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Immunology

Interleukin 2 regulates Raf-1 kinase activity through a tyrosine phosphorylation-dependent mechanism in a T-cell line

(T lymphocyte/signal transduction/phosphatase)

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ABSTRACT **Previously we found that interleukin 2 (IL-2) induces tyrosine phosphorylation and activation of the serine/threonine-specific kinase encoded by the *raf-1* protooncogene in a T-cell line, CTLL-2. Here we extended these findings by exploring the effects of selective removal of phosphate from tyrosines in p72-74-Raf-1 kinase that had been immunoprecipitated from IL-2-stimulated CTLL-2 cells. Treatment *in vitro* of IL-2-activated Raf-1 with the tyrosine-specific phosphatases CD45 and TCPTP (formerly called T-cell protein tyrosine phosphatase) reduced Raf kinase activity to nearly baseline levels. This effect was completely inhibited by the phosphatase inhibitor sodium orthovanadate. In contrast, treatment of Raf-1 with a serine/threonine-specific phosphatase, protein phosphatase 1 (PP-1), resulted in a more modest decrease in Raf *in vitro* kinase activity, and this effect was prevented by okadaic acid. Two-dimensional phosphoamino acid analysis confirmed the selective removal of phosphate from tyrosine by CD45 and from serine and threonine by PP-1. The immunoreactivity of p72-74-Raf-1 with anti-phosphotyrosine antibodies was also completely abolished by treatment with CD45 in the absence but not in the presence of sodium orthovanadate. These findings provide evidence that the IL-2-stimulated phosphorylation of Raf-1 on tyrosines plays an important role in upregulating the activity of this serine/threonine-specific kinase in CTLL-2 cells and, as such, provides a model system for studying the transfer of growth factor-initiated signals from protein tyrosine kinases to serine/threonine-specific kinases.**

Interleukin 2 (IL-2) regulates the growth, survival, and cytolytic killing activity of T lymphocytes (1). Although this lymphokine has shown some efficacy in enhancing immune responses to tumors in patients, the severe side effects of systemic IL-2 administration have drastically limited its clinical uses (2). An improved knowledge of the molecular events that occur in T cells upon binding of IL-2 thus could potentially contribute to more effective manipulation of immune-cell responses against cancer.

CTLL-2 was originally described as a tumor-specific cytolytic T cell that was isolated from mice and that could be maintained long-term in culture with IL-2 (3). This T-cell line has been used extensively as a model for investigations of the mechanisms of IL-2 signal transduction. Unlike many other growth factor receptors (4), the known subunits of the IL-2 receptor complex lack homology with protein-tyrosine kinases (PTKs) (5–7). Nevertheless, tyrosine phosphorylation of numerous intracellular proteins occurs rapidly in CTLL-2 and other T cells when stimulated with IL-2 (8–10), and pharmacological inhibitors of PTKs prevent most of the biochemical and cellular events that normally follow from exposure of T cells to IL-2 (see refs. 11 and 12 for examples). Ultimately, however, it is believed that growth factor-activated PTKs pass their information on to serine/threonine-specific kinases in a signal transduction cascade that eventually reaches the nucleus, resulting in the changes in gene expression that lead to cellular proliferation, suppression of apoptosis, and other responses.

The 72- to 74-kDa kinase encoded by the *raf-1* protooncogene is a good candidate for a serine/threonine-specific kinase that receives its activation signals directly from upstream PTKs. Although somewhat controversial, Raf-1 was originally reported to become phosphorylated on tyrosines in platelet-derived growth factor-stimulated fibroblasts and was shown to physically associate with platelet-derived growth factor receptors (13). Subsequently, the lymphokines IL-2, IL-3, and granulocyte–macrophage colony-stimulating factor were found to induce tyrosine phosphorylation and activation of Raf-1 in hematolymphoid cells (14–16). The tyrosine phosphorylation of Raf-1 occurs with high stoichiometry in some lymphokine-dependent cells, including IL-2-stimulated CTLL-2 cells where 20–50% of the total incorporated $^{32}PO_4$ was found on tyrosines (14). Furthermore, a physical association between Raf-1 and the p75 β chain of the IL-2 receptor has been described (17), suggesting that it may be a proximal player in an IL-2-regulated signal transduction cascade. An important gap in these scenarios that have placed Raf-1 in a likely position to relay information from PTKs to downstream serine/threonine-specific kinases concerns the functional significance of the tyrosine phosphorylation that has been observed in Raf-1 after stimulation of cells with lymphokines and other types of growth factors. Here we describe the results of experiments in which tyrosine-specific protein phosphatases were used to remove phosphates selectively from tyrosine residues in Raf-1 and the effects on Raf-1 *in vitro* kinase activity were then determined.

MATERIALS AND METHODS

Cell Cultures. CTLL-2 cells were adapted to growth in RPMI 1640 medium containing 1 mM L-glutamine, 10% (vol/vol) heat-inactivated fetal bovine serum, and 10% (vol/vol) conditioned medium from the IL-2-producing cell line MLA-144 (18) and were then maintained in this medium as described (14). All experiments were performed 3 days after plating CTLL-2 cells, when the cells had reached their plateau phase of growth and had consumed most of the IL-2 in the cultures. Cells were washed three times with RPMI 1640 medium to remove residual lymphokine and then re-

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Abbreviations: IL-2, interleukin 2; PTK, protein-tyrosine kinase; TCPTP, T-cell protein tyrosine phosphatase; PP-1, protein phosphatase 1.

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turned to culture for 7–10 h in medium lacking IL-2. In some cases, the cells were resuspended in phosphate-free medium and $^{32}PO_4$ (0.2 mCi/ml; 1 Ci = 37 GBq) was added to the cultures during this 7- to 10-h period to metabolically label the cells. Quiescent CTLL-2 cells were then restimulated with 100 units of recombinant human IL-2 per ml (generous gift from Cetus/Chiron) (19) for 5 min.

Antibodies, Immunoprecipitations, and Immunoblotting. The Raf-1-specific monoclonal antibody URP30S3 was used for most experiments (20). In some cases, rabbit antisera generated against the SP63 peptide corresponding to the C terminus of Raf kinases were used (21). The anti-phosphotyrosine monoclonal antibody 4G10 was purchased from Upstate Biotechnology (Lake Placid, NY). Immunoprecipitations and immunoblot analysis of Raf-1 were performed exactly as described except that 4G10 was used at a dilution of 1:50,000 (vol/vol) and an emission chemiluminescence detection system was used (Amersham). Exposure times with Kodak XRP film were typically 15–30 s.

Phosphatase Treatments and *in Vitro* Raf Kinase Assays. Raf-1-containing immunoprecipitates were prepared from either unlabeled or $^{32}PO_4$-labeled CTLL-2 cells and treated for 30–60 min at 30°C with either 100 units of CD45 per ml, 100 units of TCPTP (T-cell protein tyrosine phosphatase) per ml, or 76 units of protein phosphatase 1 (PP-1) per ml in 50 μl of a solution containing 25 mM Hepes (pH 7.2), 2 mM dithiothreitol, 5 mM EDTA, 0.05% Triton X-100, 5% (vol/vol) glycerol, 5% 2-mercaptoethanol, and 1 mg of bovine serum albumin per ml (22–24). For some samples, either 1 mM sodium orthovanadate or 1 μM okadaic acid was added before the phosphatases, but all reactions were stopped by addition of these phosphate inhibitors. For $^{32}PO_4$-labeled Raf-1, the immunoprecipitates were washed five times in modified RIPA lysis buffer (14) before analysis by SDS/PAGE using 7.5% gels. In some cases, the bands corresponding to $^{32}PO_4$-labeled Raf-1 were excised from gels, acid hydrolyzed, and analyzed by two-dimensional electrophoresis with 0.1-mm plastic-backed thin-layer cellulose plates (EM Science) as described (14). For unlabeled Raf-1, immunoprecipitates were washed twice with a buffer containing 1% Triton X-100 (14), once with 0.5 M $LiCl_2$/50 mM Tris·HCl, pH 7.4, and once with water before *in vitro* kinase assays using a synthetic peptide as substrate exactly as described (14, 25, 26).

RESULTS

To determine the functional significance of tyrosine phosphorylation in regulation of Raf-1 kinase activity in IL-2-stimulated CTLL-2 cells, we used purified tyrosine-specific phosphatases CD45 and TCP to specifically dephosphorylate tyrosines in p72-74-Raf-1 and then determined the effect of

Table 1. Effect of phosphatase treatments on *in vitro* kinase activity of Raf-1

Stimulation with IL-2	Phosphatase			Phosphatase inhibitor		Raf kinase activity		
	CD45	TCP	PPI	Na_3VO_4	Oka	Total cpm	Corr cpm	Rel. activity
				Experiment 1				
−	−	−	−	−	−	4,380	1124	1.0
+	−	−	−	−	−	7,694	4494	3.8
+	+	−	−	−	−	4,820	1620	1.3
+	+	−	−	+	−	10,024	6824	5.7
+	−	+	−	−	−	3,692	492	1.0
+	−	+	−	+	−	8,094	4894	4.4
+	−	−	+	−	−	6,100	2900	2.5
+	−	−	+	−	+	6,864	3664	3.1
				Experiment 2				
−	−	−	−	−	−	2,016	1612	1.0
+	−	−	−	−	−	6,760	6356	4.0
+	+	−	−	−	−	2,656	2252	1.4
+	+	−	−	+	−	6,736	6332	3.9
+	−	+	−	−	−	2,716	2312	1.4
+	−	+	−	+	−	5,936	5532	3.4
+	−	−	+	−	−	5,044	4640	2.9
+	−	−	+	−	+	5,476	5072	3.1
				Experiment 3				
−	−	−	−	−	−	673	—	1.0
+	−	−	−	−	−	2,903	—	4.3
+	+	−	−	−	−	998	—	1.5
+	+	−	−	+	−	2,820	—	4.2
+	−	+	−	−	−	902	—	1.3
+	−	+	−	+	−	2,571	—	3.8
+	−	−	+	−	−	2,452	—	3.6
+	−	−	+	−	+	2,963	—	4.4

CTLL-2 cells were deprived of IL-2 for 7–10 h and then restimulated for 5 min with 100 units of recombinant human IL-2 per ml (+) or left unstimulated (−) before immunoprecipitating Raf-1 with the URP30S3 monoclonal antibody. Immune complexes were then treated with various phosphatases in the presence or absence of the phosphatase inhibitor $NaVO_4$ or okadaic acid (Oka) (22–24). After stopping reactions by addition of phosphatase inhibitors to all samples, followed by washing, the immune complexes from duplicate samples were resuspended in kinase reaction buffer containing 20 mM NaF and 1 mM $NaVO_4$, [γ-^{32}P]ATP, and either a synthetic peptide substrate, IVQQFGFQRRASDDGKLTD, or a peptide lacking a serine phosphate acceptor site, IVQQFGFQRRAADDGKLTD, essentially as described (14, 25, 26). Total of ^{32}P incorporation into the substrate peptide was corrected for background by subtracting the cpm obtained when the serine-deficient peptide was used (Corr cpm). Relative kinase activity (Rel activity) was calculated by normalizing all data relative to the corrected cpm value obtained with IL-2-deprived CTLL-2 cells, which was set at 1.0. Data from three experiments are presented.

this treatment on the activity of this kinase *in vitro*. Table 1 summarizes the results of three experiments. As shown, stimulation of CTLL-2 cells for 5 min with IL-2 resulted in an ≈4-fold increase in Raf-1 kinase activity, as measured by an *in vitro* kinase assay that uses a synthetic peptide as the substrate (14, 25, 26). Treatment of Raf-1 immunoprecipitates with CD45 or TCPTP markedly reduced Raf-1 kinase activity, bringing it down to levels approaching the baseline activity seen in unstimulated CTLL-2 cells. When the tyrosine phosphatase inhibitor sodium orthovanadate was included in the phosphatase reactions, no loss of Raf-1 kinase activity occurred, thus demonstrating the specificity of this effect. Heat denaturing the phosphatase before use also abolished inhibitory activity (data not shown). Treatment of Raf-1 immunoprecipitates with the serine/threonine-specific phosphate PP-1 also reduced Raf-1 kinase activity *in vitro*, but to a lesser extent than the tyrosine-specific phosphatases (Table 1). When the phosphatase inhibitor okadaic acid was included in the reactions with PP-1, little or no reduction in Raf-1 kinase activity was observed.

As a first attempt to confirm that the CD45 and TCPTP phosphatases were effectively dephosphorylating tyrosinyl residues in Raf-1, we subjected Raf-1 immunoprecipitates to immunoblot analysis using the anti-phosphotyrosine-specific antibody 4G10. As shown in Fig. 1, Raf-1 immunoprecipitated from unstimulated CTLL-2 cells contained little or no detectable phosphotyrosine, at least as defined by this assay. Stimulation of CTLL-2 cells for 5 min produced a large increase in the immunoreactivity of Raf-1 with the 4G10 antibody, consistent with the ability of IL-2 to stimulate tyrosine phosphorylation of Raf-1 in these T cells. Increases in the binding of 4G10 to both the 72- and 74-kDa Raf-1 bands were observed. The slower-migrating form of Raf-1 with an apparent molecular mass of 74 kDa has been attributed to a phosphorylation-induced conformational change in the kinase (25). Raf-1 that had been recovered from IL-2-stimulated CTLL-2 cells and treated with CD45 or TCPTP exhibited little or no immunoreactivity with the phosphotyrosine-specific antibody (Fig. 1; data not shown), suggesting effective dephosphorylation of tyrosinyl residues in Raf-1. As expected, when sodium orthovanadate was included in the phosphatase reactions, essentially no reduction in the binding of 4G10 to Raf-1 was observed. As a control, Raf-1 immunoprecipitates were also treated with the PP-1, which produced little or no change in the immunoreactivity of Raf-1 with the 4G10 antibody.

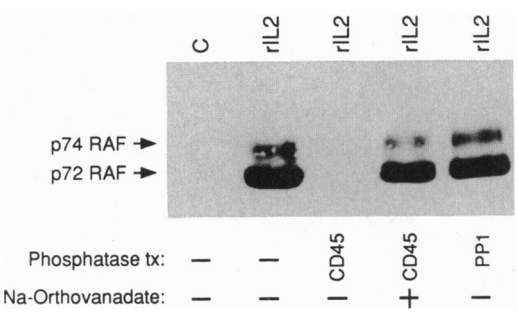

FIG. 1. Analysis of phosphatase-treated p72-74-Raf-1 by immunoblot assay using an anti-phosphotyrosine-specific antibody. Raf-1 was immunoprecipitated from IL-2-deprived CTLL-2 cells (lane C) and from CTLL-2 cells that had been restimulated for 5 min with recombinant IL-2 (rIL2). The resulting immune complexes were then treated with either CD45 or PP-1 in the presence or the absence of sodium orthovanadate as indicated. Raf-1-containing immunoprecipitates were then subjected to SDS/PAGE and transferred to nitrocellulose filters for incubation with 4G10 antibody. Blots were then washed, and the bound 4G10 monoclonal antibody was detected by incubation with a horseradish-peroxidase-conjugated antibody and the chemiluminescent substrate luciferase. Exposure to x-ray film was for 30 s. Data are representative of several experiments.

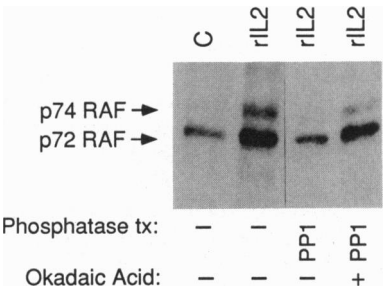

FIG. 2. Effect of PP-1 on $^{32}PO_4$ content of p72-74-Raf-1. CTLL-2 cells were metabolically labeled with $^{32}PO_4$ and Raf-1 was immunoprecipitated from these IL-2-deprived cells before (lane C) or after restimulation with recombinant IL-2 (rIL2). Immune complexes were then treated with PP-1 in the presence or absence of okadaic acid as indicated. The resulting proteins were analyzed by SDS/PAGE (7.5% gels) and autoradiography.

As an initial attempt to confirm that the serine/threonine-specific phosphatase PP-1 was dephosphorylating Raf-1, CTLL-2 cells were metabolically labeled with $^{32}PO_4$. Raf-1 was then immunoprecipitated from the cells and treated with PP-1 in the presence or absence of okadaic acid. As shown in Fig. 2, IL-2 induced a marked increase in the phosphorylation of p72-Raf-1 and led to the appearance of the slower-migrating form of Raf-1, consistent with previous reports (14, 27). Treatment of the immunoprecipitated $^{32}PO_4$-labeled Raf-1 with PP-1 resulted in a large decrease in the radioactive signal, suggesting removal of the majority of $^{32}PO_4$ from the kinase. Inclusion of the phosphatase inhibitor okadaic acid in the reaction mixtures abrogated most of this effect of PP-1.

Finally, to biochemically confirm the selectivity of the various phosphatases for dephosphorylation of the expected amino acids, Raf-1 was immunoprecipitated from $^{32}PO_4$-labeled CTLL-2 cells after IL-2 restimulation and treated with either CD45 or PP-1. The resulting Raf-1 proteins were then acid hydrolyzed and the products were compared side-by-side in the same two-dimensional plate, each sample thus serving as a control for the other. As shown in Fig. 3, CD45-treated Raf-1 kinase contained phosphoserine and phosphothreonine but very little phosphotyrosine. Conversely, PP-1-treated Raf-1 contained predominantly phosphotyrosine, but only scant amounts of phosphothreonine,

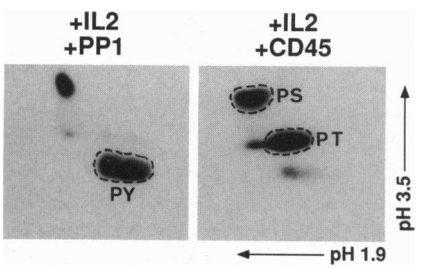

FIG. 3. Phosphoamino acid analysis of p72-74-Raf-1 after treatment with CD45 or PP-1. Raf-1 kinase was immunoprecipitated from IL-2-stimulated CTLL-2 cells that had been metabolically labeled with $^{32}PO_4$ and treated for 60 min with either PP-1 or CD45. After subjecting the immune complexes to SDS/PAGE, the p72 and p74 Raf-1 bands were excised from the gels and acid hydrolyzed, and the resulting phosphoamino acids were separated by two-dimensional electrophoresis using thin-layer cellulose plates. Electrophoresis in pH 3.5 buffer was at 1 kV for 55 min, whereas the pH 1.9 dimension was run at 1 kV for 45 min. As standards, unlabeled phosphotyrosine (PY), phosphoserine (PS), and phosphothreonine (PT) were mixed with the samples and their relative positions were revealed by treatment of the plates with ninhydrin vapor. Only selected standards are outlined in the figure.

and markedly reduced levels of phosphoserine. The results of this phosphoamino acid analysis thus confirm the specificity of the CD45 and PP-1 phosphatases for selective dephosphorylation of tyrosines and serines/threonines, respectively, in Raf-1 under the conditions used here.

DISCUSSION

Previously, we reported that IL-2 induces phosphorylation and activation of the p72-74-Raf-1 kinase in CTLL-2 cells (14). The extent of IL-2-induced phosphorylation on tyrosine varied from 20% to 50% of the total $^{32}PO_4$ incorporated into Raf-1. The relatively high stoichiometry of tyrosine phosphorylation in these T cells thus created an opportunity to assess the functional significance of this posttranslational modification in the regulation of Raf-1 kinase activity. When treated with CD45 or TCPTP, levels of Raf-1 kinase activity *in vitro* were reduced by an average of 68% (Table 1). This result is in reasonable agreement with our previous finding that about one-half of the immunodetectable Raf-1 protein in IL-2-stimulated CTLL-2 cells can be immunoprecipitated with anti-phosphotyrosine antibodies (14). This large diminution in Raf-1 kinase activity that occurred upon dephosphorylation of tyrosine strongly suggests that IL-2-induced tyrosine phosphorylation of Raf-1 plays a major role in activation of this serine/threonine-specific kinase in CTLL-2 cells. Studies to map and mutate the sites of tyrosine phosphorylation in Raf-1 in order to confirm this hypothesis remain to be done.

Although tyrosine phosphorylation appears to represent a mechanism for activating the Raf-1 kinase in IL-2-stimulated CTLL-2 cells, this kinase can also be activated by pathways involving exclusively serine and threonine phosphorylation. Phorbol esters that activate protein kinase C, for example, are potent inducers of Raf-1 kinase phosphorylation and activation (25, 27, 28). In this regard, Siegel *et al.* (29) reported that antibodies directed against the T-cell antigen receptor complex stimulated phosphorylation and activation of Raf-1 in a T-cell hybridoma through a protein kinase C-dependent mechanism that involved no detectable tyrosine phosphorylation. Similarly, phosphorylation of Raf-1 in fibroblasts stimulated with epidermal growth factor or insulin and in hematopoietic cells stimulated with colony-stimulating factor 1 appears to occur exclusively on serines and threonines (26, 30, 31). In some but not all fibroblasts, platelet-derived growth factor may be able to stimulate tyrosine phosphorylation of Raf-1 but this occurs at very low stoichiometry relative to serine/threonine phosphorylation (13, 29, 31), suggesting that the latter represents a more quantitatively important mechanism for activating Raf-1 in those cells. Similarly, although IL-3 and granulocyte–macrophage colony-stimulating factor are able to stimulate tyrosine phosphorylation of Raf-1 with relatively high stoichiometry in some myeloid cell lines (15), tyrosine phosphorylation occurs with much lower stoichiometry in others (16), again suggesting that serine/threonine phosphorylation may be a more common mechanism for achieving activation of Raf-1.

Somewhat surprising, therefore, was the less striking effect that the serine/threonine-specific phosphatase PP-1 had on Raf-1 kinase activity *in vitro* in our experiments (Table 1). This observation raises the possibility that serine/threonine phosphorylation events are relatively less important for regulating the activity of Raf-1 in IL-2-stimulated CTLL-2 cells. It should be noted, however, that PP-1 removed only ≈70% of phosphoserine from Raf-1, whereas CD45 produced nearly complete dephosphorylation of tyrosine (Fig. 3). Thus, the importance of serine phosphorylation in activating the Raf-1 kinase in IL-2-stimulated CTLL-2 cells could have been underestimated. Furthermore, we cannot from these experiments determine whether tyrosine phosphorylation can by itself activate the Raf-1 kinase independent of serine/threonine phosphorylation.

Because CTLL-2 cells differ from normal T cells in their ability to remain constantly responsive to IL-2 in the absence of periodic stimulation with antigen, the high stoichiometry of tyrosine phosphorylation of Raf-1 observed in these cells may represent a special circumstance. Regardless of how accurately the events described here in CTLL-2 cells quantitatively and qualitatively reflect the situation in normal T cells, these IL-2-dependent T cells provide a model system for molecular explorations of the mechanisms by which the activity of the serine/threonine-specific Raf-1 kinase can be regulated by tyrosine phosphorylation.

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1. Taniguchi, T. (1992) *Behring Inst. Mitt.* **91**, 87–95.
2. Rosenberg, S. A., Lotze, M. T., Muul, L. M., Leitman, S., Chang, A. E., Ettinghausen, S. E., Matory, Y. L., Skibber, J. M., Shiloni, E., Vetto, J. T., Seipp, C. A., Simpson, C. & Reichert, C. M. (1985) *N. Engl. J. Med.* **313**, 1485–1492.
3. Gillis, S. & Smith, K. (1977) *Nature (London)* **268**, 154–156.
4. Ullrich, A. & Schlessinger, J. (1990) *Cell* **61**, 203–207.
5. Greene, W. C. & Leonard, W. J. (1986) *Annu. Rev. Immunol.* **4**, 69–95.
6. Hatakeyama, M., Tsudo, M., Minamoto, S., Kono, T., Doi, T., Miyata, T., Miyasaka, M. & Taniguchi, T. (1989) *Science* **244**, 551–556.
7. Takeshita, T., Asao, H., Ohtani, K., Ishii, N., Kumaki, S., Tanaka, N., Munakata, H., Nakamura, M. & Sugamura, K. (1992) *Science* **257**, 379–382.
8. Saltzman, E. M., Thom, R. R. & Casnellie, J. E. (1988) *J. Biol. Chem.* **263**, 6956–6959.
9. Farrar, W. L. & Ferris, D. K. (1989) *J. Biol. Chem.* **264**, 12562–12567.
10. Mills, G., May, C., McGill, M., Furg, M., Baker, M., Sutherland, R. & Greene, M. (1990) *J. Biol. Chem.* **265**, 3561–3567.
11. Merida, I., Diez, E. & Gaulton, G. N. (1991) *J. Immunol.* **147**, 2202–2207.
12. Einspahr, K., Abraham, R., Dick, C. & Leibson, P. (1990) *J. Immunol.* **145**, 1490–1497.
13. Morrison, D. K., Kaplan, D. R., Escobedo, J. A., Rapp, U. R., Roberts, T. M. & Williams, L. T. (1989) *Cell* **58**, 649–657.
14. Turner, B., Rapp, U., App, H., Greene, M., Dobashi, K. & Reed, J. C. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 1227–1231.
15. Carroll, M. P., Clark-Lewis, I., Rapp, U. R. & May, W. S. (1990) *J. Biol. Chem.* **265**, 19812–19817.
16. Kanakura, Y., Druker, B., Wood, K. W., Mamon, H. J., Okuda, K., Roberts, T. M. & Griffin, J. D. (1991) *Blood* **77**, 243–248.
17. Maslinski, W., Remillard, B., Tsudo, M. & Strom, T. B. (1992) *J. Biol. Chem.* **267**, 15281–15284.
18. Rabin, H., Hopkins, R. F., Ruscetti, F. W., Neubauer, R. H., Brown, R. L. & Kawakami, T. G. (1981) *J. Immunol.* **127**, 1852–1856.
19. Rosenberg, S., Grimm, E., McGrogan, M., Doyle, M., Kawasaki, E., Koths, K. & Mark, D. (1984) *Science* **223**, 1412–1415.
20. Kolch, W., Wessinger, E., Mischak, H., Troppmair, J., Showalter, S., Lloyd, P., Heidecker, G. & Rapp, U. (1990) *Oncogene* **5**, 713–720.
21. Schultz, A. M., Copeland, T. D., Mark, G. E., Rapp, U. R. & Oroszlan, S. (1985) *Virology* **146**, 78–89.
22. Tonks, N. K., Charbonneau, H., Diltz, C. D., Fischer, E. H. & Walsh, K. A. (1988) *Biochemistry* **27**, 8695–8701.
23. Tonks, N. K., Diltz, C. D. & Fischer, E. (1988) *J. Biol. Chem.* **263**, 6722–6730.
24. Anderson, N. G., Maller, J. L., Tonks, N. K. & Sturgill, T. W. (1990) *Nature (London)* **343**, 651–653.
25. Reed, J. C., Yum, S., Cuddy, M. P., Turner, B. C. & Rapp, U. R. (1991) *Cell Growth Differ.* **2**, 235–243.

26. App, H., Hazan, R., Zilberstein, A., Ullrich, A., Schlessinger, J. & Rapp, U. (1991) *Mol. Cell. Biol.* **11**, 913–919.
27. Zmuidzinas, A., Mamon, H. J., Roberts, T. M. & Smith, K. A. (1991) *Mol. Cell. Biol.* **11**, 2794–2803.
28. Morrison, D., Kaplan, D., Rapp, U. & Roberts, T. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 8855–8859.
29. Siegel, J. N., Klausner, R. D., Rapp, U. R. & Samelson, L. E. (1990) *J. Biol. Chem.* **265**, 18472–18480.
30. Blackshear, P. J., McNeill-Haupt, D., App, H. & Rapp, U. R. (1990) *J. Biol. Chem.* **265**, 12131–12134.
31. Baccarini, M., Sabatini, D. M., App, H., Rapp, U. R. & Stanley, E. R. (1990) *EMBO J.* **9**, 3649–3657.