24*) are almost identical. This degree of sequence identity suggests that this sequence is functionally important and that the transmembrane region serves some function other than simply providing a membrane anchor.

To study the role of the transmembrane region, Escobedo *et al.* (*40*) replaced this segment of the PDGF receptor with transmembrane sequences of other receptors. The "donor" transmembrane sequences we chose in these experiments were those of the low density lipoprotein (LDL) receptor and the normal and transforming forms of the *neu* gene. The normal *neu* gene encodes a tyrosine kinase receptor–like molecule that binds an unknown ligand (*41*); a single amino acid change—valine to glutamic acid—in the transmembrane region is responsible for the transforming activity of the

**Table 1.** Comparison of amino acid sequences of five immunoglobulin-like repeats of the mouse PDGF receptor (D1 to D5) with those of immunoglobulin and neural cell adhesion molecules. Alignment scores generated by the ALIGN program are shown for pairwise comparisons between the immunoglobulin-like repeats of the mouse PDGF receptor and a human immunoglobulin μ constant domain (MHHU), human immunoglobulin K variable domain (KIHUWE), and the five domains (D1 to D5) of a chicken neural cell adhesion molecule (ChNCAM). The abbreviations are from the National Biomedical Research Foundation code (*71*). These scores are expressed in standard deviations from the mean of 300 randomized comparisons at a gap penalty of 7 with the mutation data matrix bias of +5. Scores above 3 represent significant similarities. These alignments were performed by F. Bazan at the University of California at San Francisco.

| Mouse PDGF receptor | MHHU | KIHUWE | ChNCAM | | | | |
|---|---|---|---|---|---|---|---|
| | | | D1 | D2 | D3 | D4 | D5 |
| D1 | 4.91 | 3.02 | 5.15 | 4.78 | 6.03 | 6.17 | 2.47 |
| D2 | 0.58 | 2.41 | 1.90 | 3.14 | −0.16 | 3.31 | 1.24 |
| D3 | 5.95 | 4.55 | 6.91 | 5.03 | 5.87 | 7.26 | 5.89 |
| D4 | 2.71 | 0.77 | 2.78 | 2.02 | 5.88 | 4.93 | 3.63 |
| D5 | 1.75 | 1.94 | 3.20 | 2.08 | 5.39 | 6.67 | 1.95 |

*neu* oncogene (*41*). Experimentally the switch in transmembrane regions was accomplished by constructing chimeric cDNAs and expressing each of these in Chinese hamster ovary (CHO) fibroblast cells that lack PDGF receptors (*42*). The chimeric receptors were then tested for their ability to bind PDGF and to respond with an increase in tyrosine kinase activity after PDGF stimulation. None of the PDGF receptor molecules with transmembrane region replacements responded to PDGF with an increase in tyrosine kinase, even though all of them bound PDGF with high affinity (*42*). This finding shows that the transmembrane region is not simply a membrane anchor but plays an important functional role in signal transduction.

How does the PDGF receptor transduce signals across the membrane? There are a number of possible models (Fig. 4). First, when PDGF binds to the receptor, a conformational change could be propagated by perturbing the structure of the transmembrane α-helix. However, it is conceptually difficult to envision how the structure of the transmembrane region, which is essentially insulated from the rest of the molecule, could be altered after ligand binds to the extracellular region. A second model is that PDGF binding somehow displaces the molecule a short distance in a direction perpendicular to the plane of the membrane, thereby altering intramolecular interactions in the cytoplasmic domain. There is little direct evidence for or against this mechanical model, but it does not predict that the sequences in the center of the membrane region would have a high degree of conservation. Therefore this model does not account for the identity of human and mouse PDGF receptor transmembrane sequences. In a third model, PDGF may induce receptor dimerization to form an active site that requires a dimeric structure to recognize a specific substrate for the tyrosine kinase. In a fourth model that is similar to the third model, the receptor may be a dimer in its native state in the absence of ligand. When PDGF binds, the relationship of the two extracellular components of the dimer may be altered. Since the transmembrane α-helical regions are relatively rigid, there would be a concomitant alteration in the intracellular substrate-binding regions of the dimer when PDGF binds to the extracellular domain. In a related model, the PDGF receptor could be a component of a heterodimer that includes another unidentified molecule. The heterodimer model is conceptually identical to the third and fourth homodimer receptor models described above.

Are the dimeric models (Fig. 4) consistent with the apparent requirement for specific transmembrane segments of the receptor? If the PDGF receptor molecule is relatively inflexible along an axis perpendicular to the membrane, then the rotational angle of the external domain relative to the cytoplasmic domain around this axis may be critical. The kinase domains of the receptor dimer may require a specific relative orientation to form an active site. Mutations in the transmembrane α-helix that may alter the pitch or length of the helix may significantly alter orientation of the cytoplasmic domain in relation to the extracellular domain and thus may hinder the formation of an intracellular active site after ligand binds to the extracellular region. This type of dimeric model accounts for specificity and conservation of transmembrane sequences as well as the existence of dimeric molecules in the unactivated state of the receptor. Fortunately the third and fourth models described above are experimentally testable and are under investigation in a number of laboratories. Although the ligands for the homologous PDGF and CSF-1 receptors have dissimilar sequences, they are both disulfide-linked dimers and may interact with two subunits of a receptor dimer. Both CSF-1 and PDGF receptors have immunoglobulin-like extracellular domains and also have KI regions and are likely to utilize similar signal transduction mechanisms. It would be of interest to know whether the ligand of the c-*kit* protein is also a disulfide-linked dimer.

## PDGF-Induced Change in Receptor Conformation

When PDGF binds to its receptor there may be a structural change in either the folding pattern of the cytoplasmic portion of the receptor or in the association of this region with other molecules. Keating *et al.* (*44*) prepared antisera directed at peptides from several regions of the PDGF receptor molecule and tested the hypothesis that the binding of PDGF to the receptor would alter the exposure of some epitopes by either changing intramolecular folding or intermolecular interactions. In these experiments intact fibroblasts were stimulated by PDGF, and cell extracts prepared under nondenaturing conditions were immunoprecipitated with the series of antisera. One antiserum was able to discriminate between a receptor that had been stimulated by PDGF and an unstimulated receptor. This antiserum, which was directed against sequences in the c-*tail* domain near the junction of this domain and the second kinase domain (Fig. 1), precipitated seven times more receptor from PDGF-stimulated cells than from unstimulated cells (*44*). The same antiserum recognized PDGF-stimulated and unstimulated receptors equally well under denaturing conditions. Thus under nondenaturing conditions the antiserum recognized a change in exposure of its epitope, probably caused by a PDGF-induced change in conformation of the receptor. PDGF induced this change in vitro, but only in the presence of adenosine triphosphate (ATP). Nonhydrolyzable

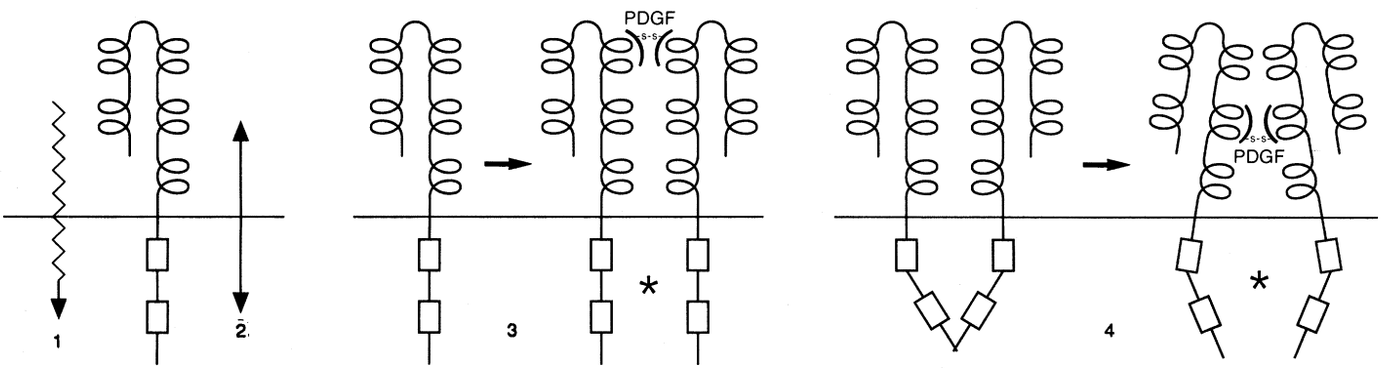



**Fig. 4.** Several possible mechanisms of signal transduction. The first mechanism involves perturbing the structure of the α-helix that spans the membrane. The second mechanism involves a displacement of the receptor molecule perpendicular to the plane of the membrane. In the third mechanism, PDGF induces dimerization of the receptor. In the fourth mechanism, the receptor is a dimer even in the absence of PDGF, and PDGF alters the relative positions of the components of the dimer.

analogs of ATP that would act as competitive antagonists of ATP blocked the conformational change induced by PDGF. The most likely explanation of these and other observations is that the conformational change is a consequence of receptor autophosphorylation (*44*). Further support for this idea has come from experiments on mutants of the PDGF receptor that lack tyrosine kinase activity and fail to undergo the conformational change in response to PDGF (*45*). However, we have constructed two receptor mutants—one with a deleted KI region and another with an altered autophosphorylation site—that have tyrosine kinase activity but do not undergo conformation change (*45*). Thus kinase activity appears to be necessary but not sufficient for the change in conformation. In all of the mutants we have tested there is a one-to-one correlation between the ability of the mutant PDGF receptors to undergo conformational change and their ability to stimulate DNA synthesis, an indication that this change may be an essential step in signal transduction (*45*). This conformational change could be a feature of any of the models described in the previous section, but might be especially relevant to the first and fourth models (Fig. 4).

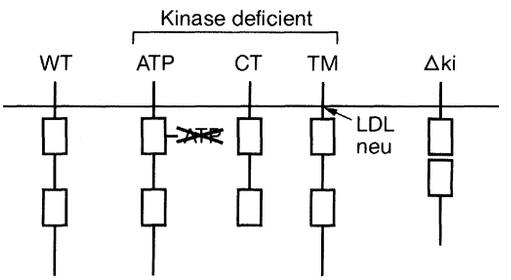
## Activation of Intracellular Pathways

When PDGF binds to its receptor it rapidly stimulates a group of "early" responses that occur in minutes. These include activation of the tyrosine kinase, hydrolysis of phosphatidylinositol (PI) (*46*), alterations in cellular *p*H (*47*), increase in cytosolic calcium levels (*48*), a dramatic change in the cytoskeleton (*49*), increased expression of a group of genes (*50–55*), elevation of cellular cyclic adenosine monophosphate (cAMP) (*56*), and internalization and degradation (downregulation) of the receptor. How do these events culminate in DNA synthesis? What other cellular reactions are involved? How are these responses interrelated? Is tyrosine receptor kinase activity required to induce PI hydrolysis? To explore these issues, we altered the structure of the receptor by site-directed mutagenesis and measured the functional consequences of the mutations. Experimentally this was achieved by expressing normal and mutated receptor cDNA sequences in CHO cells, which normally lack PDGF receptors. Stable transfectants were analyzed for their responses to PDGF.

*Role of tyrosine kinase.* We studied the role of tyrosine kinase in the cellular response to PDGF by constructing mutants of the PDGF receptor that are defective in PDGF-stimulated tyrosine kinase activity. To ensure that the alteration in PDGF responsiveness observed in the mutant receptors could be attributed to the loss of tyrosine kinase activity, and not merely to conformational changes caused by the mutation, we inactivated the kinase by three independent mutations (*40*). The first was to convert the codon for lysine at the ATP binding site (amino acid position 602) to an alanine codon. In the second mutation the coding sequences for 97 of the 145 carboxyl-terminal amino acids were deleted, thus removing most of the sequences 3′ to the kinase domain. The third group of mutations, described in the previous section, involved replacing the transmembrane sequences of the PDGF receptor with the transmembrane sequence of another molecule, such as the LDL receptor, the *neu* oncogene, or the nontransforming counterpart of the *neu* gene. The results of these studies on kinase-deficient mutants are summarized in Fig. 5.

Most responses to PDGF, including PI turnover, change in calcium levels, alteration in cellular *p*H, and DNA synthesis, could not be elicited in the transfectants expressing kinase-deficient mutants of the receptor even though the mutated receptors could bind PDGF with high affinity ($K_D$, 0.1 to 0.3 n*M*). This result was consistent with our expectation that tyrosine kinase plays a pivotal role in most of the responses to PDGF. However, one response, PDGF-stimulated receptor downregulation, occurred normally even in the kinase-deficient mutants (*40*). Thus ligand-induced internalization and degradation of the PDGF receptor do not involve tyrosine kinase activity. Recently the opposite conclusion—namely, that kinase activity is required for downregulation—was made for the insulin and EGF receptors (*57–59*).

The tyrosine kinase substrates that are important in the mitogenic response to PDGF have not yet been identified. Antibodies to phosphotyrosine detected 85-, 75-, and 34-kD proteins that are phosphorylated on tyrosine residues when fibroblasts are stimulated by PDGF. Kaplan *et al.* (*60*) suggested that the 85-kD substrate is a phosphatidylinositol kinase, although this identification has not been conclusively established. The 75-kD protein may be the *raf* proto-oncogene product that is a serine/threonine kinase (*61*). The 34-kD protein substrate of the PDGF tyrosine kinase has not been identified. Numerous other less prominent substrates of the PDGF receptor tyrosine kinase can be visualized with antibodies to phosphotyrosine. It has been difficult to assess the importance of any of these substrates as putative mediators of the biochemical responses to PDGF. Nor is the effect of receptor autophosphorylation on receptor function understood. This has been a difficult problem since there are 28 cytoplasmic residues that are potential targets of the autophosphorylation reaction. The role of one of these residues in PDGF receptor function, the tyrosine at amino acid position 825, has been examined. This tyrosine is in a position equivalent to tyrosine-416 of the v-*src* protein (*62*). In view of the apparent role of this tyrosine in regulating v-*src* tyrosine kinase activity (*62–64*), we converted tyrosine-825 to phenylalanine. This receptor mutant has a markedly attenuated ability to mediate PDGF-stimulated cell prolif-




| | WT | ATP | CT | TM | Δki |
|---|---|---|---|---|---|
| Binding | + | + | + | + | + |
| Autophosphorylation | + | − | − | − | + |
| PI hydrolysis | + | − | − | − | + |
| $Ca^{2+}$ increase | + | − | | − | + |
| pH change | + | − | | − | + |
| Downregulation | + | + | + | + | + |
| PI kinase | + | − | − | − | − |
| Conformation change | + | − | − | − | − |
| DNA synthesis | + | − | − | − | − |

**Fig. 5.** Signal transduction by PDGF receptor mutants. Mouse PDGF receptor cDNA was altered by site-directed mutagenesis, and the mutant receptors were expressed in CHO cells (*40, 66*). Stick figures represent the wild-type receptor (WT), the ATP–binding site mutant (ATP), a receptor that has a deletion of its carboxyl-terminal region (CT), the transmembrane substitution mutants (TM), and the mutant that has a deletion of its KI domain (ΔKI). The responses measured for each mutant include high affinity binding of $^{125}$I-labeled PDGF and receptor autophosphorylation in responses to stimulation of intact cells by PDGF (autophosphorylation). PDGF-stimulated phosphatidylinositol (PI) hydrolysis, increase in cellular calcium, change in *p*H, receptor downregulation, phosphatidylinositol kinase, receptor conformational change, and DNA synthesis were measured as described (*44, 66*). (+) Responses of cells were quantitatively similar to those of wild-type cells; (−) responses were significantly impaired in comparison to those of wild-type cells.

eration and does not phosphorylate the 34-kD PDGF receptor substrate, even though more than 50% of its autophosphorylating activity is preserved (*65*). Further studies will be required to determine whether this change in substrate specificity of the kinase is important in the loss of mitogenic activity by this mutant receptor.

*Kinase insert region.* The receptors in the PDGF receptor family differ from the insulin, EGF, and IGF-1 receptors in having a large sequence that interrupts the tyrosine kinase domain. The function of this KI domain is not known. However, the fact that some growth factor receptors lack a large KI region suggests that some basic functions of the receptor might not require this domain. For this reason, Escobedo and Williams (*66*) deleted 83 amino acids of this 100–amino acid region from the cDNA sequence and expressed this mutant form (ΔKI) of the receptor in CHO cells, which lack PDGF receptors. Since we anticipated that the mutant receptors might be functionally defective, we selected transfectants that had a larger number of receptors per cell than the wild-type receptor transfectants. In these experiments the cells that expressed a large number of ΔKI mutant receptors had essentially the same levels of PDGF-induced receptor autophosphorylation, PI turnover, *p*H alteration, and increase in intracellular calcium as wild-type transfectants, an indication that the KI region was not absolutely required for these responses. By contrast, the ΔKI receptor transfectants had a markedly blunted mitogenic response that was apparent in measurements of DNA synthesis or in cell number after PDGF treatment (*66*). These findings show that the early cellular responses to PDGF, including PI turnover, *p*H change, and calcium increase, are not sufficient to explain PDGF-induced cell proliferation. The results also showed that the KI domain plays an important role in PDGF-induced mitogenesis. It may be an oversimplification to state that this domain has a specific function other than to determine the substrate specificity of the tyrosine kinase domain. However, the deletion mutant has been a useful experimental tool for identification of cellular pathways that are important in the mitogenic response to PDGF.

*Stimulation of phosphatidylinositol hydrolysis and activation of phosphatidylinositol kinase.* PDGF is extremely effective in stimulating the hydrolysis of phosphatidylinositol (PI). This process produces diacylglycerol and inositol phosphate second messengers, which activate protein kinase C and calcium-mediated pathways, respectively. Measurements of PI hydrolysis by PDGF receptor mutants (Fig. 5) suggested that tyrosine kinase is required for the activation of the phospholipase C by PDGF. In addition to its stimulatory effect on PI hydrolysis, PDGF may have another role in regulating PI metabolism. When stimulated by PDGF, the receptor physically associates with a kinase that uses phosphatidylinositol as its substrate (*60, 67*). This type of PI kinase has been shown by Whitman *et al.* (*68*) to phosphorylate phosphatidylinositol on the D-3 position on the inositol ring. The predicted effect of this phosphorylation is the generation of a novel substrate of phospholipase C and consequently the production of a new set of potential second messengers produced by PDGF-induced phosphatidylinositol hydrolysis. These findings suggest that PDGF may have two effects on PI metabolism. The first is to stimulate phospholipase C activity and the second is to alter the type of inositol phosphate second messengers produced by the action of phospholipase C. The latter effect awaits experimental confirmation.

Coughlin *et al.* (*67*) examined the ability of PDGF receptor mutants to associate with PI kinase (Fig. 5) and found that the mutants that lack tyrosine kinase activity fail to associate with PI kinase activity. However, the ΔKI mutant receptor, which has autophosphorylating activity and stimulates PI hydrolysis, had no PDGF-sensitive PI kinase activity associated with it. This mutant was also defective in mediating DNA synthesis. From these data we concluded that PDGF-induced association of the receptor with PI kinase might be an important step in the mitogenic action of PDGF, but clearly is not required for PDGF-stimulated PI hydrolysis. We have speculated that the PI kinase is important in generating novel second messengers that play a role in the proliferative response to PDGF and that these messengers are not generated by the ΔKI mutant receptors. Experiments to test this hypothesis are under way.

## Induction of Gene Expression by PDGF

Upon binding PDGF, quiescent cells reenter the cell cycle, a process that requires dramatic changes in many biochemical reactions and is associated with alterations in expression of a number of genes. Among the first demonstrations of this phenomenon were the findings that PDGF increases expression of c-*myc* and c-*fos* proto-oncogenes, which encode proteins involved in the regulation of cell growth and differentiation (*50–52*). Within minutes of binding to its receptor, PDGF stimulates c-*fos* and c-*myc* transcription through at least two pathways, one that is mediated by protein kinase C and one that is not (*69*). The molecular mechanisms of these pathways have not been elucidated, but it seems likely that both pathways culminate in the covalent modification of a protein that binds directly to DNA or to another protein that is constitutively bound to a regulatory region of DNA. The intervening steps may consist of a cascade of kinase reactions that involve tyrosine, serine, and threonine kinase enzymes. Unfortunately the field of growth factor receptor studies has not yet converged with the field of transcriptional regulation to yield a cohesive picture of the intermediate steps between the cell surface receptor and nuclear transcriptional events.

Many genes other than c-*myc* and c-*fos* are regulated by PDGF (*50, 53, 55*). In no case is the molecular mechanism of this regulation clearly understood. Substrates of tyrosine kinase are likely to be intermediates in the regulation of transcription by the PDGF receptor, although these substrates have yet to be identified.

## Concluding Remarks and Summary

This year there have been a number of experimental findings that have provided insight into the mechanism of signal transduction by the PDGF receptor.

1) The extracellular region of the PDGF receptor consists of five immunoglobulin-like domains that constitute the PDGF binding site. The CSF-1 receptor, c-*kit* proto-oncogene, and at least one other receptor-like molecule, which may be the receptor for the AA form of PDGF (*70*), are structurally related to the PDGF receptor in that they have a similar extracellular domain organization and have a kinase insert region in the cytoplasmic region.

2) Signal transduction by the PDGF receptor is causally associated with a conformational change of the receptor protein in the carboxyl-terminal region of the molecule. There is a close correlation between the ability of receptor mutants to undergo this conformational change and their ability to mediate a mitogenic response to PDGF.

3) The transmembrane region of the PDGF receptor serves a specific role other than simply providing a membrane anchor for the molecule. When transmembrane regions of other receptors are substituted for the PDGF receptor transmembrane sequences, the chimeric receptor fails to transduce signals.

4) The tyrosine kinase activity of the receptor is important for most of the cellular responses to PDGF, including mitogenesis. However, tyrosine kinase activity is not required for ligand-induced

receptor downregulation. Substrates of tyrosine kinase that are important for DNA synthesis have not been identified. In particular, the link between the tyrosine kinase and the increased expression of PDGF-regulated genes has not been identified.

5) The kinase insert domain is important for the specific mitogenic action of PDGF. Experiments with a kinase insert deletion mutant have shown that PI turnover, *p*H change, and increase in cellular calcium are not sufficient to mediate the mitogenic action of PDGF. The kinase insert domain may play a role in determining the substrate specificity of the receptor tyrosine kinase and may represent a structural excursion from the kinase domain.

6) When the PDGF receptor binds its ligand, the receptor physically associates with a phosphatidylinositol kinase that may be involved in the production of novel inositol phosphate second messengers.

Despite recent progress, signal transduction by receptors for hormones, growth factors, and neurotransmitters is still poorly understood. These are especially challenging conceptual questions to be answered about the receptors that have single membrane-spanning segments. Future studies on the PDGF receptor and related molecules should provide a more complete picture of the structural changes and intermolecular associations that occur when a ligand binds to its receptor.

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