Proc. Natl. Acad. Sci. USA
Vol. 87, pp. 7270–7274, September 1990
Biochemistry

Mechanism of kinase activation in the receptor for colony-stimulating factor 1

(signal transduction/growth factor receptor/glycophorin A)

ANGEL WAI-MUN LEE* AND ARTHUR W. NIENHUIS

Clinical Hematology Branch, National Heart, Lung and Blood Institute, National Institutes of Health, Bethesda, MD 20892

Communicated by Martha Vaughan, June 7, 1990 (received for review March 29, 1990)

ABSTRACT **Receptor tyrosine kinases remain dormant until activated by ligand binding to the extracellular domain. Two mechanisms have been proposed for kinase activation: (*i*) ligand binding to the external domain of a receptor monomer may induce a conformational change that is transmitted across the cell membrane (intramolecular model) or (*ii*) the ligand may facilitate oligomerization, thereby allowing interactions between the juxtaposed kinase domains (intermolecular model). The receptor for colony-stimulating factor 1 was used to test these models. Large insertions at the junction between the external and transmembrane domains of the receptor, introduced by site-directed mutagenesis of the cDNA, were positioned to isolate the external domain and prevent transmembrane conformational propagation while allowing for receptor oligomerization. Such mutant receptors were expressed on the cell surface, bound ligand with high affinity, exhibited ligand-stimulated autophosphorylation, and signaled mitogenesis and cellular proliferation in the presence of ligand. A second experimental strategy directly tested the intermolecular model of ligand activation. A hybrid receptor composed of the external domain of human glycophorin A and the transmembrane and cytoplasmic domains of the colony-stimulating factor 1 receptor exhibited anti-glycophorin antibody-induced kinase activity that supported mitogenesis. Our data strongly support a mechanism of receptor activation based on ligand-induced receptor oligomerization.**

Growth factor receptors such as those for insulin, epidermal growth factor (EGF), platelet-derived growth factor (PDGF), and colony-stimulating factor 1 (CSF-1) are integral membrane glycoproteins sharing a number of common structural features, including an extracellular ligand-binding domain, a stretch of 20–30 hydrophobic amino acids presumed to span the membrane once, and a large intracellular domain with canonical sequences characteristic of an ATP catalytic site (1). In the normal cell, activation of the enzymatic function results only from binding of the appropriate growth factor. Recent studies have demonstrated dimer formation for the EGF (2–5) and PDGF (6–8) receptors, although the causal relationship between receptor oligomerization and kinase activation in the intact cell has not been established. In addition, some observations suggest that kinase activation can occur in the absence of oligomerization (9–11). Whatever the activation mechanism may be, ligand binding initiates a cascade of events beginning with receptor autophosphorylation and phosphorylation of intracellular substrates, culminating in DNA synthesis and cell division.

Two models can describe the mechanism of kinase activation by ligand binding: an intramolecular or an intermolecular process (2, 12). In the simplest type of intramolecular model, a single receptor monomer constitutes a fully functional independent transducing unit, and ligand binding activates the kinase function by transmitting a conformational signal across the transmembrane domain. In the simplest type of intermolecular model, ligand binding facilitates oligomerization and, by allowing critical interactions such as transphosphorylation (13, 14) to occur between the juxtaposed cytoplasmic domains, results in kinase activation. We have directly tested these two models in intact cells using the CSF-1 receptor (CSF-1R), encoded by the protooncogene c-*fms* (15). CSF-1R is a transmembrane protein of molecular weight *ca.* 160,000 expressed on cells of the monocyte/macrophage lineage and on placental trophoblasts. Our results strongly support ligand-induced oligomerization as the mechanism for kinase activation in CSF-1R.

MATERIALS AND METHODS

cDNA Cloning and Plasmid Constructions. A 600-base-pair fragment derived from the 5′ end of a partial murine CSF-1R cDNA clone (P. Browning, E. Feingold, A.W.L., and A.W.N., unpublished data) was used to screen a murine B-cell cDNA library (16), yielding a single 3.7-kilobase clone. Sequencing established that this clone encoded only amino acids 17–977 of the published sequence (17). A synthetic oligonucleotide was used to complete the coding region including the entire published signal peptide. Site-directed mutagenesis (Amersham kit) was used to create an *Ava* I site at nucleotide 1602 in M13mp19 that converted GAT (Asp-509) to GAG (Glu). Oligonucleotides encoding the helix or polyglycine sequences were synthesized with *Ava* I ends, inserted into this site, and confirmed by DNA sequencing. To construct the hybrid receptor between human glycophorin A (GpA) and CSF-1R, a two-step polymerase chain reaction procedure (18) was utilized, the details of which are available upon request. The final plasmid contained the coding sequence for the entire external region of GpA (a 19-amino acid signal peptide and a 72-amino acid extracellular domain) and the transmembrane and cytoplasmic domains of CSF-1R, a total of 558 amino acids. To obtain an intact GpA cDNA necessary as a negative control, the codons for the missing carboxyl-terminal 5 amino acids (19) were filled in by polymerase chain reaction. All amplified segments were sequenced in their entirety.

Cellular Expression. For the insertional mutants, the receptor genes were cloned into XM6-Neo, an N2-type (20) retroviral vector containing a modified neomycin-resistance gene (21), and ψ-2 retroviral producer clones were obtained by standard techniques (22). These clones were analyzed for protein expression by saturation binding (4°C) to ^{125}I-labeled human CSF-1 (Genetics Institute). Producer clones were used to infect 32D cells (23), an interleukin 3 (IL-3)-

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, colony-stimulating factor; CSF-1R, CSF-1 receptor; EGF, epidermal growth factor; GpA, glycophorin A; IL, interleukin; PDGF, platelet-derived growth factor.

*To whom reprint requests should be addressed.

dependent murine myeloid cell line devoid of CSF-1R mRNA by Northern analysis (data not shown). G418-resistant 32D clones were isolated from methylcellulose-containing medium and analyzed by the saturation binding assay. For the wild-type GpA and the GpA–CSF-1R hybrid, the cDNAs were cloned into a myeloproliferative sarcoma virus-based retroviral vector, pM5G-Neo (24). Linearized plasmid DNA was tranfected into 32D cells by electroporation and individual G418-resistant clones were isolated from methylcellulose. The clones expressing the highest levels of GpA or GPA–CSF-1R were identified by fluorescence-activated cell sorter (Coulter) analysis with an anti-GpA monoclonal antibody, 6A7 (25).

Scatchard Analysis. Purified recombinant CSF-1 (10 μg) was iodinated using 1 mCi (37 MBq) of ^{125}I-labeled Bolton–Hunter reagent (NEN). Specific activity determined by self-displacement (26) varied between 1.4 and 3.9 $\times$ 10^7 cpm/μg. Measurements were made after 16 hr of incubation at 4°C. Data were analyzed with the program LIGAND (27).

Metabolic Labeling and Stimulation with CSF-1 or Anti-GpA Antiserum. 32D cells (1–2 $\times$ 10^7) were incubated with [^{35}S]methionine (Amersham) at 100–200 μCi/ml for 4 hr at 37°C. In pulse–chase experiments, cells were incubated with [^{35}S]methionine for 20 min and then incubated in complete medium containing 2 mM L-methionine with or without 10 nM CSF-1 for the indicated lengths of time. For labeling with [^{32}P]orthophosphate, 1–2 $\times$ 10^7 32D cells or confluent 10-cm dishes of ψ-2 cells were incubated with [^{32}P]orthophosphate (Amersham) at 0.5 mCi/ml for 2 hr at 37°C. In phosphorylation experiments examining the effects of CSF-1 addition, ligand was added at 10 nM to half of the samples for 5 min at 37°C prior to rapid quenching with ice-cold phosphate-buffered saline. In experiments with anti-GpA stimulation, rabbit antiserum (28) was added at a dilution of 1:100 and the cells were incubated for an additional 15 min at 37°C before quenching.

Immunoprecipitations. A rabbit anti-Fms antiserum, R4B15 (15), was added to total cell lysates at a dilution of 1:500 and immune complexes were precipitated with protein A-Sepharose (Pharmacia). Immunoprecipitation with 1G2 (29), an anti-phosphotyrosine antibody conjugated to agarose beads (Oncogene Science), was performed according to the manufacturer's protocol. Immune complexes were eluted with 1 mM phenyl phosphate. For double immunoprecipitations, cell lysates were first incubated with 1G2, eluted with phenyl phosphate, and then incubated with R4B15 and protein A-Sepharose. Immunoprecipitates were electrophoretically separated in either 7% linear or 5–15% gradient SDS/polyacrylamide gels. Two-dimensional phospho amino acid analysis was performed as described (30).

[^{3}H]Thymidine Incorporation. 32D cells were incubated in the appropriate medium for 18 hr at 37°C and then for an additional 4 hr with 1 μCi of [^{3}H]thymidine (NEN) per sample, after which the cells were harvested onto glass-fiber filters. In competition experiments, purified glycophorin (G5017, Sigma) was incubated with anti-GpA antiserum on ice for 1 hr prior to incubation with cells.

Proliferation Assay. 32D cells were plated in methylcellulose medium with 10% fetal bovine serum at 300 or 1000 cells per dish and were examined for colony formation under various conditions.

RESULTS

Insertional Mutants Are Expressed on the Cell Surface and Exhibit Normal CSF-1 Binding. Two types of insertional sequences were utilized. The first consisted of a highly charged segment of seven Glu-Ala repeating units (helix), which, based on secondary-structure predictions, should form 3.8 turns of an α-helix (31); the second were electrically neutral segments of 6 or 12 glycines (Polygly6 or Polygly12, respectively), predicted to assume a highly flexible configuration (31, 37) (Fig. 1*A*). Scatchard analysis was performed on intact cells and showed a single class of high-affinity sites (Table 1). The binding constants for the different receptors were very similar despite the inserted sequences in the mutants, lending credence to the view that such receptors are made of distinct structural and functional domains. In addition to normal binding, results of pulse–chase experiments (data not shown) demonstrated that the biosynthesis and the CSF-1-induced downregulation of the wild-type receptor and the two types of mutant receptors followed parallel kinetics.

Insertional Mutants Demonstrate Intact Kinase Activity in ψ-2 Cells. We metabolically labeled ψ-2 clones with [^{32}P]orthophosphate and immunoprecipitated the lysates with 1G2, an anti-phosphotyrosine antibody (Fig. 2). All three mutants exhibited CSF-1-stimulated autophosphorylation. That the indicated band at 150 kDa was indeed CSF-1R was confirmed in a separate experiment with R4B15, a rabbit antiserum directed against the cytoplasmic domain of the v-*fms* gene product (data not shown). The variation in autophosphorylation should not be taken to reflect quantitative differences in kinase activity between wild type and mutants, since the precise number of receptors expressed per cell was not determined for ψ-2 clones. Phospho amino acid analysis of the receptor bands showed that both wild-type

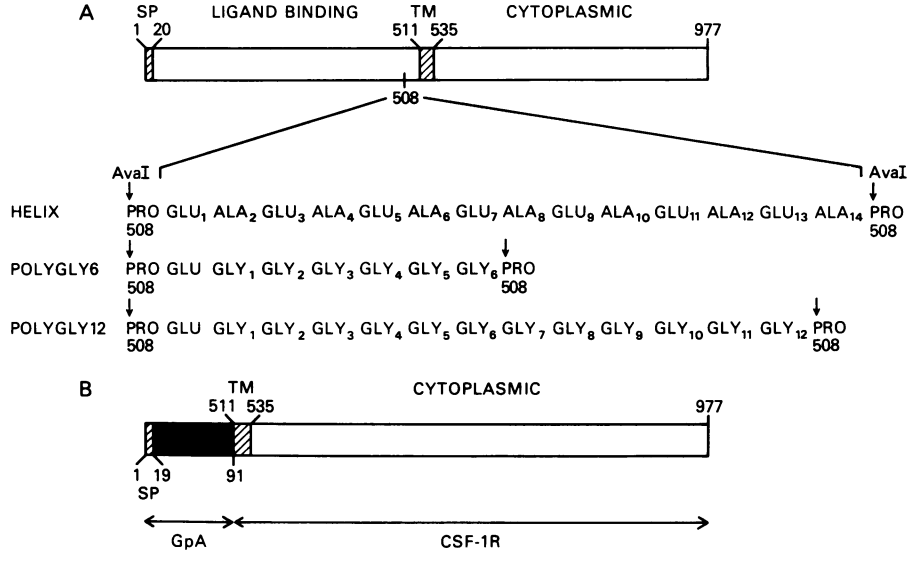

FIG. 1. Domain structure of insertional mutants and GpA–CSF-1R hybrid. (*A*) Insertional mutants. The amino acid sequences of the three insertions are given. They are just proximal to the tripeptide Pro-Asp-Glu that flanks the transmembrane domain. (*B*) GpA–CSF-1R hybrid. The signal peptide (SP) and the external domain (black reigon) originate from human GpA, while the transmembrane (TM) and cytoplasmic domains are from CSF-1R. Numbering below the bar refers to GpA, and that above to CSF-1R.

Table 1. ^{125}I-CSF-1 binding parameters for 32D clones expressing wild-type or mutant receptor

Cell line	Receptors per cell, no. × 10^{-4}	$K_a \times 10^{-9}$, M^{-1}
WT-1	1.3 ± 0.2	16.0 ± 9.0
WT-2	2.4	19.3
		(17.5 ± 8.0)
Hx-1	3.3 ± 0.2	5.7 ± 0.7
Hx-2	8.6	8.0
		(6.9 ± 1.2)
PG12-1	1.0 ± 0.1	8.7 ± 0.7
PG12-2	1.0	10.4
		(9.6 ± 1.0)

Receptor numbers and affinity constants (K_a) were derived from least-squares analysis of Scatchard data with the program LIGAND (27). All experimental points were performed in duplicate. Nonspecific binding, estimated in the presence of an at least 25-fold excess of unlabeled CSF-1, was <5% of bound counts in all cases except at concentrations >2 nM. For clone 1 of each receptor type, standard deviations were determined from the results of two independent experiments. Clones 1 and 2 were independently selected. Average values for clones expressing wild-type receptors (WT), receptors with the helix insertion (Hx), or receptors with the 12-glycine insertion (PG12) are shown in parentheses.

and mutant CSF-1Rs were phosphorylated on serines alone in the basal state, and that phosphotyrosine appeared only with CSF-1 stimulation (data not shown).

Insertional Mutants Show CSF-1-Stimulated DNA Synthesis and Proliferation. Each measurement of [^{3}H]thymidine incorporation (Fig. 3 *Upper*) was repeated at least once and for two independently selected 32D clones per construct. In the presence of CSF-1, parental 32D cells showed basal incorporation only, consistent with the absence of CSF-1R, although IL-3 in the same cells had a profound mitogenic effect. On the other hand, clones expressing either the wild-type or a mutant receptor responded to CSF-1 with an incorporation that was 75–90% of the IL-3-induced uptake, and could not be distinguished from each other. The findings of the methylcellulose proliferation assay are in agreement (Fig. 3 *Lower*). In the absence of growth factors (row A), the colonies were very small, containing mostly dead cells. In contrast, in the presence of CSF-1 (row B) or conditioned medium containing IL-3 (row C) colonies expressing either wild-type or mutant receptors were large, with hundreds of viable cells.

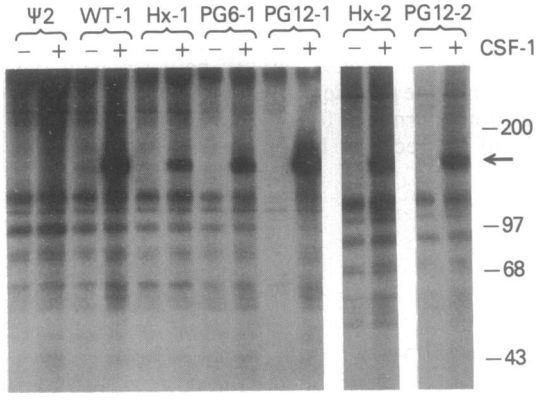

FIG. 2. CSF-1-induced autophosphorylation of wild-type and insertional mutant receptors in ψ-2 cells. Cells were metabolically labeled with [^{32}P]orthophosphate, incubated in the absence (−) or presence (+) of CSF-1, and then immunoprecipitated with 1G2, an anti-phosphotyrosine antibody. The designations 1 and 2 refer to two independently selected clones for the same construct. ψ-2, parental; WT, wild type; Hx, helix; PG6, mutant with 6 glycines; PG12, mutant with 12 glycines. WT-2 was analyzed in a separate experiment side-by-side with WT-1 and showed half as much intensity. The amount loaded per lane represents precipitated proteins in equal aliquots of lysates from confluent 10-cm dishes, not normalized for receptor content. The receptor band is indicated with an arrow. Molecular size markers (200, 97, 68, and 43 kDa) are given at right.

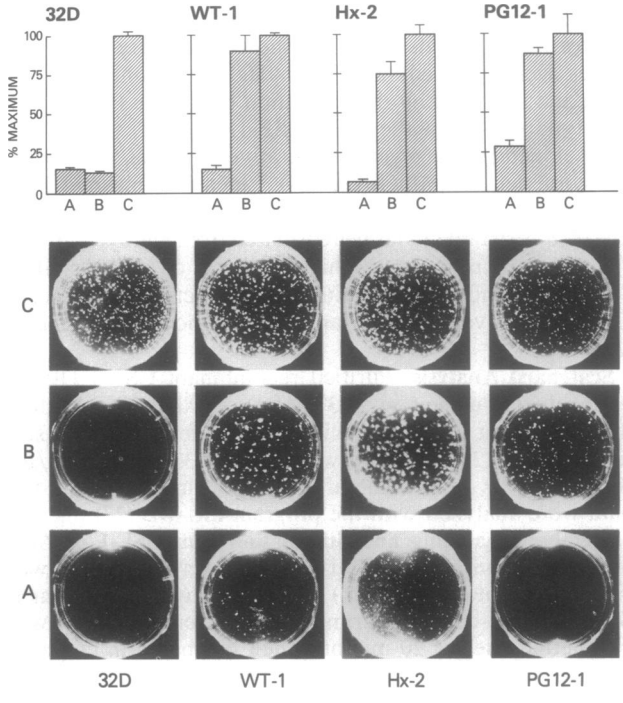

FIG. 3. (*Upper*) [^{3}H]Thymidine incorporation in 32D cells expressing wild-type and insertional mutant receptors. Conditions: bars A, regular medium; bars B, regular medium plus 3.4 nM CSF-1; bars C, regular medium plus 10% WEHI conditioned medium (source of murine IL-3). Results are given as percentages of the maximal thymidine incorporation, which for all cells occurred with WEHI conditioned medium. Each experimental point was performed in triplicate. Error bars denote 1 SD from mean. (*Lower*) Proliferation assay. Conditions: row A, regular medium; row B, regular medium plus 26 nM CSF-1; row C, regular medium plus 10% WEHI conditioned medium. Photographs were taken on day 9.

Anti-GpA Antibodies Stimulate the *in Vivo* Kinase Function of GpA–CSF-1R Hybrid in 32D Cells. The organization of the hybrid receptor is shown in Fig. 1*B*. The 32D clones expressing either the wild-type GpA (designated M or GlyM) or the GpA–CSF-1R hybrid (designated BM or Glyfms BM) were selected on the basis of their immunofluorescence intensity

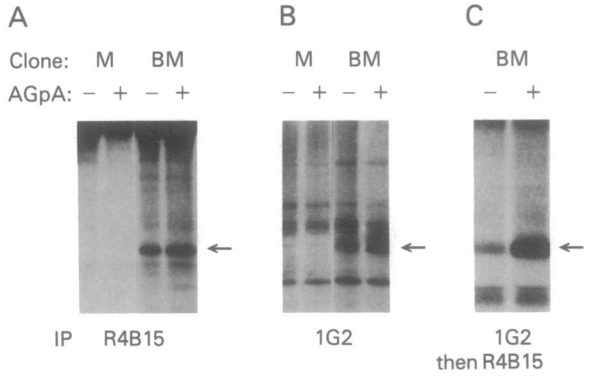

FIG. 4. Immunoprecipitation of wild-type GpA (M) and GpA–CSF-1R (BM) from 32D clones. Cells were metabolically labeled with [^{32}P]orthophosphate and incubated in the absence (−) or presence (+) of anti-GpA antiserum (AGpA). Immunoprecipitating (IP) antibody was R4B15 (anti-Fms) (*A*), 1G2 (anti-phosphotyrosine) (*B*), or 1G2 followed by R4B15 (*C*). Arrow indicates the hybrid receptor band at 84 kDa.

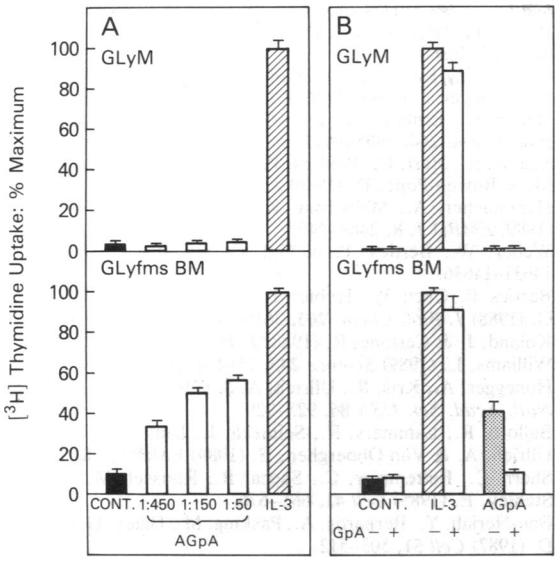

FIG. 5. [^{3}H]Thymidine incorporation in 32D clones expressing wild-type GpA (M) or GpA–CSF-1R hybrid (BM). (*A*) Titration effect of anti-GpA antiserum. CONT., regular medium only; IL-3, regular medium with 10% WEHI conditioned medium; AGpA, serial dilutions of anti-GpA antiserum in medium. (*B*) Competitive inhibition by exogenous GpA. CONT. and IL-3, as in *A*; AGpA, regular medium with 1:200 dilution of anti-GpA antiserum. Presence (+) or absence (−) of 0.32 μM GpA is indicated.

after staining with a monoclonal anti-GpA antibody (6A7) and were found to have peak intensities almost equal to that of the K562 human erythroleukemia cell line, which is known to have 10^5–10^6 GpA molecules per cell (data not shown). A polyclonal rabbit anti-GpA antiserum (28) was used to test kinase function. Immunoprecipitations of ^{32}P-labeled M and BM cells incubated with (+ lanes) or without (− lanes) anti-GpA antiserum are shown in Fig. 4. With R4B15 (anti-Fms; Fig. 4*A*), a specific band at 84 kDa was seen in both the − and + lanes. The predicted molecular mass after cleavage of the signal peptide is 60 kDa; the difference presumably comes from the addition of both O-linked (15 sites) and N-linked (1 site) oligosaccharides and is close to that estimated for GpA in K562 cells (33). With 1G2 (Fig. 4*B*), a band at the same molecular mass was more prominent in the + lane. A double immunoprecipitation was also performed (Fig. 4*C*), with 1G2 (anti-phosphotyrosine) followed by R4B15 (anti-Fms), and demonstrated unequivocally that the tyrosine kinase function of the GpA–CSF-1R hybrid can be activated by crosslinking with anti-GpA antibodies.

Anti-GpA Antibodies Induce a Mitogenic Response in the GpA–CSF-1R Hybrid. A titration effect was seen with serial dilutions of the anti-GpA antiserum (Fig. 5*A*). At the highest concentration tested, [^{3}H]thymidine uptake was almost 5 times the basal level, compared to an 8.6-fold induction with IL-3 in the same experiment. At 1:5000, induction was 75% over basal. At the same time, the anti-GpA antiserum had no effect on clone M, a 32D cell line expressing wild-type GpA only (Fig. 5*A*), or on the cell line expressing wild-type CSF-1R (data not shown). The titration curve was determined in three separate experiments and for a second independently selected M or BM clone, with similar results. The mitogenic effect is specific for the anti-GpA antibodies present in the antiserum since it was blocked by addition of purified GpA (Fig. 5*B*).

DISCUSSION

Our experiments were designed to address two specific questions. The first is whether CSF-1R transduces the ligand-binding signal by means of a conformational change propagated across the transmembrane region. The helix and polyglycine mutants contained insertions that were large (6, 12, and 14 amino acids) and heterogeneous in charge and secondary structural characteristics. To disrupt the intramolecular activation pathway, these insertions had been introduced at the junction between the extracellular ligand-binding and transmembrane domains of CSF-1R. Hydropathy plots predict only a single transmembrane domain in the tyrosine kinase receptors; both the external and the cytoplasmic domains of several receptors have been synthesized in soluble form by recombinant DNA techniques (34–36), thus excluding the existence of other significant membrane binding regions. In the presence of these insertions, ligand-induced conformational changes are unlikely to be transmitted to the transmembrane domain, since, in the case of the helix insertion, the energy cost of perturbing a thermodynamically stable α-helix would be prohibitive, and in the case of the polyglycine insertions, the flexible internal coordinates would serve as an efficient shock absorber at little energy cost. Our results show that both types of mutants are able to function normally, exhibiting CSF-1-induced kinase activation followed by DNA synthesis and cell division. This indicates that signal transduction in CSF-1R is not critically dependent on the precise nature of the link between the external and transmembrane domains. It is unlikely that our insertions were ineffective in blocking the transmission of ligand-induced conformational changes, in view of the many examples where small insertions, deletions, or even single amino acid substitutions can exert a detrimental effect if introduced at critical regions. For example, many single amino acid substitutions at the α_1/β_2 interface in hemoglobin drastically reduce cooperativity (37), and in the EGF receptor an insertion of 4 amino acids in the kinase domain renders it biologically inactive (38).

The second question we addressed is the possibility that juxtaposition of the kinase domains is a sufficient trigger for activation. To this end, we constructed a hybrid where the external domain of CSF-1R was replaced by the 72-amino acid external domain of GpA, the major sialoglycoprotein from the human erythrocyte membrane. GpA is a prototypic type I membrane glycoprotein but bears no relation to receptor tyrosine kinases. It appears to be a structural protein in red cells with no role in signal transduction; anti-GpA antibodies have no known effect on red-cell function (39, 40). Thus the GpA–CSF-1R hybrid is unlike other reported chimeras involving portions of heterologous receptors and should provide a simple system to test rigorously the crosslinking effects of anti-GpA antibodies. Our results show that binding of anti-GpA induced both tyrosine autophosphorylation of the hybrid receptor and mitogenesis in 32D cells. This mitogenic effect was (*i*) reproducibly seen with different clones expressing the hybrid receptor, (*ii*) not seen in 32D cells expressing only wild-type GpA, and (*iii*) inhibited by addition of purified GpA. Since GpA itself bears no structural similarity to CSF-1R, it is extremely unlikely that anti-GpA binding resulted in a conformational change in the external GpA domain that precisely mimicked any CSF-1-induced change in the native CSF-1R. It should be noted that a reasonably specific configuration of the receptor monomers is likely to be necessary for kinase activation, since two monoclonal antibodies that bind to GpA with high affinity (25) could not effect productive crosslinking even when second antibodies were added (data not shown). Our experimental strategy utilized the external domain of a molecule not involved in signal transduction, GpA. Our data most clearly support a model in which ligand-induced receptor oligomerization results in kinase activation. Similar conclusions have been reached by others using anti-receptor antibodies to activate native receptors (41, 42) or using chimeras

between heterologous receptors. For example, anti-T11 (CD2) antibodies activated the kinase function of a hybrid containing the external domain of the T11 antigen and the transmembrane/cytoplasmic domains of CSF-1R (43). However, anti-T11 antibodies are known to activate T cells via the T11 antigen. We believe that our studies on the GpA–CSF-1R hybrid bypass such inherent difficulties.

The receptors recently characterized for other hematopoietic growth factors (IL-3, IL-4, IL-6, IL-7, granulocyte/macrophage-CSF, granulocyte-CSF, and erythropoietin) lack sequences for any known enzymatic function in their predicted amino acid structures. It has been proposed that these receptors must interact with a second, different protein for signal transduction (44). CSF-1R is unlikely to function in this manner. It is a prototypic receptor tyrosine kinase (1) and there is no evidence that such receptors require a different protein for kinase activation. Many purified receptor tyrosine kinases exhibit ligand-induced enzymatic activation *in vitro*. Therefore, the most reasonable interpretation of our results is that CSF-1R is activated via an intermolecular mechanism involving oligomerization between identical receptor monomers. This conclusion is further supported by the recent finding that transphosphorylation between kinase-competent and kinase-defective CSF-1R can occur (45), as has been demonstrated previously for the EGF (13) and insulin/EGF hybrid (14) receptors.

Can an oligomerization model for enzyme activation be generalized to other members of the receptor tyrosine kinase class? CSF-1 is a dimer and thus clearly capable of functioning as a crosslinking agent. Similarly, PDGF, as either a homo- or a heterodimer, is also capable of inducing dimerization or oligomerization of its receptor, a molecule very similar in structure to CSF-1R. On the other hand, EGF is a monomer, as is insulin. Furthermore, the insulin receptor exists as an $\alpha_2\beta_2$ disulfide-linked heterotetramer. For the insulin receptor class, the simple model of intermolecular activation may require modification. Contacts between individual subunits could be important in kinase activation. Ligand binding may alter intersubunit interactions (which could involve any of the three domains) to facilitate closer approximation of two or more receptor molecules, leading to kinase activation. We cannot exclude the possibility that a similar phenomenon occurs when CSF-1 binds to its receptor. Indeed, transforming mutations within CSF-1R (47,48) might mimic a conformational change usually achieved only by ligand binding that favors oligomerization and thus lead to constitutive kinase activation. The ability of CSF-1 to enhance the transforming ability of v-Fms (46), the viral analog of CSF-1R, is consistent with the proposed model.

As noted, there are significant structural dissimilarities in different receptor–ligand systems. Our data are only directly applicable to CSF-1R. It may be of interest to make the type of mutants described here for the EGF and insulin receptors and test directly the role of conformational coupling between the ligand-binding and transmembrane domains.

We are indebted to G. Wong (Genetics Institute) for recombinant human CSF-1, to C. Sherr for the anti-receptor antibody, to C. Stocking for pM5G-Neo, to M. Fukuda for GpA cDNA, to R. Langlois for anti-GpA antibodies 6A7 and 10F7, and to V. Bhavanandan for anti-GpA antiserum. We are grateful to L. Davies and T. Moulton for technical assistance and to S. Anderson for flow cytometric analysis. We thank D. States, S. Brandt, I. Touw, and T. Shimada for helpful discussions, E. Feingold for help in the screening of the partial murine c-*fms* cDNA, and J. DiPersio for advice regarding the binding assay.

1. Yarden, Y. & Ullrich, A. (1988) *Annu. Rev. Biochem.* **57**, 443–478.
2. Yarden, Y. & Schlessinger, J. (1987) *Biochemistry* **26**, 1434–1442.
3. Yarden, Y. & Schlessinger, J. (1987) *Biochemistry* **26**, 1443–1451.
4. Boni-Schnetzler, M. & Pilch, P. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 7832–7836.
5. Cochet, C., Kashles, O., Chambaz, E., Borrello, I., King, C. & Schlessinger, J. (1988) *J. Biol. Chem.* **263**, 3290–3295.
6. Heldin, C., Ernlund, A., Rorsman, C. & Ronnstrand, L. (1989) *J. Biol. Chem.* **264**, 8905–8912.
7. Seifert, R., Hart, C., Phillips, P., Forstrom, J., Ross, R., Murray, M. & Bowen-Pope, D. (1989) *J. Biol. Chem.* **264**, 8771–8778.
8. Hammacher, A., Mellstrom, K., Heldin, C. & Westermark, B. (1989) *EMBO J.* **8**, 2489–2495.
9. Weber, W., Bertics, P. & Gill, G. (1984) *J. Biol. Chem.* **259**, 14631–14636.
10. Bertics, P., Chen, W., Hubler, L., Lazar, C., Rosenfeld, M. & Gill, G. (1988) *J. Biol. Chem.* **263**, 3610–3617.
11. Koland, J. & Cerione, R. (1988) *J. Biol. Chem.* **263**, 2230–2237.
12. Williams, L. (1989) *Science* **243**, 1564–1570.
13. Honegger, A., Kris, R., Ullrich, A. & Schlessinger, J. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 925–929.
14. Ballotti, R., Lammers, R., Scimeca, J., Dull, T., Schlessinger, J. Ullrich, A. & Van Obberghen, E. (1989) *EMBO J.* **8**, 3303–3309.
15. Sherr, C., Rettenmier, C., Sacca, R., Roussel, M., Look, A. & Stanley, E. (1985) *Cell* **41**, 665–676.
16. Ben-Neriah, Y., Bernards, A., Pasking, M., Daley, G. & Baltimore, D. (1987) *Cell* **51**, 503–512.
17. Rothwell, V. & Rohrschneider, L. (1988) *Oncogene Res.* **1**, 311–324.
18. Saiki, R., Scharf, S., Faloona, F., Mullis, K., Horn, G., Erlich, H. & Anaheim, N. (1985) *Science* **230**, 1350–1354.
19. Siebert, P. & Fukuda, M. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 1665–1669.
20. Armentano, D., Yu, S., Kantoff, P., von Ruden, T., Anderson, W. & Gilboa, E. (1987) *J. Virol.* **61**, 1647–1650.
21. Thomas, K. & Capecchi, M. (1987) *Cell* **51**, 503–512.
22. Wong, P., Chung, S. & Nienhuis, A. (1987) *Genes Dev.* **1**, 358–365.
23. Greenberger, J., Sakakeeny, M., Humphries, R., Eaves, C. & Eckner, R. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 2931–2935.
24. Laker, C., Stocking, C., Bergholz, U., Hess, N., De Lamarter, J. & Ostertag, W. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 8458–8462.
25. Langlois, R., Bigbee, W. & Jensen, R. (1985) *J. Immunol.* **134**, 4009–4017.
26. Calvo, J., Radicello, J. & Charreau, E. (1983) *Biochem. J.* **212**, 259–264.
27. Munson, P. & Rodbard, D. (1980) *Anal. Biochem.* **107**, 220–239.
28. Barsoum, A. & Bhavanandan, V. (1989) *Int. J. Biochem.* **21**, 635–643.
29. Frackelton, A. R., Ross, A. & Eisen, H. (1983) *Mol. Cell. Biol.* **3**, 1343–1352.
30. Cooper, J. A., Sefton, B. M. & Hunter, T. (1983) *Methods Enzymol.* **99**, 387–402.
31. Chou, P. & Fasman, G. (1978) *Annu. Rev. Biochem.* **47**, 251–276.
32. Mack, J., Torchia, D. & Steinert, P. (1988) *Biochemistry* **27**, 5418–5426.
33. Morrow, B. & Rubin, C. (1987) *J. Biol. Chem.* **262**, 13812–13820.
34. Johnson, J., Wong, M. & Rutter, W. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 7516–7520.
35. Whittaker, J. & Okamato, A. (1988) *J. Biol. Chem.* **263**, 3063–3066.
36. Wedegaertner, P. & Gill, G. (1989) *J. Biol. Chem.* **264**, 11346–11353.
37. Pettigrew, D., Romeo, P., Tsapis, A., Thillet, J., Smith, M., Turner, B. & Ackers, G. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 1849–1853.
38. Livneh, E., Reiss, N., Berent, E., Ullrich, A. & Schlessinger, J. (1987) *EMBO J.* **6**, 2669–2676.
39. Anderson, R. & Lovrien, R. (1984) *Nature (London)* **307**, 655–658.
40. Pasvol, G. (1984) *Philos. Trans. R. Soc. Lond. (Biol)* **307**, 189.
41. Schrieber, A., Libermann, T., Lux, I., Yarden, Y. & Schlessinger, J. (1983) *J. Biol. Chem.* **258**, 846–853.
42. Defize, L., Moolenaar, W., Van der Saag, P. & de Laat, S. (1986) *EMBO J.* **5**, 1187–1192.
43. Roussel, M., Transy, C., Kato, J.-Y., Reinherz, E. & Sherr, C. (1990) *Mol. Cell. Biol.* **10**, 2407–2412.
44. Itoh, N., Yoneharo, S., Schreurs, J., Gorman, D., Makuyama, K., Ishii, A., Yahano, I., Arai, K. & Miyajima, A. (1989) *Science* **247**, 324–327.
45. Ohtsuka, M., Roussel, M., Sherr, C. & Downing, J. (1990) *Mol. Cell. Biol.* **10**, 1664–1671.
46. Lyman, S., Park, L. & Rohrschneider, R. (1988) *Oncogene* **3**, 391–395.
47. Woolford, J., McAuliffe, A. & Rohrschneider, L. (1988) *Cell* **55**, 965–977.
48. Roussel, M., Downing, J., Rettenmier, C. & Sherr, C. (1988) *Cell* **55**, 979–988.