**Effects of Platelet-derived Growth Factor on Phosphorylation of the Epidermal Growth Factor Receptor in Human Skin Fibroblasts**

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Stuart J. Decker and Paul Harris

*From the Rockefeller University, New York, New York 10021*

Heterologous regulation of the epidermal growth factor (EGF) receptor by platelet-derived growth factor (PDGF) was studied in FS4 human skin fibroblasts. The addition of PDGF to FS4 cells inhibited high affinity binding of \(^{125}\)I-EGF and stimulated phosphorylation of the EGF receptor. Phosphopeptide analysis by high performance liquid chromatography revealed that PDGF treatment of cells increased phosphorylation at several distinct sites of the EGF receptor. However, PDGF did not stimulate phosphorylation of threonine 654, a residue previously shown to be phosphorylated when protein kinase C is activated. The tumor promoter 12-O-tetradecanoylphorbol-13-acetate (TPA) also stimulated phosphorylation of the same peptides from the EGF receptor as PDGF, and, in addition, induced phosphorylation of threonine 654. PDGF inhibited both high and low affinity \(^{125}\)I-EGF binding by these cells. PDGF treatment of cells had no effect on EGF-dependent, tyrosine-specific autophosphorylation of the receptor, whereas TPA treatment was inhibitory. TPA, but not PDGF, stimulated phosphorylation of a \(M_\text{r} = 80,000\) protein, known to be a substrate for protein kinase C, even though PDGF appeared to mediate breakdown of phosphorylinsitides. These data suggest that regulation of EGF receptor function by PDGF and TPA are distinct in these cells, even though some elements of regulation are shared. The results differ from those previously reported for a human lung fibroblast isolate, indicating that cell type-specific differences may exist in metabolism of the EGF receptor.

Epidermal growth factor (EGF) and platelet-derived growth factor (PDGF) are potent mitogens for a number of different cell types. Both of these ligands induce cell division through binding to specific cell surface receptors. Upon ligand binding, these receptors initiate a series of events that cumulatively lead to cell division (1, 2). Both receptors possess tyrosine-specific protein kinase activity which is dependent on ligand binding (3-8). EGF and PDGF have been shown to cause transient elevation of c-fos and c-myc transcription (6-8), and both increase turnover of phosphoinositides in certain cell types (9-11).

Several reports suggest that the PDGF receptor and EGF receptor systems may be co-ordinated in some manner. For some cells PDGF appears to be a "competence" factor which sensitizes cells to the mitogenic effects of secondary mitogens such as EGF (12, 13). Exposure to PDGF inhibits high affinity binding of \(^{125}\)I-EGF by several types of fibroblasts (14, 15). In human lung fibroblasts, PDGF-dependent inhibition of EGF binding correlates with PDGF-induced phosphorylation of threonine 654 of the EGF receptor and with inhibition of EGF receptor autophosphorylation on tyrosine residues (16, 17). Since PDGF has been shown to induce release of Ca\(^{2+}\) from intracellular stores and increase levels of diacylglycerol (9), PDGF-induced phosphorylation of threonine 654 could be due to activation of protein kinase C by these effectors.

The effects of tumor promoting phorbol diesters on EGF receptor metabolism are, in many ways, similar to those reported for PDGF in human lung fibroblasts. Treatment of cells with 12-O-tetradecanoyl-13-acetate (TPA) inhibits binding of \(^{125}\)I-EGF (1, 18) as well as the EGF-dependent tyrosine kinase activity of the EGF receptor (19-22). TPA has also been shown to stimulate protein kinase C-mediated phosphorylation of threonine 654 of the EGF receptor (22, 23). In this report, we show that the effects of PDGF and TPA on EGF receptor metabolism in human skin fibroblasts are different from results previously reported for human lung fibroblasts (16, 17).

**EXPERIMENTAL PROCEDURES**

*Materials—Tosylphenylalanyl 4-chloromethyl ketone (TPCK)-treated trypsin was obtained from Cooper Laboratories. TPA and 4-\(\alpha\)-phorbol were obtained from LC Biochemicals. Receptor grade epidermal growth factor was from Collaborative Research (Waltham, MA), and purified human PDGF was a generous gift from Dr. Thomas Deuel, Washington University or was from R and D Systems, Inc., Minneapolis, MN. \(^{125}\)I-EGF and carrier-free \[^{3}H\]myo-inositol and \[^{3}H\]inositol polyphosphate standards were from Amer sham Corp. Antiserum against the rat 80,000 protein was generously provided by Dr. James Wang, Rockefeller University, purified protein kinase C from calf brain was a kind gift from Antony Rosen, Rockefeller University, and monoclonal anti-EGF receptor antibody 526 was kindly provided by John Mendelsohn, Memorial Sloan Kettering Cancer Center.*

*Cell Culture—Human foreskin fibroblasts FS4 (kindly provided by Jan Vilecek, New York University and Pravin Sehgal, Rockefeller University) were grown in Dulbecco’s modified essential medium supplemented with 10% fetal calf serum. Cells were used 5-7 days after reaching confluence.*

*Analysis of \(^{125}\)I-EGF Binding—Confluent FS4 cells in multiwell plates were washed once with binding buffer (Dulbecco’s modified Eagle’s medium with 50 mM HEPES, pH 7.4, and 1 mg/ml bovine serum albumin) and incubated in binding buffer with or without PDGF or TPA for various periods of time. Indicated concentrations of \(^{125}\)I-EGF were then added, and the cells were incubated at 4°C for 4 h. Cells were washed four times with ice-cold binding buffer and solubilized with 0.5 ml of 0.5 M NaOH. After 5 min the radioactivity in the lysates was quantitated by liquid scintillation counting. Non-specific binding was determined by parallel incubations containing...*
500 ng/ml cold EGF. Each determination was performed in duplicate.

**Immunoprecipitation of In Vivo Phosphorylated EGF Receptor**

The EGF receptor was immunoprecipitated from detergent extracts with 0.5% fetal calf serum and 2 mCi of 32P/ml. Labeled cells were lysed directly in hot 2% sodium dodecyl sulfate and heated at 100 °C for 5 min. Lysates were diluted in RIPA buffer (20) without sodium dodecyl sulfate to an sodium dodecyl sulfate concentration of 0.1% and immunoprecipitated with polyclonal antisera prepared against the EGF receptor as previously described (20) or with rabbit antiserum against the M, the 80,000 protein shown to be a substrate for protein kinase C (25). Quantitation of immunoprecipitated 32P-labeled proteins was done by excising bands from gels, placing the gel pieces in water, and counting Cerenkov radiation in a liquid scintillation counter.

**Preparation of Tryptic Phosphopeptides**—Immunoprecipitated 32P-labeled EGF receptor was run on sodium dodecyl sulfate polyacrylamide gels and located within the gel by autoradiography. Gel slices containing EGF receptor were washed for 15 min in 5 ml of water, followed by 5 ml of 200 mM ammonium bicarbonate, pH 8.5, and incubated in 500 ml of 200 mM ammonium bicarbonate containing 30 μg of TPCK-trypsin for 5 h at 37 °C, at which time another 90 μg of trypsin was added for 16 h. This treatment resulted in the release of 90-95% of the counts from the gel slices. The digests were lyophilized prior to two-dimensional thin layer (20) or HPLC analysis.

**Phosphopeptide Mapping by Reverse-phase HPLC**—Tryptic phosphopeptides were separated by reverse-phase HPLC on a C18 column equilibrated with 0.05% trifluoroacetic acid in water. The 32P-labeled phosphopeptides were eluted from the column with a linear gradient of acetonitrile (0-66%) in 60 min at a flow rate of 1 ml/min.

**Analysis of Inositol Phosphates**—Five-day postconfluent cultures of FS4 cells in 35-mm dishes were labeled for 18-20 h with 10 μCi/ml [3H]inositol added directly to the normal growth medium. For determination of nonspecific background counts, cells were incubated with various concentrations (0.11-20 ng/ml) of 125I-EGF and the specific binding determined. Points shown represent the mean of duplicate samples. Each experiment was repeated three times with essentially the same results. In B, the effects of PDGF or TPA treatment of cells on the time course of 125I-EGF binding by FS4 human skin fibroblasts are shown. Specific binding of 125I-EGF (0.66 ng/ml) by control cells (circles). Cells incubated with 32 ng/ml PDGF (squares), or with 25 ng/ml TPA (triangles) were incubated with PDGF or TPA for 20 min prior to T-EGF addition. Incubation with PDGF or TPA for 20 min resulted in the release of 90-95% of the counts from the gel slices. The digests were lyophilized prior to two-dimensional thin layer (20) or HPLC analysis.

**Peptide Synthesis and Phosphorylation**—The peptide Lys-Arg-Thr-Leu-Arg was synthesized by the Rockefeller University Sequencing Facility. The peptide was purified by HPLC and phosphorylated by protein kinase C (purified protein kinase C was kindly provided by Anthony Rosen, Rockefeller University) according to Hunter et al. (23). The phosphorylated peptide was identified by electrophoresis at pH 8.9 (1% (NH4)2CO3) at 800 V for 45 min.

**Binding of Monoclonal EGF Receptor Antibody to FS4 Cells**—Five-day postconfluent cultures of FS4 cells in 1.5-cm wells were washed once with ice-cold Dulbecco's modified Eagle's medium containing 50 mM Hepes, pH 7.4, and 1.0% bovine serum albumin (binding buffer). 0.2 ml of binding buffer containing 10 μg of antibody (binding buffer). 0.2 ml of binding buffer containing 10 μg of antibody to FS4 cells was added to each well, and the plates were incubated for 10 min at which time additions were made for the indicated periods. Incubations were terminated by the addition of 250 μl of ice-cold 5 M perchloric acid containing 1 mM EDTA and placed on ice for 5 min. The extracts were neutralized with 50 μl of 2.5 M K2CO3 and placed on ice for at least 5 min before analysis of HPLC on a Whatman Partisil SAX 10 column (0.46 X 25 cm) equilibrated with water. Samples were loaded on the column and inositol phosphates eluted using a modification of the method of Dean and Moyer (24). Following addition of samples the column was washed with water for 5 min. The gradients used were 0-0.1 M ammonium phosphate, pH 3.8, with phosphoric acid for 15 min, 0.1-0.3 M ammonium phosphate for 10 min, and 0.3-1.0 M ammonium phosphate for 25 min. Authentic tritium-labeled inositol-1-phosphate, inositol-1,4-phosphate, and inositol-1,3,4-phosphate standards were used to calibrate the column. In this system, these standards eluted at about 21, 38-39, and 50 min, respectively.

**TABLE I**

<table>
<thead>
<tr>
<th>Addition</th>
<th>[3H]thymidine incorporation* (cpm/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>4,308</td>
</tr>
<tr>
<td>10 ng/ml EGF</td>
<td>28,438</td>
</tr>
<tr>
<td>32 ng/ml PDGF</td>
<td>26,781</td>
</tr>
<tr>
<td>10 ng/ml EGF + 32 ng/ml PDGF</td>
<td>36,725</td>
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* Confluent cells were incubated in serum free medium for 12 h, prior to incubation with the indicated additions for 24 h. Incorporation of [3H]thymidine was then measured as described in the text.
TABLE II
Effects of TPA, PDGF, and EGF on binding of EGF receptor monoclonal antibody 528 to FS4 cells.

Binding of antibody and ^125^I-protein A to FS4 cells were performed as described under "Experimental Procedures." The data presented are the mean and standard error of results obtained from three separate experiments.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% binding to control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>100</td>
</tr>
<tr>
<td>25 ng/ml TPA, 20 min</td>
<td>84.1 ± 7.7</td>
</tr>
<tr>
<td>32 ng/ml PDGF, 20 min</td>
<td>95.3 ± 4.1</td>
</tr>
<tr>
<td>100 ng/ml EGF, 40 min</td>
<td>92.7 ± 5.8</td>
</tr>
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FIG. 2. Phosphorylation of the EGF receptor in FS4 cells. 
FS4 cells were labeled in phosphate-free medium with ^32^P for 16 h. EGF receptors were immunoprecipitated from ^32^P-labeled control cells, and from cells treated for 20 min with 50 ng/ml EGF, 32 ng/ml PDGF or TPA (25 ng/ml). The cells were labeled and EGF receptors isolated by immunoprecipitation as described under "Experimental Procedures." The mobilities of known standards (×10^-3) are shown in the right-hand margin. This experiment was performed six times and a typical result is shown.

Two-dimensional thin layer phosphopeptide analysis, phosphoamino acid analysis, assay of [3H]thymidine incorporation, and SDS-polyacrylamide gel electrophoresis were performed as previously described (20).

RESULTS

The data presented are from experiments examining the effects of PDGF on metabolism of the EGF receptor in FS4 human skin fibroblasts (27), however, similar results were found with three other independent human foreskin fibroblast isolates. Both PDGF and EGF were strongly mitogenic for quiescent FS4 cells as judged by their ability to increase the incorporation of [3H]thymidine into acid precipitable counts (Table I). PDGF and EGF added together were somewhat more effective than either alone.

Comparison of PDGF and TPA Mediated Inhibition of ^125^I-EGF Binding by FS4 Cells—Since PDGF and TPA have been shown to inhibit EGF binding by a number of cell types (1, 14, 15, 18), we compared their effects on EGF binding by FS4 cells. Scatchard analysis (Fig. 1A) of ^125^I-EGF binding revealed two classes of binding sites in normal FS4 cells; about 27,300 ± 4,536 high affinity binding sites/cell (K_d = 5.1 ± 0.75 × 10^-10 M) and about 135,000 ± 17,847 low affinity binding sites/cell (K_d = 7.5 ± 0.54 × 10^-9 M). The observed PDGF-dependent inhibition of EGF binding was due primarily to loss of the high affinity EGF-binding sites with the remaining low affinity sites having an apparent K_d of 4.1 ± 0.8 × 10^-8 M (71,999 ± 9,693 binding sites/cell). TPA treatment caused an inhibition of both high and low affinity EGF binding leaving one class of very low affinity binding sites with an apparent K_d of 27,300 ± 4,536 high affinity binding sites/cell (K_d = 5.1 ± 0.75 × 10^-10 M) and about 135,000 ± 17,847 low affinity binding sites/cell (K_d = 7.5 ± 0.54 × 10^-9 M). The observed PDGF-dependent inhibition of EGF binding was due primarily to loss of the high affinity EGF-binding sites with the remaining low affinity sites having a K_d of 8.0 ± 0.9 × 10^-9 M (125,902 ± 14,548 binding sites/cell). TPA treatment caused an inhibition of both high and low affinity EGF binding leaving one class of very low affinity binding sites with an apparent K_d of 4.1 ± 0.8 × 10^-8 M (71,999 ± 9,693 binding sites/cell). Both PDGF and TPA caused prolonged inhibition of binding of ^125^I-EGF and the inhibition by TPA was more...
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FIG. 4. Confirmation that peptide z contains phosphorylated threonine 654. Peptide z was scraped from several thin layer plates on which tryptic digests of EGF receptor from TPA-treated FS4 cells had been run (as Fig. 3C). Based on previous studies and its characteristic mobility in two-dimensional analysis peptide z was believed to be Lys-Arg-ThrP-Leu-Arg containing phosphorylated threonine 654. Authentic Lys-Arg-ThrP-Leu-Arg was synthesized as described in the text. Peptide z and the authentic pentapeptide were subjected to two-dimensional thin layer analysis as described under "Experimental Procedures." In A, 100 cpm of peptide z was run, in B, 200 cpm of authentic peptide, and in C, 100 cpm of peptide z was run together with 200 cpm of authentic peptide. The origins are marked O. Electrophoresis was in the horizontal dimension (cathode on the left). Plates were exposed for 48 h at -70 °C with intensifying screens.

FIG. 5. Analysis of EGF receptor tryptic phosphopeptides by HPLC. In vivo 32P-phosphate-labeled EGF receptors were immunoprecipitated from control, PDGF (32 ng/ml) treated, and TPA (25 ng/ml)-treated FS4 cells. Gel slices containing the receptors were trypsin treated and resulting phosphopeptides resolved on a C18 reverse-phase column as described in the text. Equal amounts of tryptic peptides were analyzed for each type of sample. These experiments were repeated four times with similar results and the data shown are from a typical experiment.

FIG. 6. Phosphorylation of the Mr = 80,000 protein. FS4 cells were labeled with 32P and M r = 80,000 protein immunoprecipitated as described in the text. Immunoprecipitates from control cells (first lane on the left), cells treated for 20 min with 32 ng/ml PDGF (center lane), or with 25 ng/ml TPA (right lane) are shown. The mobilities of known standards (×10^-3) are shown in the right-hand margin. This experiment was performed twice with essentially identical results.

pronounced (Fig. 1B). These observed changes in EGF binding by PDGF were apparently not attributable to loss of cell surface EGF receptors as the specific binding of anti-EGF receptor monoclonal antibody 528 (26) was not significantly changed (Table II). TPA treatment caused a 10–20% decrease in the amount antibody specifically bound to the cell surface.

PDGF-stimulated Phosphorylation of the EGF Receptor in FS4 Cells—In addition to inhibiting EGF binding, PDGF has been shown to stimulate phosphorylation of the EGF receptor (16, 17). We also found a consistent 30–40% increase in 32P-

incorporation into the EGF receptor when FS4 cells were treated with PDGF (Fig. 2). Using WI-38 lung fibroblasts, it has been reported that residue threonine 654 of the EGF receptor is phosphorylated when cells are treated with PDGF or TPA (16, 17, 22). To examine this possibility in FS4 cells, tryptic digests of EGF receptor from control, PDGF treated, and TPA-treated cells were subjected to two-dimensional thin layer analysis (Fig. 3). Surprisingly, no PDGF-stimulated phosphorylation of the peptide containing threonine 654 was detected in cells treated with PDGF (Fig. 3B), although TPA-stimulated phosphorylation of this phospho-threonine-containing peptide was easily discernable (peptide z in Fig. 3C). Peptide z was shown to be the peptide Lys-Arg-ThrP-Leu-Arg containing phosphorylated threonine 654 (residues 652–656 of the EGF receptor) by several criteria in addition to its distinctive position in two-dimensional analysis. First, peptide z was found to comigrate in two-dimensional thin layer analysis with synthetic Lys-Arg-ThrP-Leu-Arg pentapeptide which had been phosphorylated in vitro by protein kinase C (Fig. 4). Secondly, TPA failed to stimulate phosphorylation of a peptide migrating as peptide z in tryptic digests of EGF receptor-containing alanine at position 654 which had been expressed in 3T3 cells (data not shown). The peptide map in Fig. 3B is for EGF receptor from cells treated for 20 min with PDGF. However, treatment with PDGF for 2, 5, and 60 min also did not result in detectable phosphorylation of threonine 654 (not shown).

These data suggested that PDGF-stimulated phosphoryla-
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**TABLE III**
Effects of PDGF on accumulation of inositol monophosphate ($\text{InsP}_1$), inositol bisphosphate ($\text{InsP}_2$), and inositol trisphosphate ($\text{InsP}_3$) in FS4 cells

<table>
<thead>
<tr>
<th>Addition</th>
<th>$\text{InsP}_1$ cpm</th>
<th>% Control</th>
<th>$\text{InsP}_2$ cpm</th>
<th>% Control</th>
<th>$\text{InsP}_3$ cpm</th>
<th>% Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3315 ± 436</td>
<td>100</td>
<td>500 ± 103</td>
<td>100</td>
<td>205 ± 37</td>
<td>100</td>
</tr>
<tr>
<td>32 ng/ml PDGF</td>
<td>5990 ± 719</td>
<td>181</td>
<td>788 ± 137</td>
<td>157</td>
<td>402 ± 45</td>
<td>196</td>
</tr>
</tbody>
</table>

**Fig. 7. Analysis of acid stable phosphoamino acids from $^{32}$P-labeled EGF receptors from FS4 cells.** Phosphoamino acid analysis of in vivo labeled EGF receptor from control cells (A), from cells treated with 10 ng/ml EGF for 10 min (B), with 32 ng/ml PDGF for 20 min followed by 10 ng/ml EGF for 10 min (C), or with 25 ng/ml TPA for 20 min followed by 10 ng/ml EGF for 10 min (D) are shown. EGF receptors were immunoprecipitated and phosphoamino acid analysis performed as described in the text. The position of phosphotyrosine ($pY$), phosphothreonine ($pT$), and phosphoserine ($pS$) standards are shown in lane E superimposed on a sample autoradiogram. Equal amounts of hydrolyzed receptor were analyzed. These results were reproduced in three experiments.

**DISCUSSION**

In this report we have examined the effects of PDGF on metabolism of the EGF receptor in FS4 human foreskin fibroblasts. Our data indicate that certain aspects of PDGF-mediated regulation of EGF receptor function in FS4 cells are distinct from those previously reported for WI-38 human lung fibroblast cells (16, 17).

As previously shown for a number of other cell types (1), FS4 cells exhibit high and low affinity EGF binding, and PDGF treatment results in the loss of the high affinity binding component. The addition of TPA to cultures of FS4 cells also decreases the binding of $^{125}$I-EGF, but unlike PDGF, effects both high and low affinity binding. These data reveal that EGF binding can be modulated to varying degrees by PDGF and TPA in skin fibroblasts. In contrast, PDGF and TPA-mediated regulation of EGF receptor binding affinity in WI-38 lung fibroblasts were similar with both effectors preferentially reducing high affinity binding (17).

Inhibition of EGF binding by PDGF and TPA has been shown to correlate with increased phosphorylation of the EGF receptor (1, 16-18). PDGF and TPA also stimulate phosphorylation of the EGF receptor in FS4 cells but in qualitatively different manners. A comparison of phosphopeptide maps from PDGF and TPA-treated EGF receptor showed that both increase phosphorylation of several endogenously phosphorylated peptides. Only TPA induced phosphorylation of threonine 654. These results contrast with results reported using WI-38 cells (16, 22), where in addition to increasing phosphorylation of endogenously phosphorylated peptides, both PDGF and TPA induce phosphorylation of threonine 654. Our data suggest that heterologous regulation of EGF receptor binding affinity can occur independently of phosphorylation of threonine 654. It appears that EGF binding may be regulated by EGF receptor phosphorylation through at least the
following two mechanisms: 1) by increased phosphorylation of regions of the EGF receptor represented by the endogenously phosphorylated peptides, and 2) by phosphorylation of threonine 654. These two mechanisms may act in an additive fashion.

PDGF has been shown to activate protein kinase C in a number of cell types (25, 29, 30). Since threonine 654 is known to be phosphorylated by protein kinase C (19), our data suggest that PDGF does not appreciably activate protein kinase C in FS4 cells. This idea is supported by the inability of PDGF to stimulate phosphorylation of an $M_r = 80,000$ protein, also known to be a substrate for protein kinase C in many cell types (25, 29, 30). This finding is surprising, since the PDGF-induced increase in accumulation of inositol phosphates suggests that turnover of phosphoinositides is stimulated which should lead to production of diacylglycerol, an activator of protein kinase C (28). However, Thompson et al. (32) have recently demonstrated that EGF strongly stimulates turnover of phosphoinositides in A431 cells without apparent activation of protein kinase C. It appears that the precise relationship between diacylglycerol production and activation of protein kinase C is not clear. These data indicate that activation of protein kinase C is not a prerequisite for PDGF-dependent mitogenesis, corroborating previous work examining the effects of PDGF on protein kinase C-depleted cells (33).

Moreover, PDGF treatment appears to result in activation of kinases other than protein kinase C which are able to phosphorylate the EGF receptor.

The effects of PDGF and TPA on EGF-dependent, tyrosine-specific autophosphorylation of the EGF receptor were compared in FS4 cells. As previously reported for other cell types, TPA treatment of FS4 cells blocks the EGF-stimulated increase in EGF receptor phosphorylation content. PDGF treatment, however, had no effect. These results differ from those reported for regulation of autophosphorylation of the EGF receptor (16, 22).

The data presented demonstrate that regulation of EGF receptor metabolism by PDGF in foreskin fibroblasts differs from that previously reported for WI-38 lung fibroblasts (16, 17). These differences may be cell type specific, since it has been shown that human lung fibroblast isolates (including WI-38 cells) are refractory to the mitogenic stimulation by EGF, whereas EGF is an effective mitogen for human skin fibroblasts (34, 35). The EGF receptor may be governed by different regulatory systems in these cell types. In this regard, two distinct PDGF receptor types have been reported in human fibroblasts (36), and such receptor heterogeneity could underlie the differences in response to PDGF between FS4 foreskin fibroblasts and WI-38 lung fibroblasts.

Acknowledgments—Special thanks to Thomas Deuel for providing purified PDGF, to Jan Vliek and Pravin Seghal for the FS4 cells, to James Wang for 80,000 protein antiserum, to Dr. Antony Rosen for protein kinase C, and to John Mendelsohn for antibody 528.

REFERENCES