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Differentiation of myeloid cells is accompanied by increased levels of pp60$^{c\text{-}src}$ protein and kinase activity

(myeloid leukemia/bone marrow/tumor promoter/cellular oncogene)

CONNIE E. GEE*, JAMES GRIFFIN†, LEANDRO SASTRE‡, LINDA J. MILLER‡, TIMOTHY A. SPRINGER‡, HELEN PIWNICA-WORMS*, AND THOMAS M. ROBERTS*

*Laboratory of Neoplastic Disease Mechanisms, †Division of Tumor Immunology, and ‡Laboratory of Membrane Immunohistochemistry, Dana–Farber Cancer Institute, 44 Binney Street, Boston, MA 02115

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ABSTRACT **We have detected a significant increase in the levels of pp60$^{c\text{-}src}$ kinase activity associated with the differentiation of myeloid cell lines HL-60 and U-937. The induction of pp60$^{c\text{-}src}$ kinase activity becomes apparent ≈14 hr after the addition of phorbol 12-myristate 13-acetate and increases 20-fold by 72 hr. The enhanced kinase activity can be accounted for by elevated levels of c-src protein in the differentiated cells. When nonleukemic bone marrow cells were examined, myeloid progenitor cells exhibited a low level of pp60$^{c\text{-}src}$ kinase activity. As these cells are allowed to differentiate in culture, the resulting adherent monocytes are as high in pp60$^{c\text{-}src}$ kinase activity as HL-60 cells induced to differentiate into monocytes. A strong correlation is found between the levels of pp60$^{c\text{-}src}$ kinase activity and the degree of monocytic differentiation of the cells from patients with acute myeloid leukemia. Our findings suggest that the activation of pp60$^{c\text{-}src}$ kinase activity is a normal physiological event associated with myeloid differentiation.**

The pp60$^{c\text{-}src}$ protein has been found in both normal and transformed cells of all species examined. In fibroblasts, there is relatively little pp60$^{c\text{-}src}$, roughly 1000–10,000 molecules per cell, and it is found associated with membranes exposed to the cytosol (reviewed in ref. 1). Although the role of pp60$^{c\text{-}src}$ in these cells has yet to be elucidated, there is growing evidence to suggest that it functions in the transduction of signals arising from the stimulation of cells by growth-controlling factors. It is known, for instance, that the addition of platelet-derived growth factor (PDGF) to quiescent fibroblasts in culture results in phosphorylation of pp60$^{c\text{-}src}$ on an amino-terminal tyrosine, which in turn increases its kinase activity *in vitro* (2). Two quite different tumor viruses apparently transform fibroblasts by altering pp60$^{c\text{-}src}$ activity. Polyoma virus, a DNA tumor virus, produces a transforming protein called middle-sized tumor (middle T) antigen, which forms a tight noncovalent complex with pp60$^{c\text{-}src}$ (3). The specific activity of this complex in phosphorylating tyrosine substrates *in vitro* is 50-fold higher than that of the noncomplexed pp60$^{c\text{-}src}$, and phosphorylation of the src protein is similar to that resulting from PDGF addition (4). The transforming protein of Rous sarcoma virus (RSV), denoted pp60$^{v\text{-}src}$, is a modified form of pp60$^{c\text{-}src}$, which has a higher specific activity as a tyrosine kinase (1, 5, 6). The increased kinase activity of pp60$^{v\text{-}src}$ and middle T antigen-modified pp60$^{c\text{-}src}$ correlates with their transforming ability, suggesting that they may transform cells in part by sending cellular signals corresponding to growth factor addition in the absence of growth factors.

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High levels of pp60$^{c\text{-}src}$ kinase activity have been found in neural retina cells and neurons (7, 8). Since these are nondividing cells, pp60$^{c\text{-}src}$ is presumably not functioning in proliferation in response to growth factors. One possibility is that it has an analogous role in differentiated neurons as in fibroblasts—functioning in signal transduction for various receptor molecules. Rat pheochromocytoma cell line PC12, which can be induced to differentiate into neuron-like cells by nerve growth factor, can also be induced to differentiate by RSV infection (9), further suggesting that the activation of pp60$^{c\text{-}src}$ is an integral part of the neuronal differentiation pathway.

That the activation of pp60$^{c\text{-}src}$ may play a role in cellular differentiation was also suggested by our observation of the induced differentiation of the mouse myelomonocytic cell line M1 into monocytes, as measured by the expression of the myeloid-specific differentiation marker Mac-1 (unpublished observations), when a recombinant retrovirus encoding polyoma middle T antigen was introduced. Since middle T antigen has been shown to specifically activate pp60$^{c\text{-}src}$ in fibroblasts (10), we theorized that the activation of pp60$^{c\text{-}src}$ may be a normal event during myeloid differentiation. To test this hypothesis, we measured the levels of pp60$^{c\text{-}src}$ during the induced differentiation of two human myeloid leukemia cell lines, HL-60 and U-937. HL-60 cells are promyelocytes that can be induced to differentiate into either monocytes/macrophages or granulocytes *in vitro* in response to a number of agents. U-937 cells are monoblasts that can only differentiate along the monocyte/macrophage pathway (reviewed in ref. 11).

MATERIALS AND METHODS

Cells and Culture Conditions. HL-60 and U-937 cells were grown in RPMI 1640 medium (GIBCO) supplemented with 20% or 10% heat-inactivated fetal calf serum (Hazelton, PA), respectively/2 mM glutamine/50 μM 2-mercaptoethanol/5 μg of gentamycin per ml. Cells were grown at a density of 2–8 × 10^5 per ml and were activated to differentiate with various reagents dissolved in dimethyl sulfoxide or ethanol. The final concentration of solvent in the culture medium was 0.01% or less and had no apparent effect on the cells. Psi-104 cells were derived from mouse fibroblast Psi-2 cells (12) transfected with a plasmid containing the polyoma virus early region and the neomycin-resistance gene, provided by W. Morgan (Dana–Farber Cancer Institute). GM4980 human foreskin fibroblast cells were provided by C. D. Stiles (Dana–Farber Cancer Institute).

Abbreviations: middle T antigen, middle-sized tumor antigen; RSV, Rous sarcoma virus; PMA, phorbol 12-myristate 13-acetate; GM-CSF, granulocyte/macrophage colony-stimulating factor; cfu, colony-forming unit(s).

Activation and Analysis of Cellular Differentiation. HL-60 and U-937 cells were treated with a variety of inducers. Differentiation was assayed by morphological alterations, such as adherence to plastic and formation of pseudopodia, as observed at the light microscope level and by fluorescence flow cytometry using mouse monoclonal antibody LM2/1 against the human macrophage differentiation antigen Mac-1 (Mo1) as described (13).

Analysis of Cellular src Protein Products. Immunoprecipitations, protein kinase assays, and $NaDodSO_4$/PAGE were performed as described by Kaplan *et al.* (14) using mouse monoclonal antibodies GD11 (15) and 527 (7), specific for $pp60^{c\text{-}src}$.

RESULTS

HL-60 and U-937 cells were induced to differentiate in culture with phorbol 12-myristate 13-acetate (PMA) in 0.01% dimethyl sulfoxide. After treatment, the cells became adherent to the plastic culture dish and were shown by fluorescence flow cytometry to express the myeloid differentiation antigen, Mac-1, as expected for differentiated cells. Control cells treated with 0.01% dimethyl sulfoxide alone neither became adherent nor expressed Mac-1 (data not shown). Cells were lysed 72 hr after the addition of PMA or 0.01% dimethyl sulfoxide and immunoprecipitated with a monoclonal antibody specific for $pp60^{c\text{-}src}$ (GD11) (15). The kinase assays were performed *in vitro* using rabbit muscle enolase as a phosphorylation substrate for $pp60^{c\text{-}src}$. The PMA-treated U-937 and HL-60 cells (Fig. 1*A*, lanes 3 and 5, respectively), yielded 20-fold more GD11-immunoprecipitable kinase activity than did an equal number of untreated cells (lanes 2 and 4). Polyoma-transformed fibroblasts were used as a positive control for $pp60^{c\text{-}src}$ (lane 1). Phosphoamino acid analyses have shown that enolase was phosphorylated at tyrosine (data not shown). The increase in enolase kinase activity paralleled the increase in phosphate incorporation by the $pp60^{c\text{-}src}$ protein.

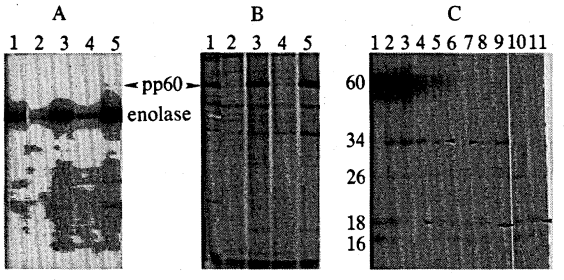

FIG. 1. Immunoprecipitation of $pp60^{c\text{-}src}$ from induced and uninduced myeloid cell lines. (*A*) Phosphorylation of exogenous substrate enolase. (*B*) [^{35}S]Methionine labeling *in vivo*. (*C*) Partial proteolytic peptide mapping. (*A* and *B*) HL-60 or U-937 cells (5×10^6) with or without PMA treatment (10 ng/ml and 2 ng/ml, respectively) for 72 hr were assayed. Cells were lysed and immunoprecipitated with $pp60^{c\text{-}src}$-specific monoclonal antibody GD11. The immunoprecipitates were assayed for the ability to phosphorylate the exogenous substrate enolase (*A*). In *B*, cells were labeled for 4 hr with [^{35}S]methionine (300 μCi/ml; 1 Ci = 37 GBq) in methionine-free medium and the amounts of $pp60^{c\text{-}src}$ were assayed. Lanes: 1, Psi-104 cells, a polyoma-transformed mouse fibroblast line used as a positive control for enolase phosphorylation; 2 and 4, U-937 and HL-60 cells, respectively, without treatment; lanes 3 and 5, U-937 and HL-60 cells treated with PMA. (*C*) HL-60 cells (1×10^7) without treatment or treated with PMA (10 ng/ml) for 15 min or 17 hr were labeled *in vivo* with [^{32}P]phosphoric acid (1 mCi/ml) in phosphate-free medium for 4 hr at 37°C before immunoprecipitation with GD11. The labeled $pp60^{c\text{-}src}$ was isolated and V8 protease mapping was performed as described by Cleveland *et al.* (16) and electrophoresed on a $NaDodSO_4$/15% polyacrylamide gel. Normal human fibroblast GM4980 without or with PMA treatment (100 ng/ml for 40 min) and digested with 0.1 μg of V8 protease per ml was used as control (lanes 10 and 11, respectively). Uninduced HL-60 cells were digested with 0.1, 0.01, and 0 μg of V8 protease per ml (lanes 7–9); HL-60 cells induced for 15 min (lanes 4–6, in increasing V8 protease concentrations); HL-60 cells induced for 17 hr (lanes 1–3, in decreasing protease concentrations).

The increase in $pp60^{c\text{-}src}$ kinase activity during PMA-induced myeloid differentiation may have resulted either from an increase in the expression of the cellular *src* gene(s) or from an increase in the kinase specific activity of the $pp60^{c\text{-}src}$ already present in the undifferentiated cells. To characterize this increase, U-937 and HL-60 cells, with or without PMA treatment for 72 hr, were labeled with [^{35}S]methionine, lysed, and immunoprecipitated with GD11. Analysis by scintillation counting of the excised 60-kDa band showed a 20-fold increase in the levels of $pp60^{c\text{-}src}$ protein in the activated U-937 and HL-60 cells (Fig. 1*B*, lanes 3 and 5) compared with the nonactivated cells (lanes 2 and 4). Lane 1 is a positive control for $pp60^{c\text{-}src}$ as in Fig. 1*A*. Thus, the increased levels of $pp60^{c\text{-}src}$ protein easily account for the increase in the phosphorylation of the protein observed in the autokinase assays.

In fibroblasts, the addition of PMA causes the appearance of a new amino-terminal serine phosphorylation without changing the specific activity of the $pp60^{c\text{-}src}$ tyrosine kinase activity *in vitro* (17). This structural alteration is manifested as a retardation in the mobility of the amino-terminal peptide of $pp60^{c\text{-}src}$ on $NaDodSO_4$/PAGE. To determine whether a similar change is occurring on PMA treatment of myeloid cells, HL-60 pretreated with PMA for 15 min and 17 hr were labeled *in vivo* with [^{32}P]phosphoric acid. The labeled $pp60^{c\text{-}src}$ was immunoprecipitated with GD11, isolated from the polyacrylamide gel, and digested with *Staphylococcus* V8 protease. There was a similar change in the mobility of the three amino-terminal V8-digested $pp60^{c\text{-}src}$ peptides (34, 18, and 16 kDa) on addition of PMA to normal human fibroblasts (Fig. 1*C*, lane 11) and to myeloid cells (lanes 1–6). The V8-digested $pp60^{c\text{-}src}$ peptides from uninduced fibroblasts and HL-60 cells are in lanes 10 and 7–9, respectively. Phosphoamino acid analyses have shown that PMA also causes a new amino-terminal serine phosphorylation of $pp60^{c\text{-}src}$ in myeloid cells (data not shown). This amino-terminal modification occurred within 15 min of PMA addition and persisted in the presence of PMA; therefore, it did not appear to affect the level of $pp60^{c\text{-}src}$ kinase activity in these cells.

The time course of $pp60^{c\text{-}src}$ expression during PMA activation was examined to determine whether the increase in $pp60^{c\text{-}src}$ kinase activity was an early or late event in myeloid differentiation. HL-60 cells were pretreated with PMA for various lengths of time and the levels of $pp60^{c\text{-}src}$ autokinase activity were measured (Fig. 2). There was no significant increase in the $pp60^{c\text{-}src}$ autokinase activity (lanes 1–3) until 14 hr after the addition of PMA. Then it increased progressively and reached a plateau at 72 hr (lanes 4–7). By 96 hr, the kinase activity began to decrease (lane 8). This is due to cell death resulting from terminal differentiation.

To ascertain whether the increase in $pp60^{c\text{-}src}$ was PMA specific or dependent on cellular differentiation, HL-60 and U-937 cells were treated with other inducers of myeloid differentiation (Fig. 3). U-937 cells were treated with human granulocyte/macrophage colony-stimulating factor (GM-CSF) (lane 8) and PMA (lane 9) for 72 hr; HL-60 cells with either human GM-CSF (lane 6), $1,25(OH)_2D_3$, a dihydroxyl derivative of vitamin D_3 (lane 5), PMA (lane 4) for 72 hr, or 1% dimethyl sulfoxide for 8 days (lane 2). By autokinase assays, $pp60^{c\text{-}src}$ levels were elevated in HL-60 and U-937 cells as they are differentiated by all of these reagents but not in an equal number of cells in the mock activations (lanes 1, 3, 7, and 10). This finding further supports the hypothesis that

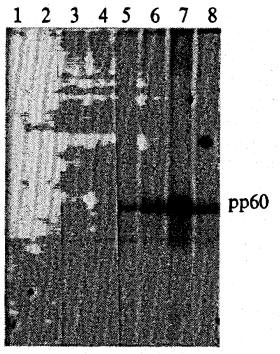

FIG. 2. Time course of PMA induction of pp60$^{c\text{-}src}$ kinase activity. HL-60 cells were treated with 10 ng of PMA per ml for various lengths of time. Mouse monoclonal antibody 527 was used to immunoprecipitate pp60$^{c\text{-}src}$ from the cell lysates. The immunoprecipitates were tested for autokinase activity. Lanes 1–8, no addition, 20 min, 10, 14, 24, 48, 72, and 96 hr of PMA treatment.

the increase in pp60$^{c\text{-}src}$ kinase activity is a generalized phenomenon associated with myeloid differentiation rather than a PMA-specific occurrence.

Because it is possible that the increase in pp60$^{c\text{-}src}$ kinase activity may be limited to the differentiation of leukemic cell lines, we measured the levels of pp60$^{c\text{-}src}$ kinase activity in normal peripheral blood leukocytes and bone marrow cells (Fig. 4 *A* and *B*). The levels of pp60$^{c\text{-}src}$ autokinase activity in total and platelet-free blood monocytes (Fig. 4*A*, lanes 1 and 2) were similar, demonstrating that the elevated level of pp60$^{c\text{-}src}$ kinase activity is endogenous to monocytes rather than due to platelet contamination. The level of pp60$^{c\text{-}src}$ kinase activity per monocyte is comparable to that found in HL-60 cells after 72 hr of PMA treatment (data not shown) and was notably higher than that found in peripheral blood granulocytes (Fig. 4*A*, lane 3), E-rosetting T lymphocytes (Fig. 4*A*, lane 4), and uninduced HL-60 cells (Fig. 4*B*, lane 3). The mixture of B lymphocytes and natural killer cells exhibited an elevated level of pp60$^{c\text{-}src}$ kinase activity, which is one-half to one-third that of the monocytes (Fig. 4*A*, lane 5). When normal bone marrow cells were fractionated into

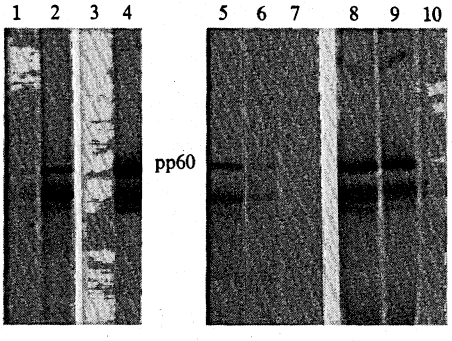

FIG. 3. Induction of pp60$^{c\text{-}src}$ kinase activity by reagents that activate myeloid differentiation. U-937 cells (5×10^6) with no treatment (lane 10) or treated with 2 ng of PMA per ml (lane 9) and human GM-CSF (lane 8) for 72 hr. The concentration of GM-CSF is in 24-fold excess of the saturating level necessary to induce colony formation. HL-60 cells (5×10^6) uninduced (lanes 3 and 7) or activated to differentiate in the monocyte pathway with 10 ng of PMA per ml (lane 4), with 0.1 μM 1,25(OH)$_2$D$_3$ (lane 5), or with human GM-CSF (lane 6) for 72 hr. HL-60 cells (1×10^7) were untreated (lane 1) or activated to differentiate in the granulocyte pathway with 1% dimethyl sulfoxide for 8 days (lane 2). Note that the relative level of increase in pp60$^{c\text{-}src}$ kinase activity between lanes 1 and 2, which correspond to the granulocyte pathway, is much less than that between lanes 3 and 4, which correspond to the monocyte pathway.

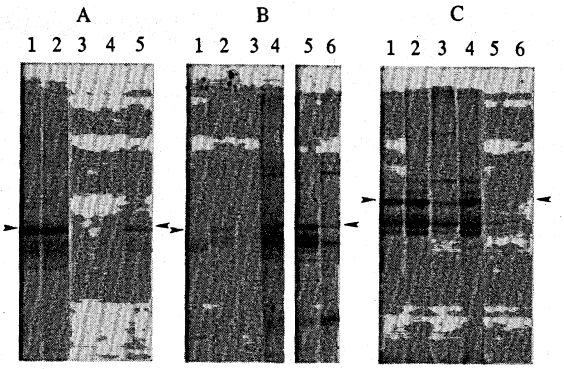

FIG. 4. The levels of pp60$^{c\text{-}src}$ kinase activity in peripheral blood leukocytes (*A*), normal bone marrow cells (*B*), and acute myeloid leukemia cells (*C*). Normal bone marrow cells were fractionated into myeloid progenitor cells and adherent monocytes (18). Granulocytes, monocytes, E-rosetting T lymphocytes, and a mixture of B lymphocytes and natural killer cells were purified from normal peripheral blood as described by Griffin *et al.* (19). Blood monocytes were rendered platelet-free by extensive washes in the presence of 5 mM EDTA. After washing, there were <1% platelets remaining on the monocytes as assayed by binding with anti-platelet antibody (anti-PLT-1) (20). Leukemic cells from patients with acute myeloblastic leukemia were cryopreserved at diagnosis and later thawed for analysis in the presence of DNase I (100 μg/ml) (19). Bone marrow cells (3×10^6, except when noted otherwise), blood leukocytes (5×10^6), and patient cells (1×10^7) were assayed for pp60$^{c\text{-}src}$ autokinase activity. (*A*) Lanes: 1 and 2, total monocytes and platelet-free monocytes, respectively; 3, granulocytes; 4, E-rosetting T lymphocytes; 5, a mixture of B lymphocytes and natural killer cells. (*B*) Lanes: 1, marrow precursor cells; 2, unseparated bone marrow cells; 3, uninduced HL-60 cells; 4, adherent bone marrow cells; 5, partially differentiated marrow precursor cells; 6, uninduced HL-60 cells. Note that there are 1.5×10^6 cells in lane 5 and 3×10^6 cells in all other lanes in *B*. (*C*) Lanes: 1, M2 leukemia cells; 2 and 4, M4 leukemia cells; 3, M5a leukemia cells; 5 and 6, M1 leukemia cells.

granuloyte-monocyte precursor cells and adherent monocytes, the results were identical. On a per cell basis, the precursor cells (Fig. 4*B*, lane 1) have the same amount of pp60$^{c\text{-}src}$ kinase activity as uninduced HL-60 cells (Fig. 4*B*, lane 3); the adherent monocytes were >10-fold higher in pp60$^{c\text{-}src}$ kinase activity (Fig. 4*B*, lane 4); and the unfractionated bone marrow cells showed an intermediate amount of pp60$^{c\text{-}src}$ (Fig. 4*B*, lane 2). The purified marrow precursor cells were allowed to differentiate in culture. After 5 days, the resulting population of cells (Fig. 4*B*, lane 5) was 5- to 10-fold higher in pp60$^{c\text{-}src}$ kinase activity than the uninduced HL-60 cells (Fig. 4*B*, lane 6). (Note that there are twice as many uninduced HL-60 cells as differentiated marrow cells.) This population of cells exhibited one-half to one-third as much pp60$^{c\text{-}src}$ as the peripheral blood monocytes (Fig. 4*B*, lane 4) because it still contains morphologically undifferentiated cells. After 2 weeks in culture, the marrow cells have differentiated into adherent monocytes. The pp60$^{c\text{-}src}$ kinase activity in these cells is >20-fold higher than that in the HL-60 cells (data not shown). These data support the notion that the increase in c-src expression is a normal physiological event associated with myeloid differentiation.

The levels of pp60$^{c\text{-}src}$ kinase activity in a panel of human myeloid leukemia cells were assayed. The acute myeloid leukemia cases were subclassified according to their stages of differentiation by standard morphological and cytochemical methods specified by FAB criteria (21). M1s are undifferentiated myeloid leukemia cells; M2s are myeloid cells with partial granulocytic differentiation; M4s are myelomonocytic cells; M5as are undifferentiated monoblasts. The levels of

$pp60^{c\text{-}src}$ autokinase activity in the leukemic cells correlate with their stages of monocytic differentiation. The M1s (Fig. 4*C*, lanes 5 and 6) and M5as (lane 3), which are both undifferentiated cells, exhibited the lowest amount of $pp60^{c\text{-}src}$ kinase activity; the M4 cells (lanes 2 and 4), which are fully differentiated, demonstrated the highest level. The M2 cells (lane 1), which are partially differentiated, exhibited slightly less $pp60^{c\text{-}src}$ kinase activity than the M4 cells. Interestingly, the M2 and M4 cells could be further distinguished by the appearance of a 64-kDa phosphoprotein specific for the M4 cells (lanes 2 and 4).

DISCUSSION

We have detected significant changes in the levels of kinase activity of $pp60^{c\text{-}src}$ during the differentiation of the myeloid cell lines HL-60 and U-937. A 20-fold increase in the $pp60^{c\text{-}src}$ kinase activity, as measured either by autophosphorylation or phosphorylation of exogenous substrate enolase, was found to accompany PMA-induced myeloid differentiation *in vitro*. This increase in kinase activity can be accounted for by an increase in the amount of $pp60^{c\text{-}src}$ protein present in the differentiated cells. The increase in $pp60^{c\text{-}src}$, which accompanies the differentiation of tumor cells in culture, mimics the normal physiological phenomenon. When nonleukemic bone marrow cells and peripheral blood leukocytes were examined, the myeloid progenitors exhibited a low level and the adherent monocytes exhibited a high level of $pp60^{c\text{-}src}$. The progenitor cells were allowed to differentiate in culture. We found the same increase in the $pp60^{c\text{-}src}$ kinase activity in the resulting monocytes/macrophages, demonstrating that the activation of $pp60^{c\text{-}src}$ kinase activity is a normal physiological event associated with myeloid differentiation. This is further supported by the data on acute myeloid leukemia. The levels of $pp60^{c\text{-}src}$ kinase activity in these tumor cells correlate with their degrees of monocytic differentiation.

The enhancement of $pp60^{c\text{-}src}$ kinase activity during myeloid differentiation was elicited by various inducers. Agents that cause differentiation in either the monocytic or granulocytic pathway increased $pp60^{c\text{-}src}$ kinase levels. The time course of PMA induction was slow, with pronounced increases seen only after 10–14 hr, reaching a plateau after 72 hr. We have not yet determined whether these changes in $pp60^{c\text{-}src}$ protein levels are controlled at the level of transcription or by some other mechanism affecting, for instance, protein stability, but Gonda *et al.* (22) have reported a high level of $pp60^{c\text{-}src}$ mRNA in avian spleens and macrophages.

These findings suggest an explanation for a previous observation that RSV transforms most cell types efficiently with the exception of macrophages (23). Lipsich *et al.* (24) have reported that mature avian macrophages are not transformed by RSV even though high levels of $pp60^{v\text{-}src}$ are produced in the infected cells. Since the activated kinase activity exhibited by $pp60^{v\text{-}src}$ has been shown to be necessary for transformation by RSV, the inability of RSV to transform macrophages may be due to the high level of $pp60^{c\text{-}src}$-related tyrosyl kinase activity already present in these cells. Studies on genetically engineered fibroblasts that overexpress the c-*src* gene show that increasing the levels of $pp60^{c\text{-}src}$ protein does not result in the same changes in phosphorylation of cellular proteins as seen in cells expressing $pp60^{v\text{-}src}$ (6). Thus, it will be interesting to see if the phosphorylation substrates of $pp60^{c\text{-}src}$ and $pp60^{v\text{-}src}$ *in vivo* are similar in myeloid cells.

The increase in $pp60^{c\text{-}src}$ protein and its associated kinase activity may have an active role in myeloid differentiation or it may be a consequence of it. Before we discuss this point, it is helpful to review other examples of regulated expression of cellular oncogenes during myeloid differentiation. Two nuclear proteins, c-myc and c-myb, are thought to be involved in the maintenance of the proliferative state of immature myeloid cells. Upon induction of differentiation, the normally high expression of the genes encoding these two proteins decreases (25). In turn, there is a rapid transient activation of the expression of the c-*fos* gene within the first 15 min of induction to differentiate in the monocyte pathway (25–27). However, this rapid activation of the c-*fos* gene appears to be PMA specific; other inducers of myeloid differentiation do not elicit the same response. Approximately 6–12 hr after induction, the proto-oncogene c-*fms*, which encodes a protein homologous with or identical to the receptor for CSF-1, is induced (28, 29). When CSF-1 binds to the receptor, it is internalized and destroyed. The expression of the *c-fms* gene product has an obvious role in myeloid differentiation, as the commitment of the monocyte-progenitor cells and the regulation of the growth, proliferation, and differentiation of monocytes may be dependent on the concentration of CSF-1 (30).

Since the enhancement of the $pp60^{c\text{-}src}$ kinase activity occurs late (10–14 hr after the addition of PMA) and since normal peripheral blood monocytes are high in $pp60^{c\text{-}src}$ activity, it seems likely that the protein is functioning in the differentiated state. Several receptors, including the *fms* gene product, the Fc receptors, and various complement receptors appear on the cell surface during monocyte differentiation, and $pp60^{c\text{-}src}$ may play a role in signal transduction for these molecules. The c-*src* gene product might also have a role at an earlier stage in the differentiation pathway. We cannot yet rule out a small transient activation of $pp60^{c\text{-}src}$ within 20 min after PMA addition. The notion that $pp60^{c\text{-}src}$ may have a role in the activation of cellular differentiation is suggested by our observation that the middle T antigen of polyoma virus, a known activator of $pp60^{c\text{-}src}$, induced the differentiation of the mouse myelomonocytic cell line M1 into monocytes as assayed by the appearance on the cell surface of the myeloid differentiation antigen Mac-1. However, the mechanism by which middle T antigen affects myeloid differentiation remains to be elucidated. Whether the c-*src* gene product plays a role in the function of monocytes or in their differentiation, the system we described should prove valuable in the study of this protein.

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