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Isolation of a Novel Receptor cDNA Establishes the Existence of Two PDGF Receptor Genes

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A genomic sequence and cloned complementary DNA has been identified for a novel receptor-like gene of the PDGF receptor/CSF1 receptor subfamily (platelet-derived growth factor receptor/colony-stimulating factor type 1 receptor). The gene recognized a 6.4-kilobase transcript that was coexpressed in normal human tissues with the 5.3-kilobase PDGF receptor messenger RNA. Introduction of complementary DNA of the novel gene into COS-1 cells led to expression of proteins that were specifically detected with antiserum directed against a predicted peptide. When the new gene was transfected into COS-1 cells, a characteristic pattern of binding of the PDGF isoforms was observed, which was different from the pattern observed with the known PDGF receptor. Tyrosine phosphorylation of the receptor in response to the PDGF isoforms was also different from the known receptor. The new PDGF receptor gene was localized to chromosome 4q11–4q12. The existence of genes encoding two PDGF receptors that interact in a distinct manner with three different PDGF isoforms likely confers considerable regulatory flexibility in the functional responses to PDGF.

SEVERAL FAMILIES OF MEMBRANE-spanning growth factor receptors with tyrosine kinase activity have been identified (*1*). One such family includes the structurally related growth factor receptors for platelet-derived growth factor (PDGF) (*2*) and colony-stimulating factor (CSF1) (*3*). The latter was initially identified as the normal cellular homolog of the v-*fms* oncogene (*4*). A third member of this family, c-*kit* (*5*), for which the ligand has yet to be identified, was also first detected as a viral oncogene (*6*). In the present studies, we describe the identification of a genomic sequence and the isolation of the cDNA of a novel gene, whose predicted product has a structure similar to other growth factor receptors of this subfamily.

Reduced stringency hybridization of human genomic DNA with DNA probes from the tyrosine kinase domain of v-*fms* or of the mouse PDGF receptor (PDGFR) led to detection of several bands in addition to those of either c-*fms* or PDGFR genes. A 12-kbp Eco RI band generated the strongest signal with either probe, while smaller size bands corresponded in size to restriction fragments reported for human c-*kit* (*6*). Thus, we cloned the 12-kbp Eco RI fragment, which we designated λT11. Regions homologous to v-*fms*/PDGFR tyrosine kinase domains within λT11 were localized by hybridization and subjected to nucleotide sequence analysis. Their discrete open reading frames were most closely related to but distinct from human c-*fms*, c-*kit*, or the PDGFR. To characterize the coding region of this gene, overlapping cDNA clones were isolated from normal human fibroblast and brain cDNA libraries and subjected to nucleotide sequence analysis. For structural and functional comparison, we also cloned and sequenced cDNAs encompassing the entire coding sequence of the human PDGF receptor (*7*).

For the novel gene, a single open reading frame of 1089 amino acids was predicted from the nucleotide sequence, which has been submitted to GenBank (*8*). According to the putative cleavage site for the hydrophobic signal peptide (*9*), the NH_2-terminus of the mature product was predicted to be glutamine at amino acid 24 followed by 1065 amino acids. This polypeptide sequence with a calculated molecular mass of around 120 kD contained all of the characteristics of a membrane-spanning tyrosine kinase receptor. A hydrophobic segment consisting of 24 amino acids (residues 525

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Fig. 1. Predicted amino acid sequence of the T11 receptor-like gene product and homology with other tyrosine kinases. The sequence of the T11 cDNA was determined from a series of overlapping clones isolated from normal human embryo fibroblast and brain cDNA libraries. Each clone was shown to hybridize a single 6.4-kb transcript in M426 human embryo fibroblasts. The predicted amino acid sequence of the T11 long open reading frame is shown and is numbered at the left. The potential NH_2-terminal signal peptide is underlined. Potential sites of N-linked glycosylation are overlined, and Cys residues are bold. The putative single transmembrane region is boxed. The potential ATP binding site (Gly at residues 600, 602, and 605 and Lys at residue 627) in the kinase domain is circled. The putative Tyr autophosphorylation site at residue 849 is underlined. A schematic diagram of the predicted protein domains shows the signal sequence (S, block box), ligand binding domain (LB), transmembrane domain (TM, black box), juxtamembrane domain (JM), tyrosine kinase domains (TK1, TK2; cross-hatched boxes), interkinase domain (IK) and COOH-terminus (C). The numbers below indicate the percentage of identical amino acids within each respective domain. IR, insulin receptor; EGFR, epidermal growth factor receptor; ND, not determined.

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0001 MGTSHPAFLV LGCLLTGLSL ILCQLSLPSI LPNENEKVVQ LNSSFSLRCF GESEVSWQYP
0061 MSEEESSDVE IRNEENNSGL FVTVLEVSSA SAAHTGLYTC YYNHTQTEEN ELEGRHIYIY
0121 VPDPDVAFVP LGMTDYLVIV EDDDSAIIPC RTTDPETPVT LHNSEGVVPA SYDSRQGFNG
0181 TFTVGPYICE ATVKGKKFQT IPFNVYAIKA TSELDLEMEA LKTVYKSGET IVVTCAVFNN
0241 EVVDLQWTYP GEVKGKGITM LEEIKVPSIK LVYTLTVPEA TVKDSGDYEC AARQATREVK
0301 EMKKVTISVH EKGFIEIKPT FSQLEAVNLH EVKHFVVEVR AYPPPRISWL KNNLTLIENL
0361 TEITTDVEKI QEIRYRSKLK LIRAKEEDSG HYTIVAQNED AVKSYTFELL TQVPSSILDL
0421 VDDHHGSTGG QTVRCTAEGT PLPDIEWMIC KDIKKCNNET SWTILANNVS NIITEIHSRD
0481 RSTVEGRVTF AKVEETIAVR CLAKNLLGAE NRELKLVAPT LRSELTVAAA VLVLLVIVII
0541 SLIVLVVIWK QKPRYEIRWR VIESISPDGH EYIYVDPMQL PYDSRWEFPR DGLVLGRVIG
0601 SGAFGKVVEG TAYGLSRSQP VMKVAVKMLK PTARSSEKQA LMSELKIMTH LGPHLNIVNL
0661 LGACTKSGPI YIITEYCFYG DLVNYLHKNR DSFLSHHPEK PKKELDIFGL NPADESTRSY
0721 VILSFENNGD YMDMKQADTT QYVPMLERKE VSKYSDIQRS LYDRPASYKK KSMLDSEVKN
0781 LLSDDNSEGL TLLDLLSFTY QVARGMEFLA SKNCVHRDLA ARNVLLAQGK IVKICDFGLA
0841 RDIMHDSNYV SKGSTFLPVK WMAPESIFDN LYTTLSDVWS YGILLWEIFS LGGTPYPGMM
0901 VDSTFYNKIK SGYRMAKPDH ATSEVYEIMV KCWNSEPEKR PSFYHLSEIV ENLLPGQYKK
0961 SYEKIHLDFL KSDHPAVARM RVDSDNAYIG VTYKNEEDKL KDWEGGLDEQ RLSADSGYII
1021 PLPDIDPVPE EEDLGKRNRH SSQTSEESAI ETGSSSSTFI KREDETIEDI DMMDDIGIDS
1081 SDLVEDSFL
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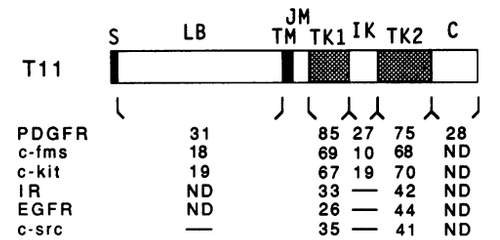

PDGFR	31	85	27	75	28	
c-fms	18	69	10	68	ND	
c-kit	19	67	19	70	ND	
IR	ND	33	—	42	ND	
EGFR	ND	26	—	44	ND	
c-src	—	35	—	41	ND	

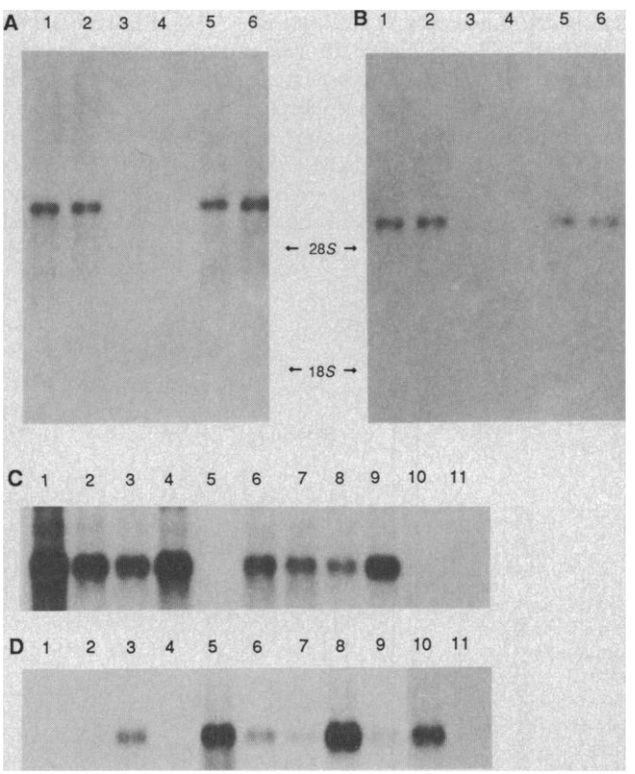

Fig. 2. Comparison of mRNA species of the T11 and PDGFR genes in normal and tumor cells. Total or polyadenylated RNA was separated by denaturing gel electrophoresis in formaldehyde (*32*), transferred to nitrocellulose, and hybridized under stringent conditions (50% formamide, 0.075*M* NaCl, 0.75*M* sodium citrate, at 42°C) with ^{32}P-labeled probes. The same filter was first hybridized with a T11 DNA probe (A and C) and then rehybridized with a PDGFR DNA probe (B and D). (**A**) and (**B**) contained poly(A) RNAs (5 μg per lane) extracted from human smooth muscle (lane 1), heart (lane 2), liver (lane 3), spleen (lane 4), and embryo (lanes 5 and 6). (**C**) and (**D**) contained total RNA (20 μg per lane) extracted from G402 leiomyoblastoma cells (lane 1), SK-LMS-1 leiomyosarcoma cells (lane 2), A1186 and A204 rhabdomyosarcoma cells (lanes 3 and 4), 8387 fibrosarcoma cells (lane 5), astrocytoma tissues (lanes 6 and 7), A1690 astrocytoma cells (lane 8), A1207 and A172 glioblastoma cells (lanes 9 and 10), and A875 melanoma cells (lane 11). Migration of 28*S* and 18*S* RNA is as indicated.

to 548) showed characteristics of a receptor transmembrane domain (Fig. 1). The region between the signal peptide and the transmembrane domain was structurally similar to the extracellular ligand binding domains of the PDGFR/CSF1R (colony-stimulating factor type 1 receptor) subfamily. Ten cysteine residues were spaced at the same positions as in the other receptors of this subfamily, and eight potential N-linked glycosylation sites were distributed in its putative extracellular domain (Fig. 1).

The cytoplasmic domain contained a conserved tyrosine kinase region and a hydrophilic COOH-terminal tail (Fig. 1). The tyrosine kinase domain included the consensus adenosine triphosphate (ATP) binding sequence (residues Gly-X-Gly-X-X-Gly . . . Lys) and a tyrosine residue at position 849 similar to the major autophosphorylation site of pp60$^{v\text{-}src}$ at position 416 (*10*). Moreover, the tyrosine kinase was divided into two domains by a hydrophilic interkinase sequence as previously shown for c-*fms*/CSF1R, PDGFR, and c-*kit* (Fig. 1). The amino acid homologies of its extracellular domain with those of the PDGFR, CSF1R, and c-*kit* were 31%, 18%, and 19%, respectively. The two kinase domains of the T11 gene were most similar to those of the human PDGF receptor (85% and 75%, respectively) as was its interkinase domain (27%).

To investigate the tissue-specific expression of our new receptor-like gene, a T11 probe was used for Northern blot hybridization experiments. Relatively high levels of a 6.4-kb transcript were found in polyadenylated RNAs from human smooth muscle, heart, and embryo, whereas liver and spleen demonstrated undetectable or barely detectable transcripts (Fig. 2A). The 5.3-kb PDGFR transcript appeared to be coexpressed at similar levels in each of these tissues (Fig. 2B). Human skeletal muscle, fetal brain, placenta, as well as cultured fibroblasts and glial cells, also expressed high levels of both transcripts (*11*). Thus, the new gene and PDGFR genes appeared to be coordinately expressed in normal tissues examined and exhibited a very different pattern from that reported for either c-*fms*/CSF1R or c-*kit* (*4, 5*). We also compared expression of the T11 and PDGFR gene in human tumor cells. Here, their patterns of expression could be readily distinguished. Several tumor cell lines were found to contain one or the other transcript but not both (Fig. 2, C and D).

Because of their structural and deduced amino acid sequence similarities as well as their coexpression by normal cell types known to respond to PDGF, we sought to determine whether the T11 gene product exhibited any functional relation to the PDGFR. Thus, we constructed long terminal repeat (LTR)–based expression vectors. We utilized COS-1 cells, which transiently amplify gene expression in SV40 promoter–based expression vectors (*12*) and lacked detectable expression of T11 or PDGFR products as described below. At 48 hours following transfection of COS-1 cells with either construct, we measured binding of ^{125}I-labeled human platelet PDGF (^{125}I-PDGF), which is thought to be an AB heterodimer (*13*).

More ^{125}I-PDGF specifically bound to COS-1 cells transfected with the T11 cDNA than to the same cells infected with the PDGF receptor gene (Fig. 3). Binding was reduced to the level of nontransfected COS-1 cells by competition with excess human PDGF, PDGF-BB, or PDGF-AA. In contrast, binding of ^{125}I-PDGF to COS-1 cells transfected with the PDGFR cDNA was blocked by human PDGF and PDGF-BB but not by PDGF-AA (Fig. 3). Thus, while both T11 and PDGFR gene products bound human PDGF, the pattern of competition by different PDGF isoforms distinguished the two receptors. These results implied that the T11 gene encoded a novel PDGF receptor with different affinities for the three dimeric forms of PDGF. We tentatively designated the T11 receptor gene product as the type α PDGFR because PDGF binding was blocked by AA as well as BB isoforms and the product of the previously cloned PDGF receptor as type β.

To confirm the expression of each construct in COS-1 cells, we utilized anti-T11 and anti-HPR sera prepared against predicted peptides of the α and β PDGFRs, respectively (*14*). Transient expression in COS-1 cells led to readily detectable type α receptor proteins of 185 and 160 kD, whereas the type β receptor appeared as 185 and 165 kD proteins (Fig. 3). Among various cells analyzed, the respective lower molecular weight forms of each receptor did not vary in size. However, we have observed some differences in sizes of the higher molecular weight species, which we consider may be due to cell-specific differences in glycosylation.

After PDGF binding to its receptor, a number of molecular events are rapidly triggered in vivo, including phosphorylation of the receptor protein on tyrosine residues (*15*). To compare the relative autophosphorylation of the products of the two PDGFR genes by each PDGF isoform, we analyzed the responses of A204 and 8387 cells that expressed only type α or type β PDGFR genes, respectively (Fig. 2). Immunoblots of A204 cells lysed 5 min after ligand exposure revealed readily detectable and similar levels of autophosphorylation of a 180-kD species in response to each of the three PDGF isoforms (Fig. 4A). As further evidence that the induced autophosphorylation was specific to the type α PDGFR, we first subjected ligand-stimulated A204 cell lysates to immunoprecipitation with anti–type α PDGFR peptide serum followed by immunoblotting with antiphosphotyrosine serum. By this approach, we could firmly establish that the 180-kD type α PDGFR was tyrosine-phosphorylated with similar intensity in response to each of the three ligands (Fig. 4A).

Exposure of 8387 cells, which expressed only the type β PDGFR gene (Fig. 2), to the same amount of each respective PDGF isoform revealed a very different pattern of receptor autophosphorylation. Here, PDGF-BB induced the highest level of autophosphorylation of the 180-kD species specifically recognized by anti–type β PDGFR peptide serum, and human PDGF induced detectable autophosphorylation as well (Fig. 4B). In contrast, PDGF-AA induced no detectable phosphorylation. Thus, while PDGF-AB and PDGF-BB triggered both receptors, the much stronger response of the

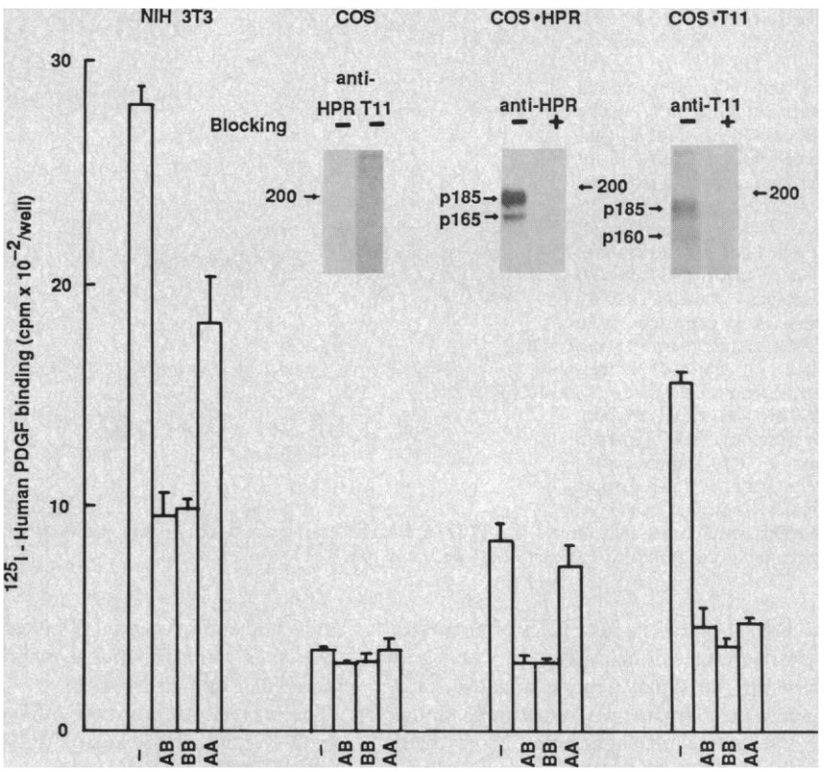

Fig. 3. Binding of ^{125}I-labeled human PDGF to NIH 3T3, control COS-1, and COS-1 cells transfected with T11 or PDGFR cDNA expression vectors. cDNA expression plasmids were constructed by introducing the T11 cDNA encompassing nucleotides 1 to 3454 or the PDGFR cDNA encompassing nucleotides 1 to 3939 into the pSV2 gpt vector into which the simian sarcoma virus LTR had been engineered as the promoter (*33*). Around 10^5 COS-1 cells per well in 12-well plates were incubated in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum 24 hours before transfection. DNA transfection was performed by the calcium phosphate precipitation method (*34*) 48 hours before binding analysis. Human PDGF was labeled with ^{125}I by the chloramine-T method to specific activities of 3.7×10^4 cpm per nanogram (*35*). The binding of ~2 ng of ^{125}I-labeled PDGF isolated from human platelets (*36*), in the absence or presence of ~100 ng of unlabeled human PDGF (PDGF-AB) (Collaborative Research), 200 ng of recombinant PDGF-BB (AmGen), or 200 ng of recombinant PDGF-AA (*27*), was carried out at 4°C for 2 hours. Unbound ^{125}I-PDGF was removed by four successive washes with binding buffer (DMEM containing 1 mg of bovine serum albumin per milliliter). The cells were then lysed in solubilizing buffer [1% Triton X-100, 20 m*M* Hepes (*p*H 7.4), and 10% (v/v) glycerol] and radioactivity was measured with a gamma counter. Results represent the mean values (+SD) of triplicate samples. Similar results were obtained in three independent experiments. To confirm receptor protein expression, COS-1 cells were also plated at 10^6 cells in 10-cm petri dishes and transfected with the same constructs. At 48 hours, cultures were lysed with staph-A buffer (10 m*M* sodium phosphate, *p*H 7.5; 100 m*M* NaCl, 1% Triton X-100, 0.1% SDS, 0.5% deoxycholate, 0.1% aprotinin, 1 m*M* PMSF, and 1 m*M* sodium orthovanadate) and clarified by centrifugation at 10,000*g* for 30 min. Proteins (100 μg per lane) were resolved by electrophoresis in 7% SDS-polyacrylamide gels, transferred to nitrocellulose filters, and probed by immunoblot analysis (with or without peptide blocking) with anti-T11 or anti-HPR peptide sera (*14*) and ^{125}I-labeled protein A (*37*).

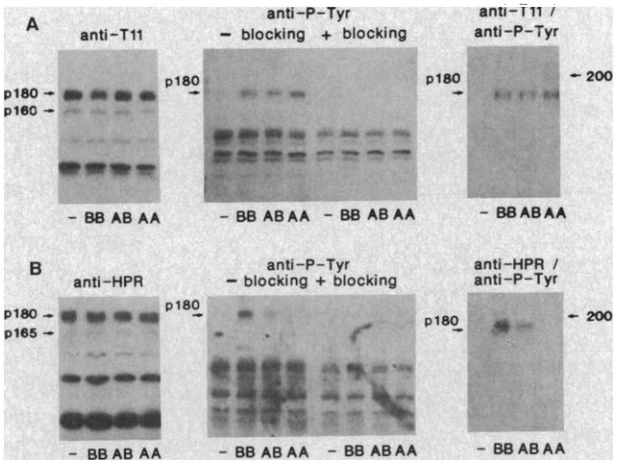

Fig. 4. Tyrosine autophosphorylation of type α and type β PDGFR gene products induced by different PDGF isoforms. A204 (**A**) or 8387 (**B**) cells were incubated with PDGF-BB (30 ng/ml) (BB), human PDGF (30 ng/ml) (AB), PDGF-AA (300 ng/ml) (AA), or 3 m*M* acetic acid vehicle control (−) for 5 min at 37°C. Cell lysates were then immunoprecipitated with anti-T11 or anti-HPR peptide sera. Total cell lysates or immunoprecipitates were analyzed by immunoblotting with antibodies to the receptors or phosphotyrosine (anti–P-Tyr) (*38*) as indicated above the blots. Anti–P-Tyr serum was preincubated with 10 m*M* phosphotyrosine for blocking. Arrows indicate the specific bands that were blocked in the presence of immunizing peptide.

β type receptor to the BB homodimer as well as its lack of detectable response to the AA homodimer readily distinguished the two receptors functionally. In NIH 3T3 cells, the 180-kD protein immunoprecipitated by the type α PDGFR antiserum was also phosphorylated by all three dimeric forms of PDGF. In contrast, the 180-kD phosphoprotein immunoprecipitated by the anti–type β receptor serum was detected only after human PDGF or PDGF-BB stimulation (*16*).

We examined 104 chromosome spreads by in situ hybridization (*17*) with a T11-DNA probe, and assigned the type α PDGFR gene to chromosome 4q11–4q12 (*16*). This localization places the new gene within the same region as the c-*kit* protooncogene (*18*). Other genes of this family have been localized on chromosome 5. These include the type β PDGFR mapped at 5q23–5q31 (*2*) and the CSF1R gene, on 5q33.2–5q33.3 (*19*). There is evidence for a common ancestral origin of human chromosomes 4 and 5 (*20*). These related receptor genes cluster near the centromere on 4q or at the distal half of 5q. Thus, if one or more of the progenitors of these genes were confined to a single ancestral chromosome, the breakup of linkage might be explained by an inversion within the long arm.

Our present studies have identified and characterized a new PDGFR gene, whose product showed functional responses to PDGF at concentrations that bound and triggered tyrosine phosphorylation of the previously identified PDGFR. The new receptor responded better to the AA homodimer, whereas the known receptor responded preferentially to the BB homodimer. Recently, two PDGFR subtypes were predicted on the basis of PDGF isoform binding and competition with mouse or human fibroblasts (*21*). These studies could not discriminate whether the subtypes represented differently processed products of a single PDGFR gene or products of distinct genes. There have also been conflicting findings concerning binding of the previously identified human PDGFR gene product by different PDGF isoforms. Introduction of expression vectors for this gene into different cell types has been reported to lead either to preferential binding of PDGF-BB (*22*) or to binding by all three isoforms (*23*). Whether this reflects differences in ligand preparations, cells utilized, or other variables is not known. In any case, our present studies establish the existence of two PDGFR genes whose products show a different pattern of responses to the three PDGF isoforms.

Receptors for insulin and insulin-like growth factor–1 (IGF-1) are 54% related in their predicted external domains and interact with both insulin and IGF-1, but with lower affinity for the heterologous ligand (*24*). The epidermal growth factor receptor (EGFR) and *erb*B-2 products, which represent another receptor family, are 43% related in their predicted external domains (*25*). In this case, EGF fails to bind the *erb*B-2 product (*26*). Although the ligand binding domains of type α and β PDGFRs are only 31% related by the same criteria, they were shown to interact with the same PDGF ligands. Thus, it is difficult to speculate concerning ligand specificity based on amino acid sequence homology alone.

The AA homodimer did not stimulate detectable tyrosine phosphorylation of the β type receptor in NIH 3T3 cells and yet is capable of inducing DNA synthesis in this cell line (*27*). Thus, it appears that the type α PDGFR can couple with mitogenic signaling pathways in fibroblasts. The type β receptor has also been reported to couple PDGF with mitogenic pathways (*23*). Recent studies have shown that introduction of an EGFR expression vector into an interleukin-3 (IL-3)–dependent hematopoietic cell normally lacking EGFR allowed EGF to couple with mitogenic and differentiation signal transduction pathways (*28*). By introducing expression vectors for each PDGFR gene into such cells, it may be possible to determine whether other known PDGF functions including chemotaxis (*29*), membrane ruffling (*30*), as well as transmodulation of a heterologous receptor (*31*), are specifically mediated by either type α or β PDGFR gene products.

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Splice Variants of the α Subunit of the G Protein G_s Activate Both Adenylyl Cyclase and Calcium Channels

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Signal transducing guanine nucleotide binding (G) proteins are heterotrimers with different α subunits that confer specificity for interactions with receptors and effectors. Eight to ten such G proteins couple a large number of receptors for hormones and neurotransmitters to at least eight different effectors. Although one G protein can interact with several receptors, a given G protein was thought to interact with but one effector. The recent finding that voltage-gated calcium channels are stimulated by purified G_s, which stimulates adenylyl cyclase, challenged this concept. However, purified G_s may have four distinct α-subunit polypeptides, produced by alternative splicing of messenger RNA. By using recombinant DNA techniques, three of the splice variants were synthesized in *Escherichia coli* and each variant was shown to stimulate both adenylyl cyclase and calcium channels. Thus, a single G protein α subunit may regulate more than one effector function.

PURIFIED PREPARATIONS OF G_s, the stimulatory regulatory component of adenylyl cyclase, or α_s, the α subunit of G_s, modulate cardiac and skeletal muscle T-tubule Ca^{2+} channels in the absence of cytoplasmic second messengers (*1*). This finding was surprising for previously there was little indication that a single G protein regulated more than one effector (*2*), and the possibility existed that the effect might have been due to a contaminating G protein. To determine whether G_s did indeed regulate more than one effector and whether G proteins are potential branchpoints for information transfer across membranes, we tested whether α_s subunits that were synthesized by recombinant methods and that are known to stimulate adenylyl cyclase (*3*) would also act on Ca^{2+} channels.

G proteins are αβγ heterotrimers that share a common set of βγ dimers and differ in the composition of their α subunits. The α subunits are similar yet distinct gene products that bind and hydrolyze guanosine triphosphate (GTP) and act on specific effectors. Because of their similarities, G proteins are often isolated together, and what appears to be a single G protein on several electrophoretic systems may in fact represent more than one. Furthermore, in the case of α_s, as many as four closely related splice variants may be expressed in a single cell (*4*). The α_s splice variants comprise a pair of shorter and a pair of longer molecules, α_s-S and α_s-L, which differ by a block of 15 amino acid residues that are encoded by exon 3 of the natural gene. Each pair in turn is formed by two polypeptides that differ by the absence (α_s-S1 and α_s-L1) or presence (α_s-S2 and α_s-L2) of a single serine residue at the insertion-deletion junction (*4*). The relative proportions in which the variants with and without serine are represented in purified G_s is unknown because they cannot be separated. Thus, we examined whether a single α_s polypeptide can activate both adenylyl cyclase and Ca^{2+} channels, and whether this property is common to all of the α_s splice variants.

Previous studies have shown that it is possible to synthesize in *Escherichia coli* biologically active α_s polypeptides as judged by their ability to activate adenylyl cyclase (*3*). To determine whether the same molecular form of α_s that stimulates adenylyl cyclase also stimulates Ca^{2+} channels, we synthesized in *E. coli* three of the four splice variants cloned previously (*4, 5*), using two of the T7 promoter–based expression systems of Tabor and Richardson (*6*), pT7-5 (*3*) and pT7-7 (*7*). For some experiments the cDNAs that encode α_s-S1 and α_s-L1 were subcloned into the Bam HI site of the cloning cassette of the pT7-7 vector. This led to the expression of fusion polypeptides with an amino-terminal extension of nine amino acid residues, termed r(+9)α_s-S1 and r(+9)α_s-L1. For other experiments, the cDNAs that encode α_s-S2 and α_s-L1, together with an appropriate ribosomal binding sequence, were inserted into plasmid pT7-5 so that rα_s-S2 and rα_s-L1 were expressed with an unaltered amino acid sequence (*3*). For control, the cDNA of the α subunit of another G protein, which does not stimulate adenylyl cyclase, human liver G_i-3 (*8*), was also subcloned into pT7-7 so as to direct the expression of the corresponding r(+9)α_i-3 (*7*). Two methods were used to induce expression of the recombinant proteins (*7*): infection with a phage containing the T7 RNA polymerase gene controlled by the *lac* promoter (plasmid-phage method) or transfection with a second plasmid that carries the T7 RNA polymerase gene under the control of the leftward λ promoter and the temperature-sensitive cI857 repressor (two-plasmid method).

When fusion proteins encoded in pT7-7 constructs were expressed by the plasmid-phage method (*7*), the recombinant polypeptides accumulated within 60 min of induction and constituted about 5 to 8% of total bacterial cell protein. However, only 5 to 10% of the recombinant α subunits could be recovered as soluble proteins after bacterial lysis with lysozyme and centrifugation (*7*). When the pT7-5 constructs were used (*3*), total expression of the recombinant polypeptides was less (about 0.5% of total cell protein), but about 50% was soluble. Thus, the final yield of activity was similar for pT7-7 and pT7-5 constructs.

Two series of experiments were performed to evaluate the effects of recombinant G protein α subunits on Ca^{2+} channel currents produced by skeletal muscle T-tubule vesicles incorporated into planar lipid bilayers (*9*). In the first, r(+9)α_s-S1 and r(+9)α_s-L1 were expressed by either the plasmid-phage or the two-plasmid method, recovered in lysates, activated with guanosine-5′-*O*-(3-thio)triphosphate (GTPγS), and tested either directly or after partial purification over DEAE Sephadex (*7*). In the latter case, about 20% of the applied r(+9)α_s was recovered with an apparent purity of about 5%, if derived from bacteria induced by the plasmid-phage method, and less if derived from bacteria induced by the two-plasmid system. In the second series of experiments, rα_s-S2 and rα_s-

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