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The product of the protooncogene c-*src* is modified during the cellular response to platelet-derived growth factor

(mitogenesis/neoplastic transformation/protein kinase/phosphotyrosine/pp60$^{c\text{-}src}$)

ROBERT RALSTON AND J. MICHAEL BISHOP

Department of Microbiology and Immunology and The Hooper Research Foundation, University of California, San Francisco, San Francisco, CA 94143

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ABSTRACT **We have observed a modification of the cellular protein kinase pp60$^{c\text{-}src}$, elicited in murine 3T3 fibroblasts by platelet-derived growth factor (PDGF). The modification occurred rapidly after addition of PDGF to the culture medium and was first detected as a reduction in the electrophoretic mobility of a portion of the pp60$^{c\text{-}src}$ molecules. A similarly modified form of the viral homologue pp60$^{v\text{-}src}$ occurs *in vivo* in the absence of stimulation by PDGF. The occurrence of modified forms of both pp60$^{c\text{-}src}$ and pp60$^{v\text{-}src}$ was associated with a novel phosphorylation at tyrosine in the amino-terminal domains of the proteins. The time-course and dose–response for this modification of pp60$^{c\text{-}src}$ paralleled PDGF-induced increases in phosphorylation of pp36, a major cellular substrate for several tyrosine-specific protein kinases. In parallel experiments, treatment of cells with PDGF increased the kinase activity of pp60$^{c\text{-}src}$ in an immunocomplex assay. These results suggest pp60$^{c\text{-}src}$ may play a role in the mitogenic response to PDGF.**

Continuous growth of cells in culture requires several protein factors present in serum. One of the major polypeptide mitogens in serum for fibroblasts is platelet-derived growth factor (PDGF) (1, 2). PDGF is released from the alpha granules of platelets during clot formation (2) and is believed to stimulate fibroblast growth in wound healing.

The cellular response to PDGF displays a number of parallels with the effects of expression of the v-*src* oncogene (the transforming gene of Rous sarcoma virus). These similarities include phosphorylation of cellular proteins at tyrosine residues (3–5), phosphorylation of the ribosomal protein S6 (6, 7), increased turnover of phosphatidylinositol (8, 9), reorganization of actin cables (2, 10), mitogenesis (2, 11, 12), and even neoplastic transformation (by v-*sis*, which encodes one subunit of PDGF) (13, 14).

Expression of v-*src* induces mitosis in fibroblasts that have been growth-arrested by serum starvation (11, 12). The v-*src* oncogene has a closely related cellular homologue, c-*src* (15). This gene is expressed in normal fibroblasts (16) and encodes a 60-kDa protein, pp60$^{c\text{-}src}$, that has been shown to possess tyrosine-specific protein kinase activity *in vitro* (17). Because of the similarities in the responses to PDGF stimulation and v-*src* expression, we investigated the possible role of pp60$^{c\text{-}src}$ in the mitogenic response to PDGF. We found that the amino-terminal domain of pp60$^{c\text{-}src}$ rapidly becomes modified when quiescent Swiss 3T3 cells are stimulated with PDGF. The modification is associated with phosphorylation of tyrosine residues in the amino-terminal portion of the protein and is accompanied by both increased kinase activity of pp60$^{c\text{-}src}$ *in vitro* and increased phosphorylation of the cellular substrate pp36 (18) *in vivo*. These changes are observed at concentrations of PDGF similar to those sufficient to induce DNA synthesis and mitosis in quiescent cells. The possible role of pp60$^{c\text{-}src}$ in the mitogenic response to PDGF and its relationship to transformation are discussed.

MATERIALS AND METHODS

Cell Culture and Radiolabeling. Swiss 3T3 cells (American Type Culture Collection CCL 92) or other cells described in the text were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum. The cells were taken from frozen stocks and used after one or two passages. The cells were grown to confluence and allowed to become stationary for 2 days prior to use. For methionine labeling, stationary cultures were incubated for 5 hr in serum-free medium 199 without methionine, supplemented with 0.3 mCi of [^{35}S]methionine (>600 Ci/mmol, Amersham; 1 Ci = 37 GBq) per ml. For phosphate labeling, stationary cultures were incubated in serum-free medium 199 without phosphate for 4 hr. The medium was then replaced with fresh medium 199 without phosphate, supplemented with 1 mCi of [^{32}P]orthophosphate (carrier-free, ICN) per ml, and the cells were incubated an additional 4 hr. Growth factors were added directly to this medium and incubation was continued for various times. The PDGF used in these experiments, purified to apparent homogeneity (19), was a gift of T. Deuel.

General Procedures. Immunoprecipitation pp60$^{c\text{-}src}$, pp60$^{c\text{-}src}$, and pp36 was performed as described (20, 21). Proteins were separated by electrophoresis in NaDodSO$_4$/9% polyacrylamide gels. For peptide mapping using *Staphylococcus aureus* V8 protease, gels were dried, without fixation, on Kimwipe tissues supported by Whatman no. 1 paper. The dried gels were removed from the Whatman paper backing, marked, and autoradiographed. Gels prepared this way are translucent and can be aligned precisely over the autoradiograms, aiding in separation of closely spaced bands. The remainder of the procedure for V8 mapping was as described (20). Phospho amino acid analysis was as described by Hunter and Sefton (3). pp60$^{c\text{-}src}$ kinase activity in immunocomplexes was determined as described (20).

RESULTS

Phosphorylation of pp36 and pp60$^{c\text{-}src}$ in Response to PDGF. To investigate the effect of PDGF on phosphorylation of pp60$^{c\text{-}src}$ and pp36, Swiss 3T3 cells were grown to confluence, incubated with [^{35}S]methionine or [^{32}P]orthophosphate, and then treated with PDGF (100 ng/ml) for 30 min. The cells were lysed and pp60$^{c\text{-}src}$ and pp36 were immunoprecipitated and analyzed by NaDodSO$_4$/PAGE. The results of two typical experiments are shown in Fig. 1. We observed no significant change in the steady-state amounts of [^{35}S]methionine-labeled pp60$^{c\text{-}src}$ or pp36 during PDGF treatment (lanes 1–4). However, treatment with PDGF increased phospho-

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Abbreviations: PDGF, platelet-derived growth factor; middle T antigen, middle-sized tumor antigen encoded by polyoma virus.

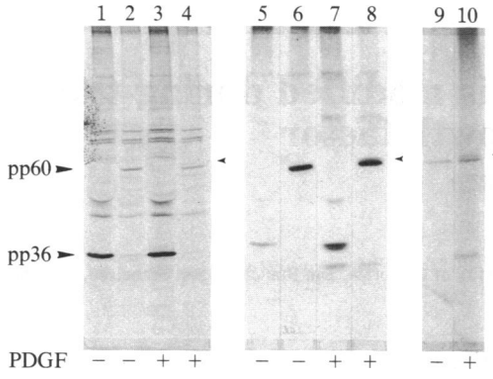

FIG. 1. Phosphorylation of pp36 and pp60$^{c\text{-}src}$ in response to PDGF. Confluent Swiss 3T3 cells were labeled with [^{35}S]methionine (lanes 1–4) or [^{32}P]orthophosphate (lanes 5–10) for 4–5 hr prior to treatment with PDGF (100 ng/ml) for 30 min. The cells were lysed, and lysates were divided for analysis of pp36 (lanes 1, 3, 5, and 7) or pp60$^{c\text{-}src}$ (lanes 2, 4, 6, 8, 9, and 10). Proteins were immunoprecipitated and analyzed by NaDodSO$_4$/PAGE and autoradiography. Lanes 1, 2, 5, and 6: pp36 and pp60$^{c\text{-}src}$ from control cells. Lanes 3, 4, 7, and 8: pp36 and pp60$^{c\text{-}src}$ from cells treated with PDGF. Results of a second experiment, in which better resolution of the two forms of pp60$^{c\text{-}src}$ was achieved, are shown in lanes 9 and 10. Lane 9: pp60$^{c\text{-}src}$ from control cells. Lane 10: pp60$^{c\text{-}src}$ from cells treated with PDGF. Small arrowheads indicate the slower-migrating form of pp60$^{c\text{-}src}$.

rylation of pp36 about 3-fold (lanes 5 and 7). pp36 precipitated by our antiserum characteristically runs as a doublet on NaDodSO$_4$/polyacrylamide gels. The faster-migrating form contains more phosphotyrosine than the slower-migrating form (data not shown). The faster-migrating form is also the predominant phosphorylated species in cells transformed by v-*src*.

PDGF treatment also increased phosphorylation of pp60$^{c\text{-}src}$. The amount of increase varied in different experiments (from 2- to 5-fold), but a characteristic feature of PDGF treatment was the appearance of a band with a slightly slower mobility than the major pp60$^{c\text{-}src}$ band (lane 8). In a second experiment, better separation of this band from the bulk of pp60$^{c\text{-}src}$ was obtained (lanes 9 and 10). A faint band at this position could be seen in the original autoradiogram of proteins from [^{35}S]methionine-labeled cells that had been treated with PDGF (lane 4), but not in untreated cells (lane 2).

To further characterize the relationship of the increases in phosphorylation of pp60$^{c\text{-}src}$ and pp36 to PDGF treatment, we determined the dose–response and time-course for their appearance. The dose of PDGF producing half-maximal increases in phosphorylation was between 3 and 10 ng/ml (Fig. 2). This is comparable to the dose required for half-maximal stimulation of DNA synthesis. The separation of the slower-migrating band from the bulk of the pp60$^{c\text{-}src}$ was poor in this experiment, but its presence was verified by partial proteolytic digestion with *S. aureus* V8 protease (see Fig. 3). The increases in phosphorylation of pp36 and pp60$^{c\text{-}src}$ occurred in parallel. The increases in phosphorylation reached their maximal level <2 min after addition of PDGF and persisted for at least 1 hr (data not shown).

Modification of pp60$^{c\text{-}src}$ in the Amino-Terminal Domain. We wished to characterize the band that migrated slightly slower than pp60$^{c\text{-}src}$. The rapidity of its appearance following PDGF stimulation suggested that it might result from posttranslational modification of pp60$^{c\text{-}src}$. We confirmed this by partial proteolytic digestion. pp60$^{c\text{-}src}$ bands and the slower-migrating band were partially digested with *S. aureus* V8 protease and the resulting fragments were separated by NaDodSO$_4$/PAGE (Fig. 3). The v-*src*-encoded protein (pp60$^{v\text{-}src}$) was digested for comparison. All lanes shown were from a single gel, but

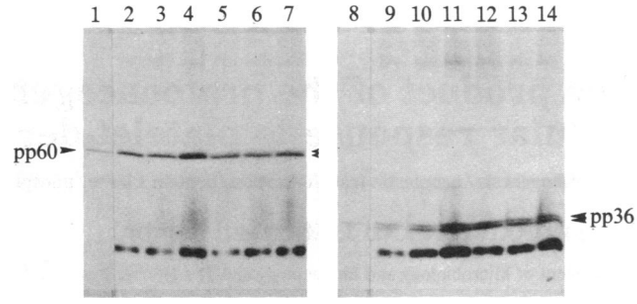

FIG. 2. Dose–response for phosphorylation of pp36 and pp60$^{c\text{-}src}$. Cells were treated with PDGF for 30 min and then lysed. pp36 (lanes 8–14) and pp60$^{c\text{-}src}$ (lanes 1–7) were immunoprecipitated and analyzed by NaDodSO$_4$/PAGE and autoradiography. Lanes 1 and 8: immunoprecipitates from control cells. Lanes 2–7 and 9–14: immunoprecipitates from cells treated with PDGF at 1, 3, 10, 30, 100, or 300 ng/ml.

exposures were adjusted to make the intensities of the fragments comparable.

The slower-migrating band yielded a pattern (Fig. 3, lane 3) that was similar to that produced by pp60$^{c\text{-}src}$ (lanes 1 and 2), but the amino-terminal fragments (N) had slower mobilities. Densitometric scanning of the V8 maps revealed that the amino-terminal fragments from the modified form of pp60$^{c\text{-}src}$ were more phosphorylated, relative to the carboxyl-terminal fragment (C) than the amino-terminal fragments of normal pp60$^{c\text{-}src}$ (data not shown). The mobilities of these modified amino-terminal fragments (N*) were also slightly slower than the amino-terminal fragments from pp60$^{v\text{-}src}$. A mixing experiment was done by cutting out some of the normal pp60$^{c\text{-}src}$ band along with the slower-migrating band (hereafter referred to as pp60$^{c\text{-}srcN^*}$). PDGF-treated RC cells, which overexpress pp60$^{c\text{-}src}$ (22), were used for the mixing experiment. The smallest identifiable fragment whose mobility was altered (N*) was ≈19 kDa. This fragment contains the protein's amino terminus (20), so that the modification could be localized to within the first 200 amino acids of the protein.

A Similar Modification of pp60$^{v\text{-}src}$. We also examined

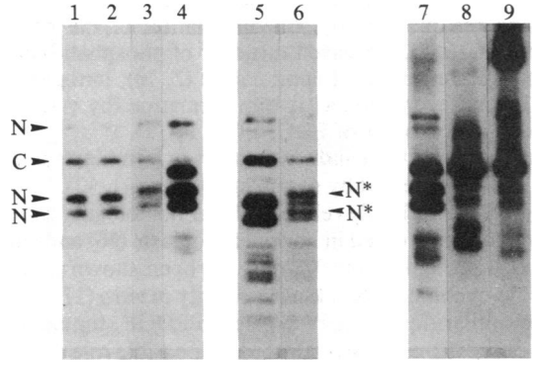

FIG. 3. Partial proteolytic mapping of pp60$^{c\text{-}src}$ and pp60$^{v\text{-}src}$. Proteins were purified by immunoprecipitation and NaDodSO$_4$/PAGE. Partial proteolytic digestion with *S. aureus* V8 protease was as described in *Materials and Methods*. Lane 1: pp60$^{c\text{-}src}$ from control Swiss 3T3 cells. Lane 2: pp60$^{c\text{-}src}$ from PDGF-treated Swiss 3T3 cells. Lane 3: modified pp60$^{c\text{-}src}$ from PDGF-treated Swiss 3T3 cells. Lane 4: pp60$^{v\text{-}src}$ from rat fibroblasts (T2 cells) transformed by Rous sarcoma virus. Lane 5: pp60$^{c\text{-}src}$ from control RC cells. Lane 6: modified pp60$^{c\text{-}src}$ mixed with normal pp60$^{c\text{-}src}$ from RC cells. Lane 7: pp60$^{v\text{-}src}$ from Rous sarcoma virus-transformed rat cells. Lane 8: the same gel (lane 7) after treatment with 1 M KOH. Lane 9: pp60$^{v\text{-}src}$ phosphorylated in membrane preparations from Rous sarcoma virus-transformed rat cells. N, amino-terminal fragment; N*, modified amino-terminal fragment; C, carboxyl-terminal fragment.

$pp60^{v\text{-}src}$ for similar modifications. Phosphorylation of tyrosine residues in the amino-terminal portion of $pp60^{v\text{-}src}$ has been reported by several groups (23–26). We wanted to determine whether such modifications could be detected under the conditions we were using for analysis of $pp60^{c\text{-}src}$. $NaDodSO_4$/polyacrylamide gels of V8-digested $pp60^{v\text{-}src}$ were treated with 1 M KOH to preferentially destroy alkyl phosphate ester bonds in the peptides (27). This treatment eliminated >95% of the [^{32}P]phosphate from phosphoserine residues, improving detection of phosphotyrosine-containing peptides. This experiment revealed that the slower-migrating portions of the amino-terminal V8 fragments of $pp60^{v\text{-}src}$ were relatively more resistant to alkali than the faster-migrating portions (Fig. 3, lanes 7 and 8). *In vitro* labeling of $pp60^{v\text{-}src}$ in membrane preparations also enhanced detection of phosphotyrosine-containing peptides, presumably because most of the serine-specific protein kinase that phosphorylates Ser-17 in $pp60^{v\text{-}src}$ was eliminated in these preparations. Membranes were prepared from rat fibroblasts transformed by Rous sarcoma virus (T2 cells) and used for phosphorylation (28). $pp60^{v\text{-}src}$ was immunoprecipitated from these preparations and analyzed by $NaDodSO_4$/PAGE. Addition of [γ-^{32}P]ATP to membranes from T2 cells resulted in phosphorylation of $pp60^{v\text{-}src}$. Partial proteolytic digestion of this protein allowed resolution of the two small amino-terminal peptides as doublets (Fig. 3, lane 9). The carboxyl-terminal peptide, which contains phosphotyrosine, was intensely labeled.

The Modifications Include Phosphorylation at Tyrosine. Analysis of the phospho amino acids from the small V8 fragments generated from $pp60^{v\text{-}src}$ immunoprecipitated from ^{32}P-labeled T2 cells (21) revealed phosphotyrosine as well as phosphoserine (Fig. 4, lane 1). To test the possibility that the phosphotyrosine we detected resulted from contamination of the amino-terminal fragments with phosphotyrosine containing peptides from the carboxyl-terminal domain, we isolated the major V8 fragments of $pp60^{v\text{-}src}$ and subjected them to further partial proteolytic digestion with V8 protease or elastase. These experiments failed to show any common phosphopeptides generated from the regions of the gel containing the major amino-terminal and carboxyl-terminal V8 fragments (data not shown).

We also analyzed the phospho amino acids from the V8 fragments of $pp60^{c\text{-}src}$ and $pp60^{c\text{-}srcN^*}$. Analysis of the small amino-terminal V8 fragments from $pp60^{c\text{-}src}$ showed only phosphoserine, but both phosphoserine and phosphotyrosine were found in the fragments from $pp60^{c\text{-}srcN^*}$ (lanes 2 and 3). The ratio of phosphoserine to phosphotyrosine in the amino-terminal V8 fragments of $pp60^{c\text{-}srcN^*}$ was 4:1. No phosphotyrosine was found in the amino-terminal V8 peptides of $pp60^{c\text{-}src}$, even though eight times more labeled $pp60^{c\text{-}src}$ than $pp60^{c\text{-}srcN^*}$ was used for analysis. The absence of phosphotyrosine in these fragments suggested that the phosphotyrosine in the adjacent V8 fragments was unlikely to be due to contamination by carboxyl-terminal peptides. This was tested as described above for $pp60^{v\text{-}src}$. Secondary digestion of the major V8 fragments of both $pp60^{c\text{-}src}$ and $pp60^{c\text{-}srcN^*}$ showed the absence of common phosphopeptides generated from the regions of the gel containing the amino-terminal and carboxyl-terminal fragments (data not shown).

PDGF May Elicit an Increase in the Kinase Activity of $pp60^{c\text{-}src}$. The correlation of increased phosphorylation of pp36 on tyrosine residues with appearance of $pp60^{c\text{-}srcN^*}$ (Fig. 1) suggested that increased phosphorylation of the amino-terminal domain of $pp60^{c\text{-}src}$ might be associated with its activation as a kinase. We therefore tested the kinase activity of $pp60^{c\text{-}src}$ *in vitro*.

$pp60^{c\text{-}src}$ was precipitated from PDGF-treated and control cells, and its kinase activity was determined in an immunocomplex kinase assay. Treatment with PDGF (100 ng/ml) for 30 min produced a 2-fold increase in kinase activity (Fig. 5). Although the increase was not large, it was consistent with the increases observed in phosphorylation of pp36 and the expected increase in specific activity for $pp60^{c\text{-}srcN^*}$ (see below).

DISCUSSION

Nature of the Modification. Stimulation of quiescent Swiss 3T3 cells with PDGF results in rapid modification of $pp60^{c\text{-}src}$ to a form that has reduced mobility on $NaDodSO_4$/polyacrylamide gels. Partial proteolytic mapping and phospho amino acid analysis revealed a tyrosine phosphorylation site in the amino-terminal portion of the $pp60^{c\text{-}src}$ molecule. We have called this species $pp60^{c\text{-}srcN^*}$.

Phosphorylation of $pp60^{v\text{-}src}$ at tyrosine in the amino-terminal domain and the associated reduction in its electrophoretic mobility have been demonstrated previously both *in vitro* and *in vivo* (23–26). This form of $pp60^{v\text{-}src}$ apparently

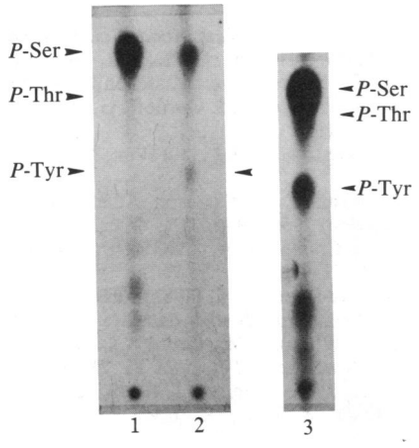

FIG. 4. Phospho amino acid analysis of amino-terminal V8 peptides from $pp60^{v\text{-}src}$ and $pp60^{c\text{-}src}$. Phospho amino acids from the small amino-terminal V8 peptides of $pp60^{v\text{-}src}$ and $pp60^{c\text{-}src}$ were analyzed as described (3). Lane 1: phospho amino acids from N plus N* fragments of $pp60^{v\text{-}src}$. Lane 2: phospho amino acids from N fragments of $pp60^{c\text{-}src}$. Lane 3: phospho amino acids from N* fragments of $pp60^{c\text{-}srcN^*}$

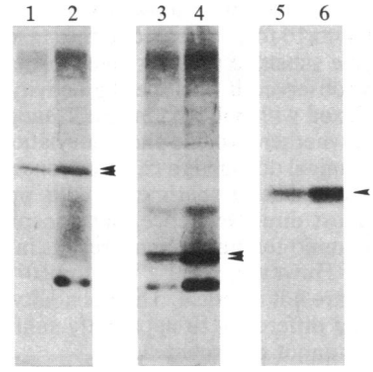

FIG. 5. Effect of PDGF on kinase activity of $pp60^{c\text{-}src}$. Cells were labeled with [^{32}P]orthophosphate and pp36 and $pp60^{c\text{-}src}$ were analyzed as in Fig. 1. Lane 1: $pp60^{c\text{-}src}$ from control cells. Lane 2: $pp60^{c\text{-}src}$ from cells treated with PDGF (100 ng/ml) for 30 min. Lane 3: pp36 from control cells. Lane 4: pp36 from cells treated with PDGF. For kinase assays, cells were treated in parallel except that no label was added. $pp60^{c\text{-}src}$ was precipitated from these cells in the same way as from the ^{32}P-labeled cells and its ability to phosphorylate bound IgG was determined as described (20). Lanes 5 and 6: phosphorylation of IgG by $pp60^{c\text{-}src}$ in the immunocomplex from control cells (lane 5) and from cells treated with PDGF (lane 6).

turns over rapidly *in vivo* (24). Altered electrophoretic mobility of $pp60^{c\text{-}src}$ associated with phosphorylation at tyrosine residues in the amino-terminal domain has been observed in cells infected with polyoma virus and in cells that express the polyoma middle-sized tumor (middle T) antigen (29, 30). The modified form of $pp60^{c\text{-}src}$ was observed only as a complex with polyoma middle T antigen (30).

It is not clear whether the phosphorylation on tyrosine described for $pp60^{c\text{-}srcN^*}$ is responsible by itself for the reduced mobility of the molecule on $NaDodSO_4$/polyacrylamide gels. Because only a small amount of $pp60^{c\text{-}src}$ is modified in response to PDGF, we were unable to obtain sufficient $pp60^{c\text{-}srcN^*}$ for two-dimensional phosphopeptide mapping. It is therefore possible that the amino-terminal domain of $pp60^{c\text{-}srcN^*}$ also contains phosphorylations on serine that are not present in $pp60^{c\text{-}src}$. Two-dimensional peptide mapping of the modified forms of $pp60^{v\text{-}src}$ described above indicated that the major additional phosphorylations occurred at tyrosine residues in the amino-terminal domain (24). However, sites of serine phosphorylation within the amino-terminal domain of $pp60^{v\text{-}src}$ in addition to the major site at Ser-17 (31) have been described (32). It is probable that PDGF treatment stimulates phosphorylation of additional serine residues on $pp60^{c\text{-}src}$, because we observed increased ^{32}P-labeling of the amino-terminal domain without alteration of its mobility on $NaDodSO_4$/polyacrylamide gels, and phospho amino acid analysis of these peptides showed only phosphoserine.

Increased phosphorylation of serine residues in the amino-terminal domain of $pp60^{c\text{-}src}$ from chicken cells stimulated with PDGF has been reported (33). These workers did not detect a form of $pp60^{c\text{-}src}$ with reduced electrophoretic mobility or phosphotyrosine in the amino-terminal domain. The reason for the differences between their results and our results is not clear.

Increased Kinase Activity of $pp60^{c\text{-}srcN^*}$. We were able to detect $pp60^{c\text{-}srcN^*}$ within minutes following PDGF treatment. The time-course and dose–response for its appearance paralleled increased phosphorylation of the cellular protein pp36 on tyrosine residues. Examination of the kinase activity of $pp60^{c\text{-}src}$ *in vitro* showed a 2-fold increase following PDGF treatment, suggesting that $pp60^{c\text{-}srcN^*}$ represents an activated form of the kinase.

Phosphorylation of $pp60^{v\text{-}src}$ at tyrosine residues in the amino-terminal domain was reported by several groups to be associated with increased kinase activity (23–26). Purification of this modified form of $pp60^{v\text{-}src}$ showed that its specific activity was increased 3- to 10-fold compared to the standard enzyme, depending on the substrate used for comparison (24). Similar increases were observed in the specific activity of modified $pp60^{c\text{-}src}$ complexed with polyoma middle T antigen (30).

It is not clear whether tyrosine phosphorylation of $pp60^{c\text{-}src}$ in the amino-terminal domain is a cause or consequence of its activation as a kinase. Experiments with $pp60^{v\text{-}src}$ have provided the most direct evidence that phosphorylation of tyrosine in the amino-terminal domain results in activation of the kinase (24). However, $pp60^{v\text{-}src}$ and $pp60^{c\text{-}src}$, although closely related, are not identical. The possibility that the two proteins respond differently to apparently similar modifications therefore cannot be excluded.

In our experiments, PDGF treatment resulted in about 10% of the $pp60^{c\text{-}src}$ being converted to $pp60^{c\text{-}srcN^*}$. If modification of $pp60^{c\text{-}src}$ results in increases in kinase activity similar to those observed for $pp60^{v\text{-}src}$ [e.g., 10-fold (24)], then the sum of the activities of $pp60^{c\text{-}srcN^*}$ and $pp60^{c\text{-}src}$ would account for the 2-fold increase in kinase activity following PDGF treatment.

Possible Role of $pp60^{c\text{-}src}$ in Mitogenic Response. We have demonstrated that stimulation of quiescent Swiss 3T3 cells with PDGF results in phosphorylation of the amino-terminal domain of $pp60^{c\text{-}src}$ on tyrosine and apparent enhancement of its kinase activity. These observations raise the question of the role of $pp60^{c\text{-}src}$ in the mitogenic response to PDGF.

There are a number of parallels between the cellular response to PDGF binding and v-*src* expression, including phosphorylation of cellular proteins on tyrosine, phosphorylation of the ribosomal protein S6, increased turnover of phosphatidylinositol, reorganization of actin cables, and mitogenesis. These similarities and the similarities in modifications of $pp60^{v\text{-}src}$ and $pp60^{c\text{-}srcN^*}$ associated with increased kinase activity suggest that modifications of $pp60^{c\text{-}src}$ may be involved in the mitogenic response to PDGF.

Studies with polyoma virus have shown that middle T antigen is capable of altering the growth properties of the cell. Middle T antigen associates with a modified form of $pp60^{c\text{-}src}$ that has increased kinase activity (30). Cells transformed by middle T antigen display many of the biochemical and morphological characteristics described above for v-*src* and PDGF (2, 5, 34). Morphological transformation by middle T antigen is dependent on serum concentration (35). This is provocative because of the similarity between the modified form of $pp60^{c\text{-}src}$ associated with middle T antigen and the $pp60^{c\text{-}srcN^*}$ produced in response to PDGF stimulation. It is possible that serum is required, at least in part, to generate a short-lived, activated form of $pp60^{c\text{-}src}$, such as $pp60^{c\text{-}srcN^*}$, which is then stabilized by middle T antigen. In this model, middle T antigen would act to block decay of a mitogenic signal.

The suggestion from our data that modification of $pp60^{c\text{-}src}$ plays some role in the mitogenic response to PDGF is reinforced by the analogies among the actions of $pp60^{v\text{-}src}$, $pp60^{c\text{-}src}$ modified by middle T antigen, and PDGF. There are caveats, however: $pp60^{v\text{-}src}$ may phosphorylate aberrant substrates; middle T antigen may affect other cellular proteins beside $pp60^{c\text{-}src}$; PDGF affects other protooncogenes—notably, it increases expression of c-*fos* and c-*myc* (36). Still, the correlations seem highly suggestive of a role for $pp60^{c\text{-}src}$ in the cellular response to PDGF. Definitive analysis of the various modifications of $pp60^{c\text{-}src}$ and $pp60^{v\text{-}src}$ and their effects will be needed to resolve the issue.

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