Proc. Natl. Acad. Sci. USA
Vol. 87, pp. 6738–6742, September 1990
Biochemistry

A point mutation at tyrosine-809 in the human colony-stimulating factor 1 receptor impairs mitogenesis without abrogating tyrosine kinase activity, association with phosphatidylinositol 3-kinase, or induction of c-*fos* and *junB* genes

(c-*fms* protooncogene/signal transduction/cell transformation)

MARTINE F. ROUSSEL*†, SHEILA A. SHURTLEFF*‡, JAMES R. DOWNING*§, AND CHARLES J. SHERR*†‡¶

Departments of *Tumor Cell Biology and §Pathology, and ‡Howard Hughes Medical Institute, Saint Jude Children's Research Hospital, Memphis, TN 38104; and †Department of Biochemistry, University of Tennessee College of Medicine, Memphis, TN 38163

Communicated by Hidesaburo Hanafusa, June 27, 1990 (received for review May 17, 1990)

ABSTRACT **Substitution of phenylalanine for tyrosine-809 in the human colony-stimulating factor 1 receptor (CSF-1R) inhibited its ability to transduce ligand-dependent mitogenic signals in mouse NIH 3T3 cells. When combined with an "activating" mutation at codon 301 that induces constitutive CSF-1R tyrosine kinase activity, the codon 809 mutation suppressed ligand-independent cell transformation. Comparative mapping of tryptic phosphopeptides from mutant and wild-type CSF-1R indicated that tyrosine-809 is a site of ligand-dependent receptor phosphorylation *in vivo*. The mutant receptor was active as a tyrosine kinase *in vitro* and *in vivo*, underwent CSF-1-dependent association with a phosphatidylinositol 3-kinase, and induced expression of the protooncogenes c-*fos* and *junB*, underscoring its ability to trigger some of the known cellular responses to CSF-1. The mutant receptor is likely to be impaired in its ability to interact with critical cellular effectors whose activity is required for mitogenesis.**

The effects of colony-stimulating factor 1 (CSF-1) on the proliferation, differentiation, and survival of mononuclear phagocytes are mediated through its binding to a single class of high-affinity receptors (CSF-1R) that have an intrinsic protein-tyrosine kinase (PTK) activity (1). CSF-1 binding leads to activation of the receptor kinase (2–4), cross-phosphorylation of receptor subunits (5), and rapid receptor downmodulation (6–8). CSF-1R PTK activity is essential for signal transduction (8) and enables mononuclear phagocytes to traverse the G_1 phase of the cell cycle (1, 9, 10). The interaction of the tyrosine-phosphorylated receptor with other cellular proteins and/or their phosphorylation on tyrosine must therefore trigger a series of biochemical events that culminate in DNA synthesis and mitogenesis. Consistent with these observations, CSF-1R variants containing "activating" mutations in their extracellular domain exhibit ligand-independent PTK activity, thereby providing sustained mitogenic signals for cell transformation (11–14).

Candidate substrates for receptor tyrosine kinases include phosphatidylinositol (PtdIns) 3-kinase (15, 16), phospholipase C-γ1 (PLC-γ1) (17–20), the *raf-1*-encoded serine kinase (21, 22), and the $p21^{ras}$ GTPase-activating protein, GAP (23–26). These proteins coprecipitate in immune complexes with the ligand-stimulated B-type platelet-derived growth factor receptor (PDGF-R), and their binding to PDGF-R can be selectively disrupted by mutation of sites of receptor "autophosphorylation" (25, 27, 28). Activation of PDGF-R might induce the translocation of these effector molecules from the cytoplasm to the plasma membrane, resulting in their allosteric activation through direct receptor binding and/or tyrosine phosphorylation.

Although CSF-1R is structurally related to B-type PDGF-R (29) and appears to be derived from a duplication of the same ancestral gene (30), it neither phosphorylates nor coprecipitates in immune complexes with PLC-γ1 (31), the *raf-1* serine kinase (J.R.D. and C.J.S., unpublished data), or GAP (26) but does undergo a ligand-dependent association with PtdIns 3-kinase (32, 33). Cross-phosphorylation of a PTK-inactive CSF-1R mutant by an enzymatically competent receptor subunit was sufficient to trigger its binding to PtdIns 3-kinase, whereas deletion of two sites of tyrosine phosphorylation (Tyr-699 and Tyr-708) within the CSF-1R "kinase insert" (KI) domain reduced but did not completely eliminate its association with this enzyme (33). Mutations at other sites of tyrosine phosphorylation within CSF-1R might therefore generate receptors that are selectively impaired in signal–response coupling. We now report that a CSF-1R mutant containing a phenylalanine-for-tyrosine substitution at codon 809 binds CSF-1, retains PTK activity, and regulates the expression of two early response genes but is severely impaired in inducing mitogenesis.

MATERIALS AND METHODS

Construction and Expression of CSF-1R[809F]. A CSF-1R (c-*fms* protooncogene) mutant containing a phenylalanine-for-tyrosine substitution at codon 809 (CSF-1R[809F]) was prepared by using the polymerase chain reaction. A *Sal* I–*Bam*HI fragment representing the 3′ end of the human c-*fms* gene (nucleotides 2398–3393; ref. 34) was cloned into M13 phage and amplified in partial reactions from two sets of primers, A to B and C to D. Primers A and D were complementary to phage sequences flanking the M13 cloning sites; primers B (antisense strand, 5′-CTTGACAATGAAGTTG-GAGTC-3′) and C (sense strand, 5′-GACTCCAACTTCAT-TGTCAAG-3′) represented complementary c-*fms* sequences bracketing Tyr-809 but programmed phenylalanine at this position (underlined). Reaction mixtures (100 μl) contained 10 mM Tris·HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% (wt/vol) gelatin, 1 μg of M13 template, 1 μM each oligonucleotide, 200 μM deoxynucleoside triphosphates, and 2.5 units of *Thermus aquaticus* (*Taq*) DNA polymerase and were incubated for 20 cycles as described (33). The products of the two reactions were then mixed and coamplified for an addi-

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: CSF-1, colony-stimulating factor 1; CSF-1R, CSF-1 receptor; PDGF, platelet-derived growth factor; PDGF-R, PDGF receptor; PtdIns, phosphatidylinositol; PLC, phospholipase C; PTK, protein-tyrosine kinase; KI, kinase insert; GAP, GTPase-activating protein.

¶To whom correspondence should be addressed.

tional 20 cycles using primers A and D. The amplified fragment was digested with *Sac* I (at c-*fms* nucleotides 2560 and 3144) and recloned into M13, and several independent clones were subjected to nucleotide sequencing analysis. A clone encoding Phe-809 was reconstructed into a full-length c-*fms* gene. Two other clones containing adventitious mutations in addition to Phe-809, involving conversion of either Val-811 or Gly-813 to serine, were assembled into intact genes and analyzed in parallel. These mutants rendered phenotypes indistinguishable from that of CSF-1R[809F].

Retroviral vectors encoding wild-type or mutant c-*fms* genes were transfected by the calcium phosphate technique into mouse NIH 3T3 cells, either alone or together with a second vector, containing human CSF-1 cDNA (11, 12). Cell lines expressing mutant receptors were obtained by cell sorting using a monoclonal antibody to human CSF-1R (12, 35). Biological and biochemical characterization of a CSF-1R mutant lacking the KI domain was described previously (33).

Other Biochemical Techniques. Procedures for metabolic labeling with [^{35}S]methionine (36) or [^{32}P]orthophosphate (4), immunoprecipitation and gel electrophoresis (36), immune complex tyrosine kinase (37) and PtdIns 3-kinase (33) assays, phospho amino acid analysis and two-dimensional mapping of tryptic phosphopeptides (8), immunoblotting with antibodies to phosphotyrosine (4), enumeration of ^{125}I-CSF-1 binding sites by equilibrium binding (12), growth of cell lines in serum-free medium (38), and Northern blot analysis of RNA (39) are described in detail in the references cited.

RESULTS

CSF-1R[809F] Is Impaired in Mitogenicity. Tyr-809 of human CSF-1R, located in an evolutionarily conserved region of the PTK domain, has a counterpart in all tyrosine kinases and is a major site of *in vivo* phosphorylation within many, but not all, family members (40). An analogous site of CSF-1-stimulated tyrosine phosphorylation was recently demonstrated in murine CSF-1R (41). We mutagenized Tyr-809 to phenylalanine and expressed CSF-1R[809F] in mouse NIH 3T3 cells that lacked endogenous CSF-1 receptors. NIH 3T3 cells expressing human CSF-1R can undergo a complete mitogenic response to CSF-1 and proliferate in serum-free medium containing purified human recombinant CSF-1 as the only added growth factor (11, 12, 38). Because mouse CSF-1 does not bind with high affinity to human CSF-1R, autocrine transformation is precluded unless human CSF-1 cDNA is coexpressed (11). Unlike results obtained with the wild-type receptor, cells cotransfected with CSF-1R[809F] and CSF-1 cDNAs did not undergo transformation (Table 1). Cell lines expressing CSF-1R[809F] at levels comparable to those expressing the wild-type gene formed at least 20-fold fewer and much smaller colonies when plated in semisolid medium containing human CSF-1 and did not proliferate in serum-free medium containing the purified growth factor (data not shown). Thus, the Phe-809 mutation severely inhibited receptor-induced signals for cell growth.

Substitution of phenylalanine for Tyr-969 in the human CSF-1R carboxyl-terminal tail enhances the efficiency of autocrine transformation (refs. 11–14 and Table 1). Tyr-969 is not a site of phosphorylation, and the manner by which sequences near the CSF-1R carboxyl terminus negatively regulate receptor function remains unclear. Cells cotransfected with CSF-1R[809F,969F] and CSF-1 cDNAs produced few foci, indicating that in combination with Phe-969, the attenuating effect of the Phe-809 mutation was incomplete. When Phe-809 was combined with an "activating" mutation (Glu-301) in the extracellular domain that enables CSF-1R to induce ligand-independent cell transformation (12, 14), the doubly mutated receptor induced no foci. Enhanced ligand-independent focus formation induced by CSF-1R[301E,969F] was also suppressed by the Phe-809 mutation (Table 1), again suggesting that it severely impaired but did not completely abrogate cell transformation.

Table 1. Biological activity of CSF-1R mutants in NIH 3T3 cells

CSF-1R mutant	Transformation efficiency, foci per 0.1 μg of input DNA	
	− CSF-1 cDNA	+ CSF-1 cDNA
Wild type	0	446
809F	0	0
969F	0	1074
809F,969F	0	14
301E	90	84
301E,809F	0	0
301E,969F	380	338
301E,809F,969F	6	2

Cells in 35-mm diameter culture dishes were transfected by the calcium phosphate technique with retroviral vector plasmids (100 ng) containing different c-*fms* genes, either alone (− CSF-1) or with a second vector (+ CSF-1) containing CSF-1 cDNA (100 ng) (11, 12). Sheared high molecular weight NIH 3T3 cell DNA (5 μg) was used as carrier. One day after transfection, the cells were trypsinized and replated in three 60-mm diameter dishes and fed every 3 days thereafter. Foci were counted 18–21 days after transfection. The data represent averages of quadruplicate experiments. Cotransfection of c-*fms*[301E] plus CSF-1 cDNA does not increase its transforming efficiency (14).

Tyrosine Phosphorylation Mediated by CSF-1R[809F]. The levels of the mature cell surface form of CSF-1R detected in a cell line expressing the mutant receptor were 1.5-fold higher than those detected in cells synthesizing wild-type CSF-1R (≈30,000 binding sites per cell by Scatchard analysis). Further studies revealed that CSF-1R[809F] was glycosylated and transported to the cell surface with normal kinetics, bound CSF-1 with high affinity, and underwent accelerated ligand-induced turnover, indicating that the mutation did not affect normal receptor trafficking (data not shown). The mutation did not appear to perturb CSF-1R PTK activity as assessed by several criteria. First, in immune complex kinase reactions in which receptors are phosphorylated only on tyrosine residues (2, 4), the activity of CSF-1R[809F] was indistinguishable from that of the wild-type receptor (Fig. 1*a*). Both receptors were also equally active in phosphorylating the heterologous substrate poly(Glu-Tyr) *in vitro* (data not shown). Second, CSF-1 stimulation of cells bearing either mutant or wild-type receptors induced similar levels of receptor tyrosine phosphorylation *in vivo* (Fig. 1*b*). Third, anti-phosphotyrosine immunoblots of lysates from CSF-1-stimulated cells showed that other cellular proteins were phosphorylated on tyrosine with a pattern, intensity, and kinetics similar to those induced by wild-type CSF-1R (Fig. 1*c*).

CSF-1R immunoprecipitated from cells metabolically labeled with [^{32}P]orthophosphate contains phosphoserine and acquires phosphotyrosine only after CSF-1 stimulation (4). Trypsin digestion of the mature wild-type and mutant CSF-1R glycoproteins recovered from unstimulated cells yielded four major phosphoserine-containing peptides (Fig. 2 *a* and *b*, peptides 1–4) as well as other, minor ones. After CSF-1 stimulation for 5 min at 37°C, at least six additional phosphopeptides were recovered from the wild-type receptor (Fig. 2*c*, peptides A–F). Phospho amino acid analysis indicated that each of these spots contained phosphotyrosine, although spots A, D, and E also contained lower levels of phosphoserine (data not shown). A new spot appearing above peptide 2 contained only phosphoserine. Peptide 4 was not separated from peptide A, whereas peptide 3 was masked by peptides D and E. Because tyrosine phosphorylation of a phosphoserine-containing peptide would alter its mobility,

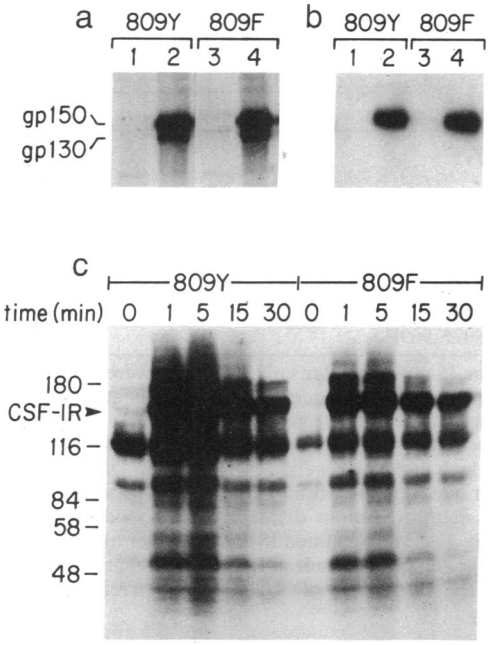

FIG. 1. Tyrosine kinase activity of CSF-1R[809F]. (*a*) Kinase assays were performed with receptor immunoprecipitates from matched lysates of cells expressing wild-type (lanes 1 and 2) or mutant (lanes 3 and 4) CSF-1R. Products of reactions with preimmune (lanes 1 and 3) or immune (lanes 2 and 4) serum were separated in denaturing polyacrylamide gels. Positions of the mature (gp150) and immature (gp130) receptor glycoproteins are indicated. (*b*) Parallel cultures expressing wild-type (lanes 1 and 2) or mutant (lanes 3 and 4) CSF-1R were stimulated for 5 min with CSF-1 (lanes 2 and 4) or were left untreated (lanes 1 and 3). Immunoprecipitated CSF-1R separated in denaturing gels was transferred to nitrocellulose and probed with an antibody to phosphotyrosine. Only gp150 contains phosphotyrosine *in vivo* (4). (*c*) Aliquots of total cell lysates from cells stimulated with CSF-1 for the indicated times were electrophoresed in a denaturing gel, transferred to nitrocellulose, and probed with antibody to phosphotyrosine. Molecular size markers (kDa) are at left.

spots A, D, and E appeared to be mixtures of phosphotyrosine- and phosphoserine-containing peptides. Peptide B was absent from maps of CSF-1R[809F] precipitated from CSF-1-stimulated cells (Fig. 2*d*), suggesting that it contained Tyr-809. Amino acid sequence data are as yet unavailable, precluding a more definitive assignment.

Because several major sites of tyrosine phosphorylation in both the murine (41, 42) and the human (33) CSF-1R occur within its KI domain, a receptor mutant lacking this region was mapped in parallel. Following CSF-1 stimulation, CSF-1R[ΔKI] was phosphorylated on tyrosine at four major sites (Fig. 2*e*, peptides B, D, F, and G). A mixing experiment confirmed that peptides B, D, and F corresponded to phosphopeptides in the wild-type receptor (Fig. 2*f*). Peptide G, present as a minor spot in maps of wild-type CSF-1R (Fig. 2*c*), was phosphorylated to a greater extent in CSF-1R[ΔKI] molecules (Fig. 2*e*). The absence of phosphopeptides A, C, and E suggested that these originated from the KI domain. Consistent with the assignment of Tyr-809 to peptide B, its phosphorylation was unaffected by the KI deletion.

CSF-1R[809F] Associates with PtdIns 3-Kinase and Regulates c-*fos* and *junB*. Ligand stimulation of CSF-1R induces an interaction with a lipid kinase that phosphorylates PtdIns at the D-3 position of inositol (32, 33). Its association is triggered by tyrosine phosphorylation of CSF-1R and is significantly reduced for mutant receptors lacking the KI domain (33). In the absence of CSF-1, increased levels of PtdIns 3-kinase were detected in immunoprecipitates containing constitutively activated CSF-1R mutants, whereas lipid kinase activity did not associate with tyrosine kinase-defective receptors unless they were phosphorylated on tyrosine in trans (33). Because the levels of PtdIns 3-kinase directly correlated with the mitogenic potential of these receptor variants, we considered that CSF-1R[809F] might not associate with this enzyme. However, following CSF-1 stimulation and cell lysis, immunoprecipitates containing CSF-1R[809F], prepared with antibodies directed to receptor epitopes or to phosphotyrosine, contained as much PtdIns 3-kinase activity as those from cells expressing wild-type CSF-1R (Table 2). Thus, the association of CSF-1R with PtdIns 3-kinase is not sufficient for mitogenesis.

Because the failure of CSF-1R[809F] to induce mitogenesis was not manifested by a loss of receptor PTK activity, the expression of several "immediate early response" genes was evaluated after stimulation of quiescent cells with saturating concentrations of CSF-1. Ligand treatment rapidly induced the transient expression of both c-*fos* and *junB* mRNAs with kinetics similar to those seen in cells expressing wild-type CSF-1R (Fig. 3), underscoring the ability of the mutant receptor to transduce functional signals. Expression of these genes has been implicated in mitogenesis, but cells expressing CSF-1R[809F] remained unable to proliferate in spite of their induction.

DISCUSSION

Transduction of human CSF-1R into mouse NIH 3T3 fibroblasts can replace their PDGF and insulin-like growth factor

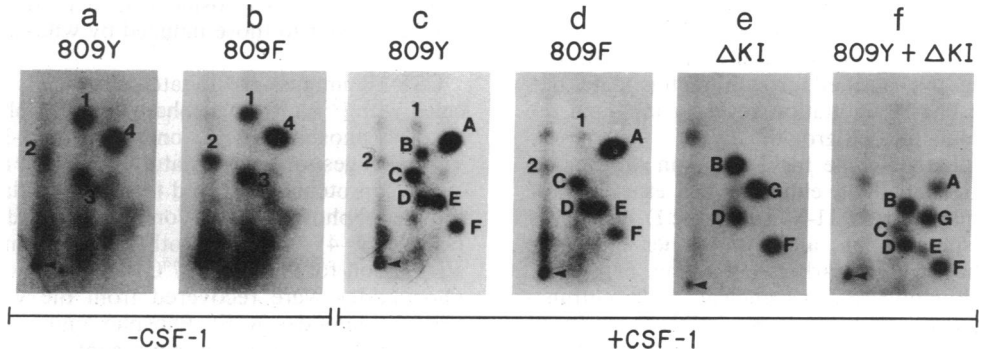

FIG. 2. Two-dimensional maps of tryptic phosphopeptides from wild-type (809Y) and mutant (809F) CSF-1R. Parallel cultures were metabolically labeled for 2 hr with [^{32}P]orthophosphate (1 mCi/ml) and stimulated where indicated for 5 min with 4 nM CSF-1. Immunoprecipitated receptors recovered from cell lysates were resolved in denaturing polyacrylamide gels, eluted, and digested with trypsin. Aliquots of the digests were applied to thin-layer plates (*a* and *b*, 1400 cpm; *c–f*, 2000 cpm). The peptides were separated by electrophoresis at pH 1.9 from left (anode) to right followed by ascending chromatography (bottom to top) (8). Arrowhead in each panel indicates the origin. Autoradiographic exposure times were 40 hr for *a* and *b* and 19 hr for *c–f*. Peptides 1–4 and A–F are discussed in the text.

Table 2. CSF-1R-associated PtdIns 3-kinase activity in immune complexes

Cell line	CSF-1 stimulation	Anti-phosphotyrosine PtdIns-3-[^{32}P]*P*, cpm	Anti-phosphotyrosine Relative activity	Anti-CSF-1R PtdIns-3-[^{32}P]*P*, cpm	Anti-CSF-1R Relative activity
Wild type	−	186	1.0	513	1.0
	+	3418	18.3	5222	10.2
809F	−	286	1.5	542	1.1
	+	3396	18.2	3554	6.9

Receptor-bearing cells were plated at 2×10^6 cells per 100-mm dish in medium containing 10% calf serum and were transferred to medium containing 0.1% serum to render them quiescent. Cells starved for serum for 24 hr were stimulated for 5 min by addition of 4.4 nM purified recombinant human CSF-1 to the same medium. Cells expressing CSF-1R[809F] expressed about 1.5-fold more CSF-1 binding sites than those expressing wild-type CSF-1R. Cell lysates adjusted for equal amounts of protein were precipitated with antibodies to phosphotyrosine or to CSF-1R, and immune complexes were assayed for PtdIns 3-kinase activity by using thin-layer chromatography to resolve the product (16, 33). Levels of enzyme activity (cpm of chromatographed product generated in a 10-min reaction at 30°C) were normalized for cell surface receptor number (33). Enzyme activities in precipitates from cells expressing wild-type CSF-1R were assigned a relative specific activity of 1.

requirements and enables the cells to proliferate in medium containing CSF-1 (11, 38). When expressed in the same cells, CSF-1R[809F] was unable to induce cell growth in response to CSF-1, even though it demonstrated ligand-dependent PTK activity, associated with PtdIns 3-kinase, and triggered expression of c-*fos* and *junB* mRNAs. Because the latter elements of the signal-transduction pathway were unperturbed by the mutation, they in themselves must be insufficient to induce mitogenesis in response to CSF-1. Conversely, CSF-1R mutants containing deletions of the KI domain, although significantly impaired in their lipid kinase binding (33), were only moderately inhibited in their ability to induce cell growth (33, 43). The pleiotropic effects of CSF-1 on mononuclear phagocyte proliferation, differentiation, and survival suggest that multiple second-messenger pathways act in concert to mediate the growth factor response (1). We propose that CSF-1R[809F] is uncoupled in its ability to interact with as yet unidentified effector molecules that transduce signals required for cell proliferation.

Major sites of ligand-induced phosphorylation within murine CSF-1R (41, 42) correspond to Tyr-699 and Tyr-708 within the KI domain of the human receptor and to Tyr-809. Comparative mapping of tryptic phosphopeptides from wild-type and mutant human CSF-1 receptors similarly suggested that Tyr-809 is a site of ligand-induced phosphorylation. Of six major phosphotyrosine-containing phosphopeptides detected in wild-type CSF-1R, only peptide B was undetected in maps of CSF-1R[809F]. Conversely, phosphopeptides A, C, and E were absent from CSF-1R[ΔKI], whereas peptide B was retained. In addition to Tyr-699 and Tyr-708, the KI domain of human CSF-1R includes another tryptic peptide, which contains Tyr-723 (34). However, tryptic peptides containing Tyr-699 and Tyr-708 are each flanked by tandem basic amino acids, suggesting that they could yield partial cleavage products accounting for additional spots. In contrast, the presence of phosphopeptides D and F in maps of both receptor mutants implies that at least one tyrosine residue other than those now mapped within murine CSF-1R is phosphorylated *in vivo* in response to ligand.

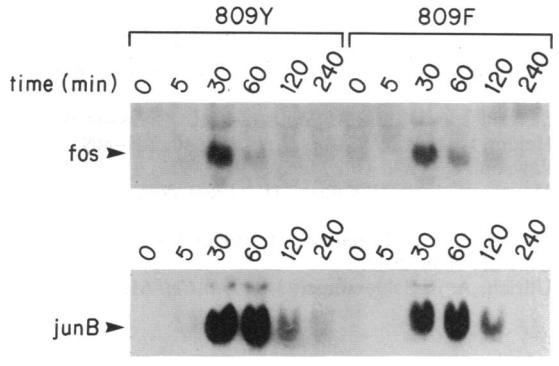

FIG. 3. Induction of c-*fos* and *junB* mRNAs in response to CSF-1. Quiescent cells maintained in medium containing 0.1% serum were treated for various times with recombinant human CSF-1. Total cellular RNA purified from cell lysates was subjected to Northern blotting analysis (39) using the indicated probes. RNAs from cells expressing wild-type or mutant CSF-1R were applied to the same gel, and equal amounts were run in each lane. Hybridization was performed for 48 hr in buffer containing 0.45 M Na^+ and 50% formamide at 37°C; filters were washed at progressively increasing stringency, ending with a 50°C wash in buffer containing 0.015 M Na^+.

How might mutation of a single tyrosine residue determine the specificity by which CSF-1R interacts with particular effectors? One possibility is that mutations within the CSF-1R intracellular domain differentially affect the catalytic efficiency of the enzyme for certain substrates. Because the patterns of ligand-induced tyrosine phosphorylation of cellular proteins were not detectably altered by the Tyr-809 mutation, putative critical substrates would have to be relatively nonabundant or phosphorylated at low stoichiometry. Alternatively, phosphorylation of Tyr-809 might trigger an association of CSF-1R with particular enzymes that are translocated to the membrane and allosterically activated through receptor binding. Their phosphorylation on tyrosine might simply result from their interaction with the receptor kinase or could, in turn, regulate their effector functions.

Tyr-809 in CSF-1R corresponds to Tyr-857 in human PDGF-R, which together with Tyr-751 in the KI domain represent major sites of PDGF-induced phosphorylation *in vivo* (27). Mutation of Tyr-857 (or its Tyr-825 homolog in murine PDGF-R) reduced PDGF-R binding to GAP by 80% (25) but did not affect its association with PtdIns 3-kinase (27, 28), whereas mutation of Tyr-751 (25) or deletion of the PDGF-R KI domain (24, 44) inhibited its binding to both molecules. Both classes of PDGF-R mutants were impaired in their ability to stimulate DNA synthesis but mediated some early responses to PDGF (44–46), consistent with the hypothesis that autophosphorylation provides signals that facilitate interactions with cellular proteins, only some of which directly contribute to mitogenesis.

What motifs in candidate effector molecules might facilitate their binding to phosphorylated receptors? Both GAP and PLC-γ1 (but not the *raf-1*-encoded kinase) contain SH2 and SH3 domains (47) that might enable them to interact with phosphotyrosine residues within particular sequence contexts. Moreover, the *crk* oncogene product, which lacks a PTK catalytic domain, contains SH2 and SH3 sequences and associates with endogenous protein kinases to induce cell

transformation (48, 49). Although proteins such as PLC-γ1, GAP, and the *raf-1* kinase bind to ligand-activated PDGF-R and might potentially interact with each other within a signal-transduction complex (26, 28, 50), the lack of direct CSF-1R binding or phosphorylation of these proteins implies that their activities are coupled to CSF-1R by an indirect mechanism or, alternatively, are not required for CSF-1-induced mitogenesis.

We thank Dr. Steven C. Clark (Genetics Institute, Cambridge, MA) for providing human recombinant CSF-1, Dr. John Cleveland for probes for c-*fos* and *junB*, Dr. Hitoshi Matsushime for assistance with RNA analysis, and Shawn Kramer, Carol Bockhold, Virgil Holder, John Kayoma, and Pamela Torrance for excellent technical assistance. This work was supported in part by U.S. Public Health Service Grant CA47064 (C.J.S.), Grant CA01429 (J.R.D.), and Cancer Center Core Grant CA21765; by the Howard Hughes Medical Institute; and by the American Lebanese Syrian Associated Charities of Saint Jude Children's Research Hospital.

1. Sherr, C. J. (1990) *Blood* **75,** 1–12.
2. Sherr, C. J., Rettenmier, C. W., Sacca, R., Roussel, M. F., Look, A. T. & Stanley, E. R. (1985) *Cell* **41,** 665–676.
3. Yeung, Y. G., Jubinsky, P. T., Sengupta, A., Yeung, D. C. Y. & Stanley, E. R. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 1268–1271.
4. Downing, J. R., Rettenmier, C. W. & Sherr, C. J. (1988) *Mol. Cell. Biol.* **8,** 1795–1799.
5. Ohtsuka, M., Roussel, M. F., Sherr, C. J. & Downing, J. R. (1990) *Mol. Cell. Biol.* **10,** 1664–1671.
6. Wheeler, E. F., Rettenmier, C. W., Look, A. T. & Sherr, C. J. (1986) *Nature (London)* **324,** 377–380.
7. Guilbert, L. J. & Stanley, E. R. (1986) *J. Biol. Chem.* **261,** 4024–4032.
8. Downing, J. R., Roussel, M. F. & Sherr, C. J. (1989) *Mol. Cell. Biol.* **9,** 2890–2896.
9. Tushinski, R. J. & Stanley, E. R. (1985) *J. Cell. Physiol.* **122,** 221–228.
10. Jackowski, S., Rettenmier, C. W. & Rock, C. O. (1990) *J. Biol. Chem.* **265,** 6611–6616.
11. Roussel, M. F., Dull, T. J., Rettenmier, C. W., Ralph, P., Ullrich, A. & Sherr, C. J. (1987) *Nature (London)* **325,** 549–552.
12. Roussel, M. F., Downing, J. R., Rettenmier, C. W. & Sherr, C. J. (1988) *Cell* **55,** 979–988.
13. Woolford, J., McAuliffe, A. & Rohrschneider, L. R. (1988) *Cell* **55,** 965–977.
14. Roussel, M. F., Downing, J. R. & Sherr, C. J. (1990) *Oncogene* **5,** 25–30.
15. Kaplan, D. R., Whitman, M., Schaffhausen, B., Pallas, D. C., White, M., Cantley, L. & Roberts, T. M. (1987) *Cell* **50,** 1021–1029.
16. Whitman, M., Kaplan, D., Roberts, T. & Cantley, L. (1987) *Biochem. J.* **247,** 165–174.
17. Wahl, M. I., Daniel, T. O. & Carpenter, G. (1988) *Science* **241,** 968–970.
18. Wahl, M. I., Olashaw, N. E., Nishibe, S., Rhee, S. G., Pledger, W. J. & Carpenter, G. (1989) *Mol. Cell. Biol.* **9,** 2934–2943.
19. Meisenhelder, J., Suh, P.-G., Rhee, S. G. & Hunter, T. (1989) *Cell* **57,** 1109–1122.
20. Margolis, B., Rhee, S. G., Felder, S., Mervic, M., Lyall, R., Levitzki, A., Ullrich, A., Zilberstein, A. & Schlessinger, J. (1989) *Cell* **57,** 1101–1107.
21. Morrison, D. K., Kaplan, D. R., Rapp, U. & Roberts, T. M. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 8855–8859.
22. Morrison, D. K., Kaplan, D. R., Escobedo, J. A., Rapp, U. R., Roberts, T. M. & Williams, L. T. (1989) *Cell* **58,** 649–657.
23. Molloy, C. J., Bottaro, D. P., Fleming, T. P., Marshall, M. S., Gibbs, J. B. & Aaronson, S. A. (1989) *Nature (London)* **342,** 711–714.
24. Ellis, C., Moran, M., McCormick, F. & Pawson, T. (1990) *Nature (London)* **343,** 377–381.
25. Kazlauskas, A., Ellis, C., Pawson, T. & Cooper, J. A. (1990) *Science* **247,** 1578–1581.
26. Kaplan, D. R., Morrison, D. K., Wong, G., McCormick, F. & Williams, L. T. (1990) *Cell* **61,** 125–133.
27. Kazlauskas, A. & Cooper, J. A. (1989) *Cell* **58,** 1121–1133.
28. Morrison, D. K., Kaplan, D. R., Rhee, S. G. & Williams, L. T. (1990) *Mol. Cell. Biol.* **10,** 2359–2366.
29. Yarden, Y., Escobedo, J. A., Kuang, W. J., Yang-Feng, T. L., Daniel, T. O., Tremble, P. M., Chen, E. Y., Ando, M. E., Harkins, R. N., Francke, U., Fried, V. A., Ullrich, A. & Williams, L. T. (1986) *Nature (London)* **323,** 226–232.
30. Roberts, W. M., Look, A. T., Roussel, M. F. & Sherr, C. J. (1988) *Cell* **55,** 655–661.
31. Downing, J. R., Margolis, B. L., Zilberstein, A., Ashmun, R. A., Ullrich, A., Sherr, C. J. & Schlessinger, J. (1989) *EMBO J.* **8,** 3345–3350.
32. Varticovski, L., Druker, B., Morrison, D., Cantley, L. & Roberts, T. (1989) *Nature (London)* **342,** 699–702.
33. Shurtleff, S. A., Downing, J. R., Rock, C. O., Hawkins, S. A., Roussel, M. F. & Sherr, C. J. (1990) *EMBO J.* **9,** 2415–2421.
34. Coussens, L., Van Beveren, C., Smith, D., Chen, E., Mitchell, R. L., Isacke, C. M., Verma, I. M. & Ullrich, A. (1986) *Nature (London)* **320,** 277–280.
35. Ashmun, R. A., Look, A. T., Roberts, W. M., Roussel, M. F., Seremetis, S. T., Ohtsuka, M. & Sherr, C. J. (1989) *Blood* **73,** 827–837.
36. Anderson, S. J., Gonda, M. A., Rettenmier, C. W. & Sherr, C. J. (1984) *J. Virol.* **51,** 730–741.
37. Roussel, M. F., Rettenmier, C. W., Look, A. T. & Sherr, C. J. (1984) *Mol. Cell. Biol.* **4,** 1999–2009.
38. Roussel, M. F. & Sherr, C. J. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 7924–7927.
39. Matsushime, H., Jinno, A., Takage, N. & Shibuya, M. (1990) *Mol. Cell. Biol.* **10,** 2261–2268.
40. Hanks, S. K., Quinn, A. M. & Hunter, T. (1988) *Science* **241,** 42–52.
41. Tapley, P. A., Kazlauskas, A., Cooper, J. A. & Rohrschneider, L. R. (1990) *Mol. Cell. Biol.* **10,** 2528–2538.
42. Van Der Geer, P. & Hunter, T. (1990) *Mol. Cell. Biol.* **10,** 2991–3002.
43. Taylor, G. R., Reedijk, M., Rothwell, V., Rohrschneider, L. & Pawson, T. (1989) *EMBO J.* **8,** 2029–2037.
44. Coughlin, S. R., Escobedo, J. A. & Williams, L. T. (1989) *Science* **243,** 1191–1194.
45. Escobedo, J. A. & Williams, L. T. (1988) *Nature (London)* **335,** 85–87.
46. Severinsson, L., Ek, B., Mellström, K., Claesson-Welsh, L. & Heldin, C.-H. (1990) *Mol. Cell. Biol.* **10,** 801–809.
47. Pawson, T. (1988) *Oncogene* **3,** 491–495.
48. Mayer, B. J. & Hanafusa, H. (1988) *Nature (London)* **332,** 272–275.
49. Mayer, B. J. & Hanafusa, H. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 2638–2642.
50. Ullrich, A. & Schlessinger, J. (1990) *Cell* **61,** 203–212.