Journal of Cellular Biochemistry 39:129–137 (1989)
Growth Factors and Their Receptors: Genetic Control and Rational Application 107–115

Identification of Tyrosine-Phosphorylated Colony-Stimulating Factor 1 (CSF-1) Receptor and a 56-Kilodalton Protein Phosphorylated in Intact Human Cells in Response to CSF-1

Richard D. Huhn, Mary Elizabeth Cicione, and A. Raymond Frackelton, Jr.

Department of Medicine, Roger Williams General Hospital, Brown University, Providence, Rhode Island 02908

Colony-stimulating factor 1 (CSF-1) selectively supports the survival, proliferation, and maturation of hemopoietic cells of the monocyte/macrophage lineage. Although the cellular receptor for CSF-1, (the c-*fms* protein) is a protein-tyrosine kinase activated by the binding of CFS-1, the role of phosphorylation of cellular proteins in CSF-1 signal transduction is poorly understood. Therefore, we examined the CSF-1-stimulated phosphorylation of cellular proteins in human BeWo choriocarcinoma cell line (known to express the c-*fms* protein). BeWo cells were metabolically labeled with $^{32}P_i$, stimulated with recombinant human CSF-1, and extracted with detergent. Phosphotyrosyl proteins were isolated from detergent extracts by affinity chromatography on a highly specific antibody to phosphotyrosine. Rapid phosphorylation of 170-kd protein, followed closely by the phosphorylation of a 56-kd protein, was observed in response to CSF-1. The 170-kd phosphotyrosyl protein bound to wheat germ agglutinin and was secondarily immunoprecipitated with a specific anti-*fms* serum, consistent with its identity as the CSF-1 receptor. Although purified human macrophages that proliferate in culture in response to CSF-1 are not generally accessible, CSF-1 did stimulate the phosphorylation of a 56-kd protein in intact mononuclear leukocytes from human peripheral blood. Thus, the BeWo cell line may represent a good model for the study of CSF-1-stimulated cellular protein phosphorylation.

Key words: colony-stimulating factor 1, protein phosphorylation, hematopoietic growth factors, c-*fms*, phosphotyrosyl proteins, tyrosine kinases

The survival, proliferation, and maturation of hemopoietic cells of the monocyte/macrophage lineage are selectively supported by the hematopoietic growth fac-

Richard D. Huhn's present address is Department of Biochemistry, St. Jude Children's Research Hospital, Memphis, TN 38101.

Received February 1, 1988; accepted August 9, 1988.

tor, colony stimulating factor 1 (CSF-1, M-CSF) [1,2]. Interestingly, roles for CSF-1 in pregnancy and embryogenesis have also been proposed since levels of CSF-1 in blood and other tissues are elevated during pregnancy and since receptors for CSF-1 are expressed both in normal placental tissues and in the human choriocarcinoma cell lines BeWo and JEG-3 [3–5].

The receptor for CSF-1 is identical to the product of c-*fms*, the cellular homologue of the v-*fms* oncogene (the transforming gene of the McDonough strain of feline sarcoma virus) [6,7]. The CSF-1 receptor is a ligand-activated protein-tyrosine kinase, analogous to the receptors for epidermal growth factor, platelet-derived growth factor, insulin, and other peptide growth factors [8–15]. The protein-tyrosine kinase activity of the activated receptors for these growth factors has been strongly implicated in the stimulation of cellular growth, although the specific substrates of the activated receptors, as well as their physiological roles, are generally undefined.

Human CSF-1 is a dimeric glycoprotein highly homologous to the murine factor and is thought to function in similar physiologic role in human hematopoiesis [16–19]. While methods for the culture of murine bone marrow-derived CSF-1-dependent macrophages have been developed for study of the action of CSF-1 in vitro, purified human bone marrow-derived macrophages are not generally accessible [20]. Thus, examination of the role of CSF-1 in human hemopoiesis and the mechanisms of CSF-1 signal transduction in human cells has been difficult. In this study, we have utilized a highly specific monoclonal antibody to phosphotyrosine to affinity purify phosphotyrosyl proteins from CSF-1-stimulated human BeWo cells and normal human peripheral blood monocytes to study the phosphorylations of cellular proteins that may be involved in transducing the CSF-1 growth signal. Utilizing this technique, we have isolated the CSF-1 receptor and 56-kd proteins from BeWo cells and peripheral blood monocytes that become phosphorylated on tyrosine in response to CSF-1.

MATERIALS AND METHODS

Cells and Materials

The human BeWo choriocarcinoma cell line [21] was obtained from the American Type Culture Collection (Rockville, MD) and was grown in RPMI-1640 medium supplemented with non-essential amino acids (Flow Laboratories, McLean, VA), and 1 mM sodium L-pyruvate, 2 mM L-glutamine, 500 U/ml penicillin, 500 μg/ml streptomycin, and 10% fetal bovine serum (GIBCO, Grand Island, NY).

Platelet-free human peripheral blood monocytes were isolated by sedimentation on a cushion of Ficoll/metrizoate, sequential washes in Ca^{++}-free, Mg^{++}-free Puck's saline G containing 0.3 mM EDTA followed by serum containing 5 mM EDTA, and plastic adherence in tissue culture dishes, essentially as described by Pawlowski et al. [22].

Metabolic labeling, growth factor stimulation, and immunoprecipitations. Confluent cultures of BeWo cells or the adherent monocytes derived from 2×10^7 washed mononuclear cells in 35-mm culture dishes were incubated with $^{32}P_i$ at 1 mCi/ml for 3 h at 37°C, then transferred to ice and treated with 10^3 U/ml recombinant human CSF-1 (rhCSF-1, Cetus lot #A422, a gift of P. Ralph) or control medium (without growth factor) for 30 min. Cells were then extracted on ice with 1% Triton X-100 in buffer containing protease, phosphatase, and kinase inhibitors, as described [11]. Tyrosine- phosphorylated proteins were isolated from the extracts by microbatch

immunoaffinity chromatography on monoclonal 1G2 antiphosphotyrosine antibody linked to Sepharose beads, competitively eluted with the hapten phenyl phosphate, and resolved by reducing SDS-PAGE, as described [11,23]. The 1G2 monoclonal antiphosphotyrosine antibody is highly specific for the phenyl phosphate moiety of phosphotyrosine: phosphoserine, phosphothreonine, phosphohistidine, and mono- and tri-phosphonucleotides are not recognized [13,24] (Frackelton AR Jr, Posner MR, Mermelstein F, manuscript in preparation).

^{32}P-labeled *fms*-related proteins were isolated from cell extracts or antiphosphotyrosine-purified phosphotyrosyl proteins by reacting them with specific rabbit antisera v-*fms* (a gift of C. Rettenmier) followed by adsorption on protein A-Sepharose (Pharmacia) as described [24]. Isolated proteins were solubilized by heating SDS sample buffer and were resolved by SDS-PAGE and autoradiography.

Isolation of glycosylated phosphotyrosyl proteins. Hapten-eluted phosphotyrosyl proteins from 1G2-immunoaffinity chromatography were applied to 25 μl of wheat germ agglutinin-Sepharose (WGA-Sepharose) (Pharmacia) and incubated 60 min at 2°C. After unbound proteins were collected, the beads were washed and the glycosylated proteins were eluted with 40 μl of 400 mM N-acetylglucosamine in 1% Triton X-100 extraction buffer. The unbound and eluted proteins were analyzed by reducing SDS-PAGE.

Staphylococcal V8 protease peptide mapping. Proteins were extracted from individual gel bands, precipitated with trichloroacetic acid, and digested with staphylococcal V8 protease type XVIII (Sigma) as described [25]. The resulting peptide fragments were resolved by SDS-PAGE (12.5% acrylamide) and visualized by autoradiography.

Phosphoamino acid analysis. Phosphoproteins in individual bands were extracted from polyacrylamide gels and then subjected to partial acid hydrolysis and two-dimensional thin-layer electrophoresis, as described [24,26,27]. Labeled phosphoamino acids were detected by autoradiography.

RESULTS

Cellular proteins phosphorylated on tyrosine in response to CSF-1 would be candidates for signal-transducing proteins, possibly substrates of CSF-1 receptor kinase itself. As an initial approach to studying CSF-1 signal transduction, we isolated phosphotyrosyl proteins from ^{32}P-labeled, CSF-1-stimulated BeWo cells by affinity chromatography on antiphosphotyrosine antibody-linked Sepharose. Phosphorylations of 170-kd and 56-kd phosphotyrosyl proteins were dramatically stimulated by CSF-1 (Fig. 1A). Phosphorylation of a less prominent 50-kd phosphotyrosyl protein in response to CSF-1 was also seen. Phosphopeptide mapping with staphylococcal V8 protease indicated that the 56-kd and 170-kd proteins of BeWo cells were unrelated (data not shown). Phosphoamino acid analysis of the 56-kd gel band of BeWo cells revealed that it contained substantial amounts of phosphotyrosine as well as phosphoserine and a trace of phosphothreonine (Fig. 1B).

The BeWo cell line was chosen for study of CSF-1-stimulated protein phosphorylations because of its simple maintenance in culture, but since no proliferative or maturational responses of this cell line to CSF-1 have been observed, we also examined adherent peripheral blood mononuclear cells, which are known to express the c-*fms* product and to bind ^{125}I-CSF-1 [4,5]. Mononuclear leukocytes were isolated

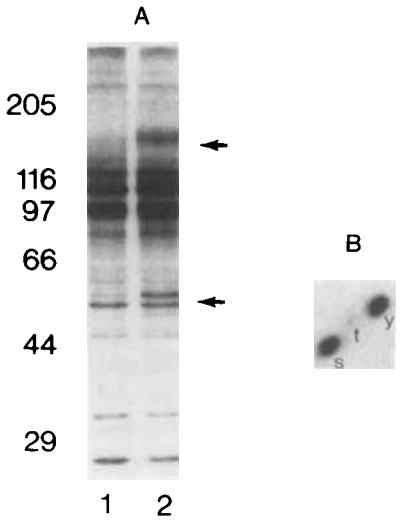

Fig. 1. **A:** Phosphorylation of 170-kd and 56-kd proteins in response to CSF-1. BeWo cells were metabolically labeled for 3 h with a mCi/ml $^{32}P_i$ and treated with 10^4U/ml rhCSF-1 **(Lane 2)** or control medium **(Lane 1)** for 30 min at 0–4°C prior to extraction with 1% Trition X-100 buffer containing inhibitors of proteases, phosphatases, and kinases. Phosphotyrosyl proteins were isolated by antiphosphotyrosine affinity chromatography and analyzed by SDS-PAGE. Autoradiogram exposure was 60 h. **B:** Phosphotyrosine content of 56-kd protein of CSF-1 stimulated BeWo cells. The 56-kd SDS-PAGE gel band of figure 1 was excised and the eluted protein was subjected to partial acid hydrolysis and 2-dimensional thin-layer electrophoresis. Phosphoamino acids were visualized by autoradiography. Exposure was 13 days with intensifying screen at −70°C.

from peripheral blood of a healthy human donor by Ficoll/metrizoate centrifugation and enriched for mature monocytes by plastic adherence. Brief metabolic labeling of these cells with $^{32}P_i$ followed by stimulation with rhCSF-1 also resulted in the phosphorylation of 170-kd and 56-kd proteins isolated by antiphosphotyrosine affinity chromatography (Fig. 2). Phosphorylations of 95-kd and 78-kd antiphosphotyrosine-isolated proteins were also enhanced in peripheral blood monocytes by CSF-1 (not observed in BeWo). Thus the phosphorylation of a 56-kd protein in response to CSF-1 appears not to be a peculiarity of the BeWo cell line, but rather to be a phenomenon common to human cells responding to CSF-1.

To explore further the relationship of the 56-kd and 170-kd proteins, we examined the kinetics of their phosphorylations in response to CSF-1. BeWo cells metabolically labeled with $^{32}P_i$ were exposed to rhCSF-1 at 0–4°C (to inhibit membrane protein internalization/degradation and protein-tyrosine phosphatases) for various times up to 30 min prior to extraction and isolation of phosphotyrosyl proteins (Fig. 3). ^{32}P-labeling of the 170-kd protein was first detected at one minute of rhCSF-1 stimulation and reached peak intensity by 5 min. Phosphorylation of the 56-kd protein could not be detected until 5 min and was still increasing in intensity at 30 min. This result is consistent with a mechanistic relationship between the phosphorylations of the 170-kd and the 56-kd proteins, possibly as kinase and substrate, respectively.

We suspected the 170-kd phosphoprotein to be the c-*fms* (CSF-1 receptor) since its molecular weight was in the appropriate range and its phosphorylation occurred specifically in response to CSF-1. To further characterize the 170-kd protein, we

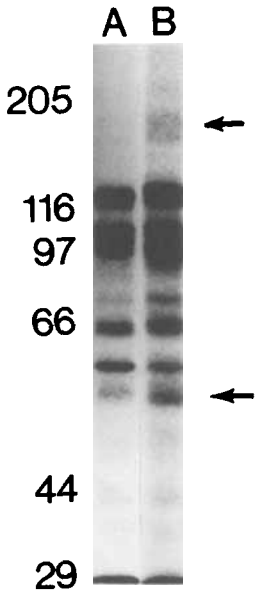

Fig. 2. Phosphorylation of 170-kd and 56-kd proteins in monocytes in response to CSF-1. Peripheral blood monocytes were isolated by Ficoll/metrizoate centrifugation and plastic adherence, then metabolically labeled with $^{32}P_i$ for 3 h and treated with 10^4 U/ml rhCSF-1 (**Lane B**) or control medium (**Lane A**). The cells were extracted with 1% Triton X-100 in buffer containing inhibitors of proteases, phosphatases, and kinases, then subjected to antiphosphotyrosine affinity chromatography and SDS-PAGE as described in the text. Autoradiogram was exposed for 16 h with intensifying screen at −70°C.

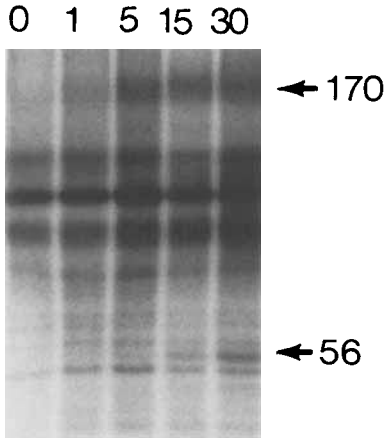

Fig. 3. Time dependence of 170-kd and 56-kd protein phosphorylations in response to CSF-1. BeWo cells were incubated with $^{32}P_i$ for 3 h, then treated with 10^4 U/ml rhCSF-1 on ice for various times from 0 to 30 min (as shown) prior to extraction for antiphosphotyrosine affinity chromatography and SDS-PAGE. Autoradiogram was exposed for 72 hr.

examined whether it was adsorbed by wheat germ agglutinin, since the CSF-1 receptor is known to be a glycosylated membrane protein [4,5,28]. Phosphotyrosyl proteins were isolated from BeWo cells that were metabolically labeled with $^{32}P_i$ and stimulated with rhCSF-1. The eluted phosphotyrosyl proteins were partitioned on WGA-Sepharose beads, then eluted with N-acetylglucosamine (Fig. 4A). A 170-kd phosphotyrosyl protein with affinity for wheat germ agglutinin was isolated only from the CSF-1-stimulated cells, consistent with its identity as the autophosphorylated c-*fms* product. Direct immunoprecipitation of detergent extracts of ^{32}P-metabolic-labeled BeWo cells with anti-*fms* serum demonstrated a phosphoprotein of identical molecular weight and similar appearances on SDS-PAGE to the 170-kd phosphoprotein isolated by antiphosphotyrosine affinity chromatography (data not shown). Final determination of the identity of the CSF-1-stimulated 170-kd protein was achieved by its ability to be secondarily immunoprecipitated from hapten eluates by the anti-*fms* serum (Fig. 4B).

DISCUSSION

In this study, we have isolated proteins phosphorylated on tyrosine from cells expressing the CSF-1 receptor to examine cellular protein phosphorylations in re-

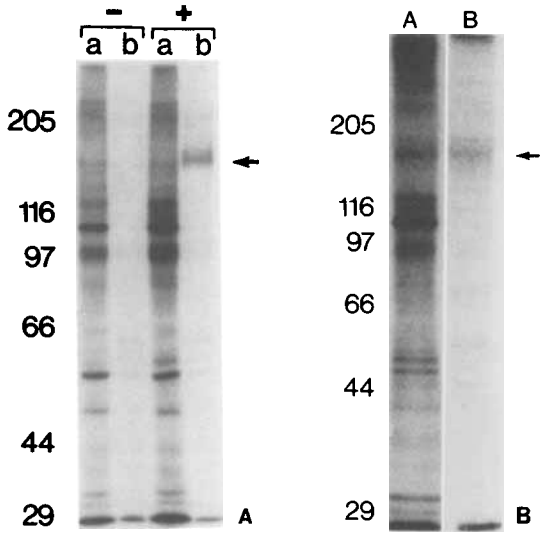

Fig. 4. **A:** Identification of glycosylated 170-kd CSF-1-stimulated phosphotyrosyl protein from BeWo cells. BeWo cells were incubated for 3 h with $^{32}P_i$, and then either stimulated (+) or not stimulated with rhCSF-1 (—) (for 30 min at 0-4°C). Phosphotyrosyl proteins were isolated from detergent extracts, as described in Materials and Methods. Hapten-eluted phosphotyrosyl proteins were partitioned on wheat germ agglutinin-Sepharose (WGA-Sepharose); bound proteins were eluted with N-acetylglucosamine. **a lanes:** Phosphotyrosyl proteins that did not bind to WGA-Sepharoses (supernatants of incubation step). **b lanes:** Phosphotyrosyl proteins eluted from WGA-Sepharose by N-acetylglucosamine. Autoradiogram exposure was 24 h with intensifying screen at −70°C. **B:** Identification of tyrosine-phosphorylated c-*fms* protein from CSF-1 stimulated BeWo cells. BeWo cells were incubated for 3 h with $^{32}P_i$ and stimulated with rhCSF-1. Phosphotyrosyl proteins isolated from detergent extracts by antiphosphotyrosine affinity chromatography (**Lane A**) were reacted with 5 μl anti-*fms* serum and immunoprecipitated on protein A-Sepharose (**Lane B**). Phosphoproteins were analyzed by SDS-PAGE and visualized by autoradiography. Exposure was 8 h with intensifying screen at −70°C.

sponse to CSF-1 stimulation. We observed phosphorylations of a 170-kd glycoprotein and 56-kd protein in response to rhCSF-1 in cells of the BeWo choriocarcinoma cell line. We believe the 170-kd phosphotyrosyl protein is the c-*fms* product (CSF-1 receptor) for several reasons: its size is in the appropriate range by SDS-PAGE; its phosphorylation occurs rapidly in response to rhCSF-1; it binds to wheat agglutinin, consistent with its being glycosylated (as the CSF-1 receptor is known to be); its electrophoretic appearance is similar to a 170-kd phosphoprotein that is immunoprecipitated by *fms* antiserum from BeWo cells and whose phospholabeling is enhanced in vivo by rhCSF-1; and it can be secondarily immunoprecipitated from hapten eluates by *fms* antiserum. A similarly rapid phosphorylation on tyrosine of the CSF-1 receptor in vivo in response to CSF-1 has recently been demonstrated in the murine BAC1.2F5 cell line by Downing et al. [29].

The previously published size of the human c-*fms* phosphoprotein detected by immune complex kinase reactions is approximately 150 kd [4,5]. The discrepancy in molecular weights between this protein and the protein we see in CSF-1-stimulated cells could be due to the effects of glycosylation or phosphorylation differences on electrophoretic behavior, or could reflect limited proteolysis. This small discrepancy is presently unresolved.

The time course of appearance of the phosphorylated 56-kd protein following the CSF-1-stimulated phosphorylation of the 170-kd protein is consistent with the activation of a 170-kd CSF-1 receptor kinase by autophosphorylation, much like the insulin receptor [30]. Only after autophosphorylation would the kinase be able to phosphorylate a cellular substrate. However, this time course may reflect the activation of secondary kinases or inhibition of phosphotyrosine phosphatases. The 56-kd protein does not appear to be a component of a macromolecular complex with the 170-kd protein, since WGA-Sepharose isolated the 170-kd protein independent of the 56-kd protein. Further studies of noncovalent associations between the 170-kd and 56-kd proteins and assays in vitro of phosphorylations of cellular proteins by purified c-*fms* kinase may help answer the question of whether the 56-kd protein is a substrate of the c-*fms* protein.

The identity of the 56-kd protein phosphorylated in response to CSF-1 has yet to be determined. It is intriguing to consider the possibility that it may be related to the *src* family of protein-tyrosine kinases. However, attempts to isolate it by immunoprecipitation either directly from detergent extracts of ^{32}P-labeled CSF-1-stimulated cells or from the mixture of hapten-eluted phosphotyrosyl proteins with monoclonal 327 *src* antibody [31] were unsuccessful (data not shown). Thus, it does not appear to be the c-*src* protein itself. Whether it may be one of the other *src*-family proteins, such as *yes, hck,* or *syn/slk,* remains to be determined [32–34]. It is especially interesting that proteins of similar sizes, as yet unidentified, or phosphorylated on tyrosine (along with other cellular proteins) in response to other peptide growth factors (epidermal growth factor in A431 cells, platelet-derived growth factor in 3T3 cells, and interleukin 3 in murine hematopoietic progenitor cells) and constitutively in v-*abl*-transformed cells and in chronic myelogenous leukemia cells [11,24,26,35]. The possible relationships between these proteins are currently under investigation.

From the study of BeWo cells, the 56-kd protein appeared to be the major candidate for a molecule that could transduce the CSF-1 signal from the CSF-1 receptor. However, no proliferative or morphologic effects of CSF-1 on BeWo cells have been observed. Therefore, since we were primarily interested in the regulatory

actions of CSF-1 on growth and differentiation of hemopoietic cells, it was important that a similar 56-kd protein phosphorylation was found in response to CSF-1 in normal human monocytes. The phosphorylations of two additional proteins appears to be enhanced in peripheral blood monocytes by CSF-1. The observation of different spectra of phosphotyrosyl proteins between the peripheral blood monocytes and BeWo cells is not especially surprising, though, since these cells are of such disparate lineages. Unfortunately, peptide mapping of the 56-kd proteins from the BeWo cells and peripheral blood monocytes has been precluded by technical difficulties such as very limited digestion by staphylococcal V8 protease or chymotrysin and low signal strength in the 56-kd band of peripheral blood monocytes. Thus, while the relationship between the two 56-kd proteins remains to be firmly established, the BeWo cell line in culture may nevertheless represent a good model for some biochemical aspects of the CSF-1 response.

The results of this present study do not necessarily implicate the phosphorylation of the 56-kd protein in cell proliferation per se. As mentioned, the growth of BeWo cells is not affected by CSF-1 (although the fetal bovine serum in which they are cultured would be expected to contain a small concentration of CSF-1). Furthermore, peripheral blood mononuclear leukocytes isolated by plastic adherence are enriched for mature monocytes that are well along the pathway of differentiation to macrophages [36]. These cells may have very limited potential for proliferation in response to CSF-1. Maturing monocyte/macrophage cells do require CSF-1 for survival, however. Thus, the phosphorylation of the 56-kd protein could conceivably be involved in transducing a "viability" signal, or perhaps signals for differentiation-related processes. The development of culture methods for human monocyte/macrophage precursors that proliferate in response to CSF-1 will enable investigations to help answer this question. The correlation of CSF-1-stimulated proliferation with the phosphorylation of an homologous protein in murine systems would help to evaluate its potential role in growth signal transduction.

ACKNOWLEDGMENTS

We wish to thank Peter Ralph (Cetus Corporation) for the generous gift of rhCSF-1, Carl Rettenmier (St. Jude Children's Research Hospital) for the gift of anti-*fms* serum, Joan Brugge (SUNY Stonybrook) for the monoclonal 327 *src* antibody, and E. Richard Stanley (Albert Einstein College of Medicine) for many helpful discussions. This study was supported by grants from the Rhode Island Foundation and the Ocean State Charities Trust to R.D.H. and from the NIH (R01-CA39235 to A.R.F. and CA13943 to the Cancer Research Center at Roger Williams General Hospital). R.D.H. is a fellow of the Pharmaceutical Manufacturer's Association Foundation.

REFERENCES

1. Stanley ER, Guilbert LJ, Tushinski RJ, Bartelmez SH: J Cell Biochem 21:151–159, 1983.
2. Stanley ER, Heard PM: J Biol Chem 252:4305–4312, 1977.
3. Bartocci A, Pollard JW, Stanley ER: J Exp Med 164:956–961, 1986.
4. Woolford J, Rothwell V, Rohrschneider L: Mol Cell Biol 5:3458–3466, 1985.
5. Rettenmier CW, Sacca R, Furman WL, Roussel MF, Holt JT, Nienhuis AW, Stanley ER, Sherr CJ: J Clin Invest 77:1740–1746, 1986.

6. Sherr CJ, Rettenmier CW, Sacca R, Roussel MF, Look AT, Stanley ER: Cell 41:665–676, 1985.
7. Sacca R, Stanley ER, Sherr CJ, Rettenmier CW: Proc Natl Acad Sci USA 83:3331–3335, 1986.
8. Hunter T, Cooper JA: Cell 24:741–752, 1981.
9. Bertics PJ, Gill GN: J Biol Chem 260:14642–14647, 1985.
10. Bishayee S, Ross AH, Womer R, Scher CD: Proc Natl Acad Sci USA 83:6756–6760, 1986.
11. Frackelton AR Jr, Tremble PM, Williams LT: J Biol Chem 259:7909–7915, 1984.
12. Pang DT, Sharma BR, Shafer JA, White MF, Kahn CR: J Biol Chem 260:7131–7136, 1985.
13. Tornqvist HE, Gunsalus JR, Nemenoff RA, Frackelton AR Jr, Pierce MW, Avruch J: J Biol Chem 263:350–359, 1988.
14. Goustin AS, Leof EB, Shipley GD, Moses HL: Cancer Res 46:1015–1029, 1986.
15. Yeung YG, Jubinsky PT, Sengupta A, Yeung DCY, Stanley ER: Proc Natl Acad Sci USA 84:1268–1271, 1987.
16. Das SK, Stanley ER, Guilbert LJ, Forman LW: Blood 58:630–641, 1981.
17. Das SK, Stanley ER: J Biol Chem 257:13679–13684, 1982.
18. Kawasaki ES, Ladner MB, Wang AM, Van Arsdell J, Warren MK, Coyne MY, Schweickart VL, Lee M, Wilson KJ, Boosman A, Stanley ER, Ralph P, Mark DF: Science 230:291–296, 1985.
19. Wong GG, Temple PA, Leary AC, Witek-Giannotti JS, Yang Y, Cialetta AB, Chung M, Murtha P, Kriz R, Kaufman RJ, Ferenz CR, Sibley BS, Turner KJ, Hewick RM, Clark SC, Yanai N, Yokota H, Yamada M, Saito M, Motoyoshi K, Takaku F: Science 235:1504–1508, 1987.
20. Guilbert LJ, Stanley ER: J Biol Chem 261:4024–4032, 1986.
21. Patillo R, Gey GO: Cancer Res 28:1231–1236, 1968.
22. Pawlowski NA, Kaplan G, Hamill AL, Cohn ZA, Scott WA: J Exp Med 158:393–412, 1983.
23. Laemmli UK: Nature 227:680–685, 1970.
24. Huhn RD, Posner MR, Rayter SI, Foulkes JG, Frackelton AR Jr: Proc Natl Acad Sci USA 84:4408–4412, 1987.
25. Cleveland DW, Fischer SG, Kirschner MW, Laemmli UK: J Biol Chem 252:1102–1106, 1977.
26. Frackelton AR Jr, Ross AH, Eisen HN: Mol Cell Biol 3:1343–1352, 1983.
27. Cooper JA, Sefton BM, Hunter T: Methods Enzymol 99:387–402, 1983.
28. Rettenmier CW, Chen JH, Roussel MF, Sherr CJ: Science 228:320–322, 1985.
29. Downing JR, Rettenmier CW, Sherr CJ: Mol Cell Biol 8:1795–1799, 1988.
30. Petruzelli LM, Ganguly S, Smith CR, Cobb MH, Rubin CS, Rosen OM: Proc Natl Acad Sci USA 79:6792–6796, 1982.
31. Lipsich LA, Lewis AJ, Brugge JS: J Virol 48:352–360, 1983.
32. Sudol M, Hanafusa H: Mol Cell Biol 6:2839–2846, 1986.
33. Quintrell N, Lebo R, Varmus H, Bishop JM, Pettenati MJ, LeBeau MM, Diaz MO, Rowley JD: Mol Cell Biol 7:2267–2275, 1987.
34. Kawakami T, Pennington CY, Robbins KC: Mol Cell Biol 6:4195–4201, 1986.
35. Morla AO, Schreurs J, Miyajima A, Wang JYJ: Mol Cell Biol 8:2214–2218, 1988.
36. Tushinski RJ, Oliver IT, Guilbert LJ, Tynan PW, Warner JR, Stanley ER: Cell 28:71–81, 1982.