# A Common PDGF Receptor Is Activated by Homodimeric A and B Forms of PDGF

JAIME A. ESCOBEDO, SUTIP NAVANKASATUSSAS, LAWRENCE S. COUSENS, SHAUN R. COUGHLIN, GRAEME I. BELL, LEWIS T. WILLIAMS

**The human platelet-derived growth factor (PDGF) receptor complementary DNA was cloned and expressed by transfection of Chinese hamster ovary (CHO) fibroblasts. The ability of CHO cells expressing the human receptor complementary DNA (CHO-HR5) to interact with different recombinant forms of PDGF (AA and BB homodimers) was tested. Both forms of PDGF bind to the transfected receptor, stimulate the receptor tyrosine kinase activity, and elicit a mitogenic response in a manner that was indistinguishable from the responses of Balb/c 3T3 cells. The data indicate that the tyrosine kinase and mitogenic responses of the Balb/c 3T3 cells to AA and BB forms of PDGF can be attributed to a single type of receptor and show that the AA form, like the BB form, is a true mitogen.**

PLATELET-DERIVED GROWTH FACTOR, a major mitogen for cells of mesenchymal origin, is a 32-kD protein heterodimer composed of two polypeptide chains, A and B, linked by disulfide bonds (*1*). In addition to the PDGF AB heterodimer, two homodimeric forms of PDGF, denoted AA and BB, have been identified (*2*). There has been no direct proof that the AA form of PDGF can bind to PDGF receptors and stimulate the receptor tyrosine kinase activity. Although the mouse receptor that binds $^{125}$I-labeled PDGF in the AB form has been identified, purified (*3*), and sequenced (*4*), it has not been clear whether this receptor is capable of binding and mediating the actions of the AA and BB homodimeric forms. In this study, we cloned the human PDGF receptor cDNA and expressed the cDNA in Chinese hamster ovary (CHO) cells. Our results show that AA as well as BB homodimeric forms of human PDGF bind to the transfected receptor, activate its tyrosine kinase, and stimulate DNA synthesis.

The first event in PDGF-mediated mitogenesis is the binding of PDGF to its receptor at the cell membrane (*5*). This interaction triggers a diverse group of early cellular responses including the activation of receptor tyrosine kinase (*6*), increased phosphatidylinositol turnover (*7*), enhanced expression of a group of genes (*8, 9*), activation of phospholipase A2 (*10*), changes in cell shape (*11*), increase in cellular calcium concentration (*12*), changes in intracellular *p*H (*12*), and internalization and degradation of $^{125}$I-labeled PDGF (*13*). These changes are followed by an increase in the rate of proliferation of the target cells. The 180-kD mouse receptor was purified on the basis of its ability to become tyrosine phosphorylated in response to AB human platelet form of PDGF (*3*), and the receptor amino acid sequence was determined from a full-length cDNA clone (*4*). The extracellular portion of this mouse receptor consists of a series of five immunoglobulin-like domains (*14*) that contain the PDGF binding region (*15*). The intracellular tyrosine kinase domain is split

J. A. Escobedo, S. Navankasatussas, S. R. Coughlin, L. T. Williams, Department of Medicine, University of California, San Francisco, the Cardiovascular Research Institute, and Howard Hughes Medical Intitute, San Francisco, CA 94143-0724.
L. S. Cousens, Chiron Corporation, Emeryville, CA 94608.
G. I. Bell, Department of Biochemistry and Molecular Biology, University of Chicago and Howard Hughes Medical Institute, Chicago, IL 60637.

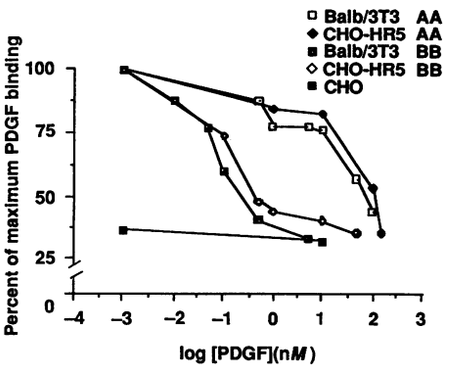


**Fig. 1.** Competitive binding of the different forms of PDGF to its receptor. Balb/c 3T3 cells and CHO transfectants (CHO-HR5) were incubated with $^{125}$I-labeled PDGF (*25*) isolated from human platelets (*26*) in the presence of increasing concentrations of AA or BB. Binding was carried out at 37°C for 45 min in whole-cell suspension. Unbound labeled PDGF was removed by centrifugation on a Ficoll gradient (*27*). The nonspecific binding determined by incubating CHO cells with $^{125}$I-labeled PDGF accounted for 25% of the bound $^{125}$I. The results are expressed as percentage of maximal binding. Each value represents the mean of three independent measurements. The standard deviation does not exceed 10% of the mean value.

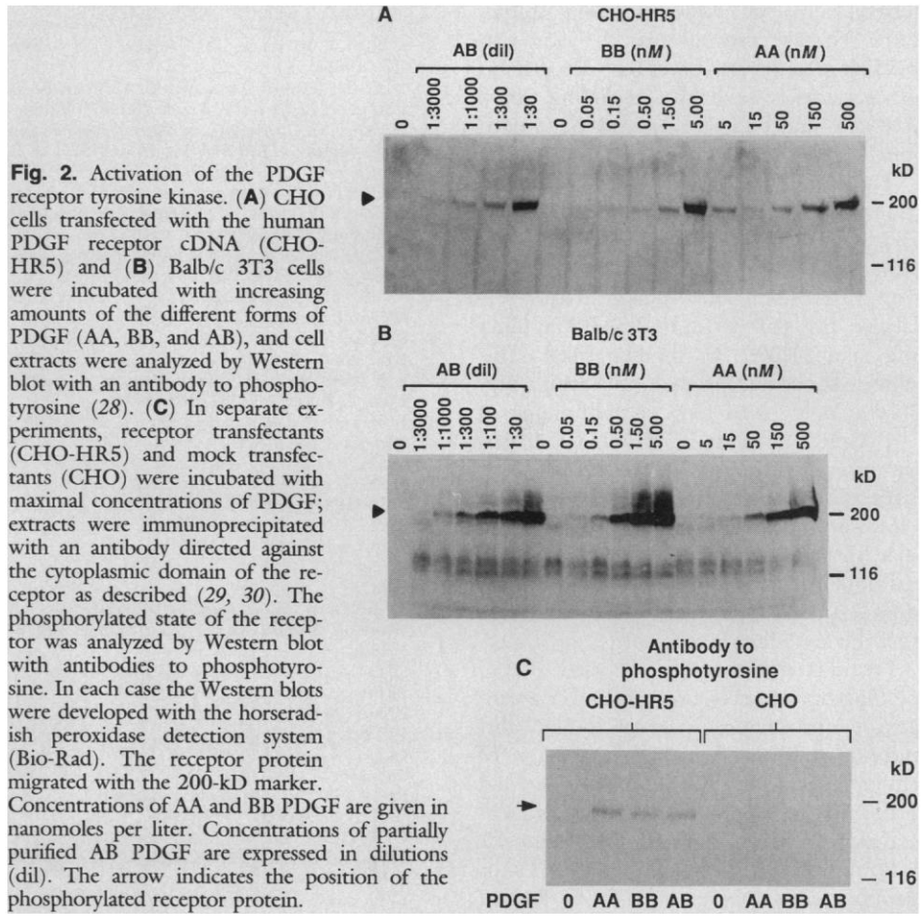


**Fig. 2.** Activation of the PDGF receptor tyrosine kinase. (**A**) CHO cells transfected with the human PDGF receptor cDNA (CHO-HR5) and (**B**) Balb/c 3T3 cells were incubated with increasing amounts of the different forms of PDGF (AA, BB, and AB), and cell extracts were analyzed by Western blot with an antibody to phosphotyrosine (*28*). (**C**) In separate experiments, receptor transfectants (CHO-HR5) and mock transfectants (CHO) were incubated with maximal concentrations of PDGF; extracts were immunoprecipitated with an antibody directed against the cytoplasmic domain of the receptor as described (*29, 30*). The phosphorylated state of the receptor was analyzed by Western blot with antibodies to phosphotyrosine. In each case the Western blots were developed with the horseradish peroxidase detection system (Bio-Rad). The receptor protein migrated with the 200-kD marker. Concentrations of AA and BB PDGF are given in nanomoles per liter. Concentrations of partially purified AB PDGF are expressed in dilutions (dil). The arrow indicates the position of the phosphorylated receptor protein.

into two portions by a 107–amino acid "kinase insert" (KI) region that is essential for PDGF-induced DNA synthesis (*16*). We have now obtained the full-length human cDNA clone (*17*). The sequences of the human and mouse receptors are highly conserved in the intracellular region (96% identity) and very similar in the extracellular domain (77% identity). The human receptor, like its murine homolog, contains five immunoglobulin-like domains in the extracellular region as well as kinase and kinase insert regions in the intracellular region.

To determine whether A chain and B chain homodimers can activate a common receptor and whether they can elicit similar responses in the same target cells, we expressed the human receptor cDNA in CHO cells, which normally lack PDGF receptors. The expression of the receptor protein in the transfectants was demonstrated by using antibodies that recognize an intracellular sequence in the receptor (*18*). The clone that had the highest level of human receptor expression was chosen for further study. This transfectant had receptors that could be labeled with $^{125}$I-labeled PDGF (Fig. 1). We tested the ability of the human recombinant AA and BB homodimers to compete for the receptor sites and displace $^{125}$I-labeled PDGF. Each homodimer was produced selectively by a yeast expression system that uses a hybrid promoter (regulatory region of the alcohol dehydrogenase II promoter (ADH-II) and the 3′ region of the glyceraldehyde 3-phosphate promoter) and the alpha-factor leader sequence to allow secretion of the recombinant protein (*19, 20*). The expressed A chain in this system contains 110 amino acids, starting at a serine residue that defines the amino terminus of the mature form of A chain PDGF produced by human umbilical vein endothelial cells (*21, 22*). The B chain expressed in yeast starts at the serine residue that defines the amino terminus of the mature form of B chain PDGF and includes 109 amino acids from the PDGF B form (*23*). Each homodimer was purified from yeast medium that is devoid of other mesenchymal cell growth factors, thus avoiding the artifact of contamination by factors that might be present in mammalian expression systems. Both AA and BB forms of PDGF competed for the sites labeled with $^{125}$I-PDGF in the human receptor transfectants. For the transfected human receptor as well as the native mouse receptor, the BB form was of higher affinity than the AA form (Fig. 1). It is possible that when expressed in yeast, the AA form of PDGF is processed aberrantly, thus giving it a low affinity. Regardless, the consistency of the pattern of competition shows that the AA form interacts with the transfected human receptor in the same way as it does with the native mouse 3T3 receptor and suggests that these receptors are functionally identical.

We also tested the ability of recombinant AA and BB homodimers and of human partially purified AB PDGF to activate the receptor tyrosine kinase. The yeast-derived AA and BB homodimeric forms and the platelet-derived AB form stimulated autophosphorylation of the transfected human receptor (Fig. 2A). The concentration of each form that was effective in stimulating autophosphorylation of the transfected human receptor was identical to the concentration that gave a similar autophosphorylation to the native mouse 3T3 receptor (Fig. 2B) or the transfected mouse receptor (*24*). These results show that the AA form of PDGF activates the receptor tyrosine kinase and that the human cDNA encodes a receptor that is functionally equivalent to the wild-type receptor that is responsible for PDGF-stimulated tyrosine kinase activity in mouse 3T3 cells. In addition, immunoprecipitation of lysates from cells stimulated with the different forms of PDGF (AA, BB, and AB) followed by immunoblot with an antibody to phosphotyrosine indicates that all forms of PDGF induced receptor autophosphorylation (Fig. 2C).

Transfection of CHO cells with either human or mouse PDGF receptor confers a PDGF-sensitive mitogenic response (Fig. 3). All forms of PDGF stimulated DNA synthesis in both the human receptor transfectant and the mouse cells bearing the native receptor (Fig. 3). These data show that the A chain homodimer and the B chain homodimer, like the AB platelet-derived form, are mitogens that can act through the receptor encoded by this human cDNA sequence. The mitogenic action of these forms of PDGF on mouse 3T3 cells appears to be mediated by a receptor that is functionally identical to the transfected human receptor. The apparent affinity of the A chain homodimer for both 3T3 and the transfectants is lower than the affinity of the B chain homodimer. All of the effects of the A chain homodimer exhibit this low apparent affinity, an indication that the A chain homodimer used for these experiments might be aberrantly processed in the yeast system. Although we cannot exclude the possibility that additional forms of PDGF receptor exist in other cell types, all of the responses we have measured in Balb/c 3T3 cells can be attributed to a single type of receptor.

These experiments were made possible by the availability of growth factor preparations devoid of contamination with other growth factors and by the use of a receptor expression system in which all of the measured PDGF responses could be attributed to this single transfected receptor cDNA. Our study indicates that in 3T3 cells and in the CHO transfectant (CHO-HR5) a single receptor type can account for the binding of

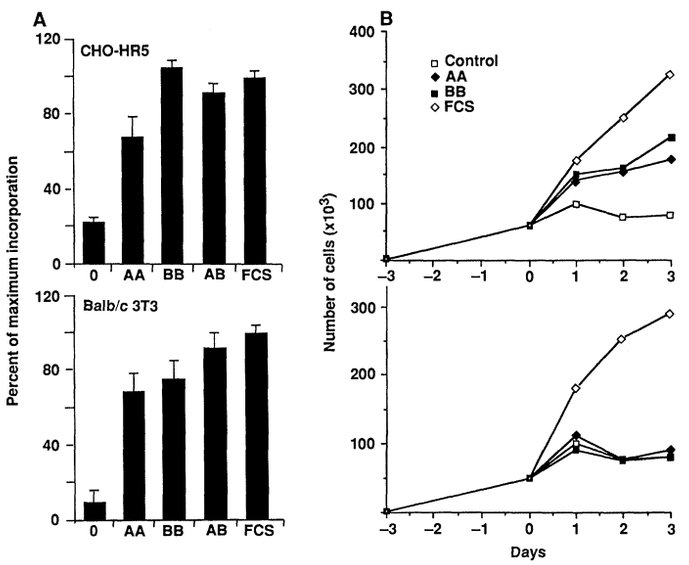


**Fig. 3.** Rate of DNA synthesis and cell proliferation in CHO-transfected cells. (**A**) Balb/c 3T3 cells and CHO cells transfected with the human PDGF receptor cDNA (CHO-HR5) were grown in quiescent media [Ham's F-12 supplemented with 0.1% bovine serum albumin (BSA) (Sigma) and transferrin (5 μg/ml)]. Then cultures were incubated with saturating concentrations of the three forms of PDGF (AA, 30 n*M*; BB, 3 n*M*; and AB, 1:100 dil). Untreated cells and cells treated with 10% fetal calf serum (FCS) were used as negative and positive controls, respectively. The level of [$^3$H]thymidine incorporation into DNA was determined by measuring the radioactivity of the acid-precipitable material as described (*21*). The result is expressed as percentage of maximum thymidine incorporation. Means (+ SD) of three independent determinations are shown. (**B**) CHO cells and CHO-HR5 cells transfected with the human cDNA were plated in 24-well plates at a cell density of 2500 cells per well. After 3 days in quiescent media (as above), the cells were stimulated with a saturating concentration of PDGF (AA, BB, or AB) as indicated. Cell number was determined by counting the cells at 24-hour intervals. The values in this figure represent the means of three independent determinations.

ligand to its receptor, activation of tyrosine kinase, and the stimulation of DNA synthesis by both the AA and BB forms of PDGF. The native mouse receptor and the human receptor encoded by the cDNA sequence reported here are functionally identical in mediating these activities. These findings show that the AA form of PDGF is an authentic growth factor that acts through the same mitogenic pathways as previously studied BB and AB forms.


REFERENCES AND NOTES

1. T. F. Deuel *et al.*, *J. Biol. Chem.* **256**, 8896 (1981).
2. T. F. Deuel, *Annu. Rev. Cell Biol.* **3**, 443 (1987).
3. T. O. Daniel, P. M. Tremble, A. R. Frackelton, Jr., L. T. Williams, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 2684 (1985).
4. Y. Yarden *et al.*, *Nature* **323**, 226 (1986).
5. B. Ek, B. Westermark, A. Wasteson, C.-H. Heldin, *ibid.* **295**, 419 (1982).
6. A. R. Frackelton, Jr., P. M. Tremble, L. T. Williams, *J. Biol. Chem.* **259**, 7909 (1984).
7. M. J. Berridge, *Biochem. J.* **212**, 849 (1983).
8. M. E. Greenberg and E. B. Ziff, *Nature* **311**, 433 (1983).
9. K. Kelly *et al.*, *Cell* **35**, 603 (1983).
10. W. T. Shier, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 137 (1980).
11. B. J. Bockus and C. D. Stiles, *Exp. Cell Res.* **153**, 186 (1984).
12. H. E. Ives and T. O. Daniel, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 1950 (1987).
13. C.-H. Heldin, B. Ek, L. Ronstrand, *J. Biol. Chem.* **258**, 10054 (1983).
14. L. M. Amzel and R. J. Poljak, *Annu. Rev. Biochem.* **48**, 961 (1979).
15. F. J. Bazan, R. J. Fletterick, J. A. Escobedo, L. T. Williams, in preparation.
16. J. A. Escobedo and L. T. Williams, unpublished results.
17. A human kidney λgt11 cDNA library [G. I. Bell *et al.*, *Nucleic Acids Res.* **14**, 8427 (1986)] screened with a full-length mouse PDGF receptor cDNA probe yielded a 2.3-kb clone (HK-6) containing sequences that encode amino acid residues 977 to 1106 and 2237 nucleotides from the 3′ untranslated region. A probe derived from this clone was used to screen a λgt10 human placenta cDNA library (provided by J. Edman and W. Rutter, University of California, San Francisco) from which four independent clones were selected. The largest cDNA insert (clone HP1-7, 4.6-kb) contained all of the nucleotides encoding the receptor protein. It lacks only the last 1204 nucleotides from the 3′ untranslated region.
18. An expression vector containing the human cDNA sequence under transcriptional control of the SV40 early promoter and the expression vector pSV2neo carrying the gene that confers resistance to neomycin were used to cotransfect CHO cells by the calcium precipitate technique. After transfection, the cells were cultured in the presence of 400 μg/ml of G-418 (a neomycin analog). G-418–resistant colonies were picked after 2 weeks in selection media. Each colony was assayed as described (*30*) for the expression of PDGF receptor by Western blot with an antibody to the receptor that specifically recognizes sequences in the carboxyl-terminal region of the receptor (29). A clone expressing the highest amount of receptor (CHO-HR5) was used in these experiments. The 195-kD protein recognized by this antibody has a size of 195 kD.
19. L. S. Cousens *et al.*, *Gene* **61**, 265 (1987).
20. A. J. Brake *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 4642 (1984).
21. T. Collins, D. T. Bonthron, S. H. Orkin, *Nature* **328**, 621 (1987).
22. B. D. Tong *et al.*, *ibid.*, p. 619.
23. C. Betsholtz *et al.*, *ibid.* **320**, 695 (1986).
24. J. A. Escobedo and L. T. Williams, unpublished results.
25. L. T. Williams, P. M. Tremble, H. N. Antoniades, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 5067 (1982).
26. H. N. Antoniades *et al.*, *ibid.* **76**, 1809 (1979).
27. P. Orchansky, M. Rubinstein, D. G. Fischer, *J. Immunol.* **136**, 169 (1986).
28. J. Y. J. Wang, *Mol. Cell. Biol.* **5**, 3640 (1985).
29. M. T. Keating and L. T. Williams, *J. Biol. Chem.* **262**, 7932 (1987).
30. J. A. Escobedo, M. T. Keating, H. E. Ives, L. T. Williams, *ibid.* **263**, 1482 (1988).


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# Chloroplast Transformation in *Chlamydomonas* with High Velocity Microprojectiles


JOHN E. BOYNTON,* NICHOLAS W. GILLHAM, ELIZABETH H. HARRIS, JONATHAN P. HOSLER,† ANITA M. JOHNSON, ALLAN R. JONES, BARBARA L. RANDOLPH-ANDERSON, DOMINIQUE ROBERTSON, TED M. KLEIN,‡ KATHERINE B. SHARK, JOHN C. SANFORD



**Bombardment of three mutants of the chloroplast *atpB* gene of *Chlamydomonas reinhardtii* with high-velocity tungsten microprojectiles that were coated with cloned chloroplast DNA carrying the wild-type gene permanently restored the photosynthetic capacity of the algae. In most transformants of one of the mutants, a fragment with a 2.5-kilobase deletion was restored to normal size by a homologous replacement event; in about 25 percent of the transformants the restored restriction fragment was 50 to 100 base pairs smaller or larger than that of wild type. About one-fourth of the transformants of this mutant contained unintegrated donor plasmid when first examined. This plasmid persisted in four different transformants after 65 cell generations of continuous liquid culture but was lost from all transformants maintained on plates of selective medium. The restored wild-type *atpB* gene remains in all transformants as an integral part of the chloroplast genome and is expressed and inherited normally.**


IN VIEW OF THE EASE WITH WHICH nuclear genes of plant and animal cells can be transformed, the lack of reliable methods for introducing genes into mitochondria or chloroplasts is noteworthy. While mitochondrial transplantation by microinjection has been successful (*1*), previous accounts of DNA mediated transformation of yeast mitochondria (*2*) and tobacco chloroplasts (*3*) have not been verified in the literature. However, indirect "transformation" of chloroplasts or mitochondria has been accomplished by introducing constructs into the nucleus carrying an organelle gene with an added amino-terminal transit sequence to target the protein to the organelle (*4*). Direct transformation of chloroplasts and mitochondria has been difficult because both organelles are bounded by double membrane envelopes that may hinder nucleic acid transport (*5*) and because many genetically polyploid organelles are present in most eukaryotic cells. We report here that the newly developed "biolistic" DNA delivery process utilizing high velocity microprojectiles (*6*) circumvents these problems, probably by direct introduction of many copies of a gene into the chloroplast. This process is also proving effective for introducing DNA into the nuclei of higher plants (*7*) and for nuclear (*8*) and mitochondrial (*9*) gene transformation of yeast.

The single cup-shaped chloroplast of the unicellular green alga *Chlamydomonas reinhardtii* is a favorable target for bombardment by DNA-coated microprojectiles, since it lies adjacent to the plasma membrane along most of the periphery of this 10-μm diameter cell (*5*). For transformation recipients, we used three photosynthetically defective mutants (Fig. 1A) with lesions in the *ac-u-c* locus (*10–12*), which defines the chloroplast *atpB* gene encoding the β subunit of the $CF_1$ complex of the chloroplast adenosine triphosphate (ATP) synthase. The mutant *ac-u-c-2-21* (CC-373) has a 2.5-kb deletion in the Bam 10 restriction fragment spanning this gene; the deletion extends from the 3′ half of *atpB* into the 22-kb inverted repeat of the chloroplast genome.


J. E. Boynton, E. H. Harris, J. P. Hosler, A. M. Johnson, A. R. Jones, B. L. Randolph-Anderson, Department of Botany, Duke University, Durham, NC 27706.
N. W. Gillham and D. Robertson, Department of Zoology, Duke University, Durham, NC 27706.
T. M. Klein, K. B. Shark, J. C. Sanford, New York State Agricultural Experiment Station, Cornell University, Geneva, NY 14456.

*To whom correspondence should be addressed.
†Present address: Department of Biochemistry, Michigan State University, East Lansing, MI 48824.
‡Present address: Plant Gene Expression Center, USDA-ARS, 800 Buchanan Street, Albany, CA 94710.