The human BCR gene encodes a protein with serine/threonine kinase activity and regulatory domains for the small G-proteins RAC and CDC42. Previous work in our laboratory has established that BCR is a substrate for c-FES, a non-receptor tyrosine kinase linked to myeloid growth and differentiation. Tyrosine phosphorylation led to the association of BCR with the RAS guanine nucleotide exchange complex GRB2-SOS via the GRB2 SH2 domain, linking BCR to RAS signaling (Maru, Y., Peters, K. L., Afar, D. E. H., Shibuya, M., Witte, O. N., and Smithgall, T. E. (1995) Mol. Cell. Biol. 15, 835–842). In the present study, we demonstrate that BCR Tyr-246 and at least one of the closely spaced tyrosine residues, Tyr-279, Tyr-283, and Tyr-289 (3Y cluster), are phosphorylated by FES both in vitro and in 32P-labeled cells. Mutagenesis of BCR Tyr-177 to Phe completely abolished FES-induced BCR binding to the GRB2 SH2 domain, identifying Tyr-177 as an additional phosphorylation site for FES. Co-expression of BCR and FES in human 293T cells stimulated the tyrosine autophosphorylation of FES. By contrast, tyrosine phosphorylation of BCR by FES suppressed BCR serine/threonine kinase activity toward the 14-3-3 protein and BCR substrate, BAP-1. These data show that tyrosine phosphorylation by FES affects the interaction of BCR with multiple signaling partners and suggest a general role for BCR in non-receptor protein-tyrosine kinase regulation and signal transduction.

The human BCR gene encodes a 160-kDa protein (BCR) with multiple biochemical functions. The N-terminal portion of BCR is a structurally distinct protein kinase capable of autophosphorylation on serine and threonine residues (1, 2). This region of BCR also binds and phosphorylates BAP-1, a member of the 14-3-3 protein family that has been implicated in BCR and BCR-ABL function (3). The central domain of BCR is homologous to guanine-nucleotide exchange factors for RHO-related GTPases (4), while the C-terminal region exhibits GTPase-activating protein activity toward these small G-proteins (5, 6). Thus, BCR may regulate multiple small GTPases involved in mitogenic signaling, cytoskeletal organization, and regulation of NADPH oxidase activity in phagocytes (7–10).

BCR was first discovered in the context of BCR-ABL, the transforming tyrosine kinase associated with chronic myelogenous leukemia (11). N-terminal, BCR-derived sequences are essential for BCR-ABL transforming activity and serve several functions. The extreme N-terminal portion of BCR-ABL encodes a coiled-coil oligomerization domain that may promote BCR-ABL activation and is indirectly required for BCR-ABL cytoskeletal localization (12). The C-terminal portion of the BCR kinase domain binds to the ABL SH2 domain in a phosphotyrosine-independent manner (13). This interaction has been proposed to release the ABL tyrosine kinase from negative regulation within BCR-ABL. BCR-derived sequences are also involved in BCR-ABL signal transduction. For example, tyrosine phosphorylation of BCR Tyr-177 within BCR-ABL leads to direct interaction with the RAS guanine nucleotide exchange complex GRB2-SOS via the GRB2 SH2 domain (14, 15). BCR-ABL has also been linked to the SHC adaptor protein (15, 16), although the specific mechanism of BCR-ABL/SHC interaction is unknown. Both pathways may contribute to the activation of RAS, which is required for transformation by BCR-ABL (17).

Accumulating evidence implicates normal BCR as a tyrosine kinase substrate and possible signaling intermediate. For example, BCR forms heteromeric complexes with BCR-ABL and is phosphorylated by BCR-ABL on multiple tyrosine residues including Tyr-177, the GRB2 binding site (18–20). Recent work from our laboratory has shown that BCR is a major transformation-related substrate for the v-FPS tyrosine kinase and its normal human homolog, c-FES (21). Tyrosine phosphorylation led to the association of BCR with GRB2-SOS in v-FPS-transformed fibroblasts via the GRB2 SH2 domain. Furthermore, tyrosine phosphorylation of BCR by c-FES strongly enhanced the binding of BCR to multiple SH2 domains in vitro, including those from GRB2, RAS GTPase-activating protein, phospholipase C-γ, and the p85 subunit of phosphatidylinositol 3'-kinase (21). These data strongly suggest that tyrosine phosphorylation of BCR induces interaction with downstream effectors that contain SH2 domains and implicate BCR as a key intermediate in signaling pathways regulated by BCR-ABL, FPS/FES, and possibly other non-receptor tyrosine kinases.

In the present study, we have mapped the specific tyrosine residues that are phosphorylated by c-FES within the BCR N-terminal region both in vitro and in vivo. We observed that tyrosine phosphorylation of BCR creates specific binding sites for the GRB2 and SHC SH2 domains, suggesting that BCR may couple FES to RAS signaling in a manner analogous to BCR-
ABL. Unexpectedly, we observed that co-expression of FES and BCR in human cells stimulated FES tyrosine kinase activity while inhibiting BCR serine/threonine kinase activity toward the 14-3-3 protein, BAP-1. By contrast, co-expression of FES with a BCR mutant lacking the FES tyrosine phosphorylation sites completely blocked FES autophosphorylation in human cells. These data provide new evidence for BCR as an effector and regulatory protein for tyrosine kinases of the FES/BCR family and show that BCR is subject to regulation by tyrosine kinases in vivo.

**EXPERIMENTAL PROCEDURES**

**Mutagenesis of the BCR 162–413 Region and Expression of GST-BCR** Fusion Proteins in Escherichia coli—DNA encoding BCR N-terminal amino acids 162–413 was amplified by polymerase chain reaction and cloned into pGEX-2T (Pharmacia Biotech Inc.). Tyr to Phe point mutants Y177F, Y231F, Y246F, Y316F, Y328F, Y360F, and deletion mutant ΔY3 (deletion of amino acids 276–283 containing Tyr-276, Tyr-279, and Tyr-Y283) were introduced into the pGEX-2T/BCR 162–413 construct using standard polymerase chain reaction-based techniques (23). Procedures for bacterial expression and glutathione-agarose affinity purification of GST fusion proteins are described in detail elsewhere (21, 24, 25).

**Phosphorylation, Tryptic Phosphopeptide Mapping, and Phosphoamino Acid Analysis of GST-BCR Fusion Proteins in Vitro**—Recombinant FES was expressed as a C-terminal FLAG fusion protein using a baculovirus/S9 cell system and purified using the anti-FLAG M2 affinity gel (21). Phosphorylation of GST-BCR 162–413 fusion proteins by recombinant FES was conducted in 40 μl of kinase buffer (50 mM HEPES, pH 7.4, and 10 mM MgCl2) containing 1 μg of GST-BCR fusion protein and 10 μCi of [γ-32P]ATP (3000 Ci/mmol, DuPont NEN). Phosphorylation reactions were incubated for 10 min at 30°C and stopped by heating at 95°C for 5 min in SDS-PAGE sample buffer. Phosphoproteins were resolved by SDS-PAGE and visualized by storage phosphor technology (Molecular Dynamics PhosphorImager). Two-dimensional tryptic phosphopeptide mapping and phosphoamino acid analysis are described elsewhere (24, 25).

**Construction and Expression of Full-length BCR Mutants**—The cDNA encoding full-length BCR was subcloned into pSP70 (Promega). A unique SacI-Stul BCR fragment was cut from pSP70/BCR and subcloned into pLSMA4 (a gift of Dr. Solon Rhode, Eppley Institute, University of Nebraska Medical Center). A unique BamHI-BgII BCR fragment was cut from pLSMA4/BCR and subcloned into pSP70 (Promega). BCR sequences encoding Tyr mutations in the 162–413 region were cut from the pGEX-2T constructs described above with NcoI and BglII and swapped with the corresponding wild-type BCR fragment in the pSP70/BCR construct. The resulting mutant fragments were cloned back through pLSMA4/BCR and pSP70/BCR to generate the full-length mutants. Full-length BCR wild-type or single Tyr to Phe mutants were then subcloned into the baculovirus transfer vector pVL1393 (Pharmingen) and the mammalian expression vector pSP72 (Promega). The combination mutant Y177F/Y246F was made by replacing the StuI RsrII fragment of full-length BCR Y246F with the corresponding fragment of BCR Y177F. BCR Y177F/Δ3Y, Y246F/Δ3Y, and Y177F/Y246F/Δ3Y were made by replacing the Δ3Y BosHI/SfiI fragment with the corresponding Y177F, Y246F, or Y177F/Y246F fragment. Preparation of recombinant baculoviruses and expression of BCR in Sf9 cells are described elsewhere (21, 23).

**In Vitro SH2 Domain Binding Assay**—pGEX vectors containing the coding sequences of the GRB2 and SHC SH2 domains were provided by Dr. Yoshiro Maru (Institute of Medical Science, University of Tokyo). pGEX vectors for expression of the ABL and phosphatidylinositol 3-kinase p85 subunit SH2 domains and the anti-BCR antibody Rb-1 were provided by Dr. Owen Witte (Howard Hughes Medical Institute, UCLA). Details of the BCR-SH2 domain binding assay are described elsewhere (21). Briefly, subconfluent monolayers of Sf9 cells were infected with recombinant BCR wild-type or mutant baculoviruses either alone or with a FBS baculovirus. Forty-eight hours postinfection, the cells were sonicated in 0.5 ml of lysis buffer (20 mM HEPES, pH 7, 0.15 M NaCl, 5 mM EDTA, 1% Triton X-100, 1.0 mM Na3VO4, 0.05 mM Na3MoO4, 20 mM NaF, 1 mM phenylmethylsulfonyl fluoride, 50 μg/ml aprotinin, and 25 μg/ml leupeptin). Cell lysates were clarified by microfuge centrifugation for 10 min at 4°C, and 0.1 ml aliquots were diluted with 0.9 ml incubation buffer (20 mM HEPES, pH 7.0, containing 150 mM NaCl, 0.1% Triton X-100, 10% glycerol, 1.0 mM Na3VO4, 0.05 mM Na3MoO4, 20 mM NaF, 1 mM phenylmethylsulfonyl fluoride, 50 μg/ml aprotinin, and 25 μg/ml leupeptin) and mixed with 100 μl immobilized GST-SH2 fusion protein. After 2 h of incubation at 4°C and then washing, the SH2-BCR fusion complexes were separated on SDS-PAGE, transferred to polyvinylidene difluoride membranes, and immunoblotted with the anti-BCR antibody Rb-1.

**RESULTS**

**Phosphorylation of the BCR 162–413 Region by FES in Vitro**—Recent work in our laboratory established that BCR is a target for the v-FPS and c-FES tyrosine kinases (21). Tyrosine phosphorylation by these kinases occurs within a region of the BCR N-terminal Ser/Thr kinase domain defined by amino acids 162–413 (21). This BCR region contains nine tyrosine residues, which represent potential phosphorylation sites, including Tyr-177, which is the presumptive GRB2 binding site (14, 15, 20, 21). To determine which of these Tyr residues are targeted by FES, we created a family of GST-BCR 162–413 fusion proteins with individual mutations of tyrosines 177, 231, 246, 316, 328, and 360 as well as a deletion of the closely spaced tyrosines 276, 279, and 283 (3Y cluster; see Fig. 1). The GST-BCR 162–413 fusion proteins were phosphorylated in vitro with recombinant FES and [γ-32P]ATP. As shown in Fig. 2, the wild-type GST-BCR 162–413 fusion protein was readily phosphorylated by FES as observed previously with the FES hom-
Tyrosine Phosphorylation of BCR by FES

**Fig. 2.** Phosphorylation of GST-BCR 162–413 fusion proteins in vitro. Wild-type (WT) and mutant GST-BCR 162–413 fusion proteins were expressed in *E. coli* and purified using glutathione-agarose. GST-BCR 162–413 proteins were phosphorylated with recombinant FES and [γ-32P]ATP and separated by SDS-PAGE. A, phosphorylated GST-BCR 162–413 proteins were detected by storage phosphor imaging. Autophosphorylated FES is also visible. B, Coomassie Blue stain of gel in A. C, relative 32P incorporation from A was corrected for protein levels (determined by laser densitometry of the stained gel shown in B) and plotted as phosphorylation/unit of protein relative to the wild-type control.

Tyrosine Phosphorylation of BCR by FES in Intact Cells—To verify that the same BCR sites phosphorylated by FES in vitro are also utilized in living cells, full-length BCR was expressed either alone or with FES in human 293T cells and labeled with [32P]P. Labeled BCR was immunoprecipitated and subjected to phosphoamino acid analysis and two-dimensional tryptic phosphopeptide mapping. As shown in Fig. 4A, BCR was phosphorylated primarily on Ser when expressed alone but was additionally phosphorylated on tyrosine when co-expressed with FES. Fig. 4B shows that BCR alone gives rise to nine Ser/Thr phosphopeptides, which are likely to arise from BCR autophosphorylation. Co-expression of BCR with FES gave rise to three additional phosphopeptides (Fig. 4B, peptides a, b, and c). Phosphoamino acid analysis of these three new phosphopeptides showed that they contain phosphotyrosine (data not shown).

To identify the BCR tyrosines phosphorylated by FES in vitro, FES was co-expressed with full-length BCR proteins containing the same series of tyrosine mutations shown in Fig. 1. The co-transfected cells were labeled with [32P]P, and BCR was immunoprecipitated and analyzed by two-dimensional tryptic mapping. As shown in Fig. 4B, mutation of Tyr-246 caused the loss of peptide b, while deletion of the 3Y cluster (Tyr-276, Tyr-279, and Tyr-283) caused the loss of peptide a. None of the point mutants in the BCR 162–413 region affected the phosphorylation of peptide c, indicating that this phosphorylation site falls outside of the 162–413 region (data not shown).

Two-dimensional tryptic mapping also showed that co-expression of BCR with FES caused the loss of serine phosphopeptide 9 from BCR (Fig. 4B). This observation suggests that tyrosine phosphorylation of BCR by FES may affect BCR autophosphorylation in vivo. As described in more detail below, Tyr phosphorylation of BCR by FES also reduced the Ser/Thr kinase activity of BCR toward the 14-3-3 protein, BAP-1. Alternatively, phosphorylation of this peptide on tyrosine may affect its position in the two-dimensional map.

Characterization of BCR-SH2 Domain Binding Specificity in Vitro—Previous work from our laboratory has shown that transformation of 3Y1 cells with the FES homolog v-FPS led to BCR/GRB2-SOS interaction via the GRB2 SH2 domain (21).
Tyrosine Phosphorylation of BCR by FES

Furthermore, tyrosine phosphorylation of BCR by FES strongly enhanced BCR binding to the SH2 domains of GRB2, ABL, p85, and other signaling proteins in vitro (21). To identify the tyrosine residues responsible for recruitment of specific SH2 domains, SH2 binding assays were conducted with wild-type and tyrosine phosphorylation site mutants of BCR. Recombinant SH2 domains from ABL, GRB2, p85 (C-terminal), and SHC were incubated with Sf9 cell lysates expressing full-length BCR or the Y177F, Y246F, and Δ3Y mutants either alone or with FES. Following incubation and washing, bound BCR proteins were visualized by immunoblotting. As shown in Fig. 5, tyrosine phosphorylation induced strong association of BCR with all of these GST-SH2 fusion proteins. Note that the concentrations of the SH2 fusion proteins used in these experiments was 100 nM, which is within the range of binding constants for physiological SH2-target protein interactions (27).

Mutagenesis of Tyr-177 to Phe completely abolished the FES-induced binding of BCR to the SH2 domain of GRB2 or ABL but did not affect binding to the other SH2 domains (Fig. 5). This result clearly identifies BCR Tyr-177 as the FES-induced site of BCR-GRB2 interaction and indicates that Tyr-177 is an in vivo phosphorylation site for FES. Deletions of the 3Y cluster specifically abolished binding of BCR to the SHC SH2 domain, suggesting that phosphorylation of this BCR region by FES creates a binding site for the SHC SH2 domain. By contrast, all of the single tyrosine mutants as well as Δ3Y bound to the ABL and p85 SH2 domains following FES-mediated phosphorylation, indicating that more than one phosphotyrosine residue or a phosphotyrosine residue outside of the BCR 162–413 region mediates these binding interactions.

Data shown in Fig. 5 demonstrate that tyrosine phosphorylation of BCR by FES greatly enhanced ABL and p85 SH2 domain binding, possibly by creating multiple phosphotyrosine-dependent sites. To test this hypothesis, SH2 binding assays were conducted using BCR proteins with all possible combinations of mutations of the FES phosphorylation sites (Y177F/Y246F, Y177F/Δ3Y, Y246F/Δ3Y, and Y177F/Y246F/Δ3Y mutants). As shown in Fig. 6, all of the BCR double mutants exhibited diminished binding to the ABL and p85 SH2 domains while the triple mutant (Y177F/Y246F/Δ3Y) did not bind to either of these SH2 domains. These results demonstrate that FES-mediated phosphorylation of multiple BCR tyrosine residues in the 162–413 region is required for maximal ABL and p85 SH2 domain binding.

Stimulation of FES Tyrosine Kinase Activity by BCR in Vivo—During the analysis of BCR phosphorylation in vivo, we observed that co-expression of BCR with FES enhanced the phosphorylation content of FES. This finding suggested that FES-BCR interaction may activate FES in vivo. To test this idea directly, FES was expressed alone or with BCR in 293T cells, and tyrosine autophosphorylation was assessed both in anti-FES immunoprecipitates and clarified cell lysates by anti-phosphotyrosine immunoblot analysis. As shown in Fig. 7, autophosphorylation of FES is very weak in vivo when expressed alone, consistent with published findings from other systems (28, 29). However, co-expression of FES with BCR strongly activated FES autophosphorylation, leading to extensive BCR phosphorylation. The BCR mutants Y177F, Y246F, Δ3Y, Y177F/Y246F, and Y177F/Δ3Y, all of which are phosphorylated by FES, activated FES autophosphorylation to...
almost the same extent as wild-type BCR. However, co-expression with Y246F/D3Y, which is weakly phosphorylated by FES, did not activate FES. Co-expression with Y177F/Y246F/D3Y mutant, which lacks all known Tyr phosphorylation sites for FES, completely suppressed FES autophosphorylation. These results suggest that BCR can stimulate FES tyrosine kinase activity in vivo and that tyrosine phosphorylation of BCR is required for this effect.

To determine if the effect of BCR on FES is unique to mammalian cells, the same experiment was conducted in Sf9 insect cells. As shown in Fig. 8, FES autokinase activity was very strong when expressed alone, and co-expression with wild-type and mutant forms of BCR had no additional activating effect. These findings suggest that BCR can stimulate FES tyrosine kinase activity in vivo and that tyrosine phosphorylation of BCR is required for this effect.

To verify that the effect of BCR on FES tyrosine phosphorylation was direct and not mediated by activation of another tyrosine kinase, we co-expressed BCR and a kinase-defective mutant of FES (K590E mutant) (24) in 293T cells. No SH2 domain binding of these BCR mutants was observed in the absence of FES co-expression (data not shown). However, our data cannot rule out the possibility that BCR could inhibit a phosphotyrosine phosphatase unique to mammalian cells.

Tyrosine Phosphorylation of BCR by FES

Tyrosine Phosphorylation by FES Suppresses BCR Ser/Thr Kinase Activity Toward the 14-3-3 Protein, BAP-1—Tryptic phosphopeptide analysis shows that tyrosine phosphorylation may affect BCR autophosphorylation (Fig. 4). To determine whether tyrosine phosphorylation of BCR affects its Ser/Thr kinase activity toward a substrate, BCR was expressed either alone or with FES in 293T cells followed by in vitro kinase assay with the 14-3-3 protein and BCR substrate, BAP-1 (3). As shown in Fig. 9, co-expression with FES suppressed BAP-1 phosphorylation by BCR by more than 60%. Control experiments showed that equal amounts of BCR are present in each immunoprecipitate and that the end point of the reaction shown falls on the linear portion of the progress curve for the phosphorylation reaction (data not shown). We also verified that BAP-1 is not a substrate for FES and does not bind to FES.
Tyrosine Phosphorylation of BCR by FES

Tyrosine phosphorylation is also likely to influence the serine/threonine kinase activity of BCR in vivo. Results shown in Fig. 9 demonstrate that tyrosine phosphorylation of BCR by FES suppresses BCR serine/threonine kinase activity toward the 14-3-3 protein BAP-1 (8). The mechanism of this suppression may involve decreased affinity of BCR for BAP-1 as a result of tyrosine phosphorylation. A recent study has shown that 14-3-3 proteins bind to serine-phosphorylated sequences within target proteins with high affinity and specificity but show no affinity for the unphosphorylated sequence (31). Tyrosine phosphorylation of BCR by FES may inhibit the serine autophosphorylation of BCR in vivo (Fig. 4), resulting in the loss of a binding site for BAP-1 and other 14-3-3 proteins. The 14-3-3 proteins have been shown to link BCR and RAF in vivo, which may alter the subcellular localization and function of these kinases (22). Decreased phosphorylation of BAP-1 as a result of FES-induced tyrosine phosphorylation may influence signal transduction by both BCR and BCR-ABL and affect their ability to interact with RAF or other signaling partners via 14-3-3 in vivo.

Acknowledgments—We thank Y. Maru (University of Tokyo), O. N. Witte (Howard Hughes Medical Institute, UCLA), and D. E. H. Afar (UCLA) for reagents and for helpful discussions.

REFERENCES
1. Timmons, M. S., and Witte, O. N. (1989) Oncogene 4, 559–567
2. Maru, Y., and Witte, O. N. (1991) Cell 67, 459–468
3. Reuther, G. W., Fu, H., Cripe, L. D., Collier, R. J., and Pendergast, A. M. (1994) Science 266, 129–133
4. Chuang, T. H., Xu, X., Kaartinen, V., Heisterkamp, N., Groffen, J., and Bokoch, G. M. (1995) Proc. Natl. Acad. Sci. U.S.A. 92, 10282–10286
5. Dickmann, D., Brill, S., Garrett, M. D., Totty, N., Hsuan, J., Monfries, C., Hall, C., Lim, L., and Hall, A. (1991) Nature 351, 400–402
6. Hart, M. J., Maru, Y., Leonard, D., Witte, O. N., Evans, T., and Cerione, R. A. (1992) Science 256, 812–815
7. Abo, A., Pick, E., Hall, A., Totty, N., Teahan, C. G., and Segal, W. (1991) Nature 353, 668–670
8. Vajkoczy, P., and Cooper, J. A. (1995) Cell 82, 527–529
9. Chant, J., and Stowers, L. (1995) Cell 81, 1–4
10. Vojtek, A. B., and Cooper, J. A. (1995) Science 266, 129–133

DISCUSSION

Using a combination of tryptic phosphopeptide mapping and SH2 domain binding assays, we have identified BCR Tyr-177, Tyr-246, and one or more of three closely spaced tyrosine residues (Tyr-276, Tyr-279, Tyr-283, the 3Y cluster) as phosphorylation sites for FES in living cells. Phosphorylation of Tyr-177 and the 3Y cluster creates specific bindings sites for the SH2 domain of GRB2 and SHC, suggesting that BCR may serve as an intermediate linking FES to the RAS signal transduction pathway. These results are consistent with our previous finding that tyrosine-phosphorylated BCR complexes with GRB2-SOS in fibroblasts transformed with the FES homolog, v-FPS (21). BCR also possesses regulatory domains for small G-proteins of the RHO family, including RAC and CDC42 (5, 6). Recent studies have shown that activation of these GTPases is required for transformation by RAS and for normal and oncogenic signal transduction by tyrosine kinases (reviewed in Ref. 8). In this regard, BCR may serve to integrate tyrosine kinase signaling through the RAS and RHO signaling pathways. A recent study has mapped several BCR tyrosine residues that are phosphorylated within BCR-ABL (20). Although FES shares some of these phosphorylation sites (Tyr-177 and possibly Tyr-283), both kinases phosphorylate unique BCR sites as well. For example, BCR-ABL but not FES phosphorylates BCR Tyr-360 (20), while FES uniquely phosphorylates BCR Tyr-246. Although the result of specific phosphorylation of BCR Tyr-246 by FES is unclear at present, it may create an additional site for the recruitment of SH2 domain effectors to the BCR N-terminal domain. Alternatively, phosphorylation of this site could affect SH2 domain binding indirectly by altering the conformation of the BCR N-terminal domain or influence BCR Ser/Thr kinase activity (see below).

Data presented here suggest that BCR may serve as a positive regulator of FES tyrosine kinase activity in vivo. Like other cytoplasmic protein-tyrosine kinases, FES has tyrosine autokinase activity that is readily detectable in an in vitro immune complex kinase assay. Autophosphorylation is also observed in vivo when FES is expressed in non-mammalian systems, such as Sf9 cells (Fig. 7). However, Tyr autophosphorylation of FES is strongly inhibited in mammalian cells (26, 28, 29), suggesting that it is regulated in trans by a factor unique to mammalian cells. Our results show that co-expression of FES with BCR strongly stimulated FES tyrosine kinase activity in human cells, suggesting that BCR is a positive regulatory factor for FES. Previous work from our laboratory has shown that the FES unique N-terminal and SH2 domains bind to BCR (21). FES-BCR interaction may lead to displacement of a negative regulatory protein and release of FES tyrosine kinase activity. The ability of BCR to activate FES is in some ways analogous to the activation of the ABL tyrosine kinase within BCR-ABL. Like FES, the c-ABL tyrosine kinase may be negatively regulated by non-covalent association with a cellular factor, such as the recently described ABL binding protein, Abi-2 (30). Fusion to BCR releases the tyrosine kinase activity of ABL, an effect that may be dependent upon direct interaction of the ABL SH2 domain with BCR-derived sequences (13). Such an interaction may prevent interaction with the ABL regulatory factor.

Tyrosine phosphorylation of BCR by FES suppresses BCR serine/threonine kinase activity toward the 14-3-3 protein BAP-1 (3). The mechanism of this suppression may involve decreased affinity of BCR for BAP-1 as a result of tyrosine phosphorylation. A recent study has shown that 14-3-3 proteins bind to serine-phosphorylated sequences within target proteins with high affinity and specificity but show no affinity for the unphosphorylated sequence (31). Tyrosine phosphorylation of BCR by FES may inhibit the serine autophosphorylation of BCR in vivo (Fig. 4), resulting in the loss of a binding site for BAP-1 and other 14-3-3 proteins. The 14-3-3 proteins have been shown to link BCR and RAF in vivo, which may alter the subcellular localization and function of these kinases (22). Decreased phosphorylation of BAP-1 as a result of FES-induced tyrosine phosphorylation may influence signal transduction by both BCR and BCR-ABL and affect their ability to interact with RAF or other signaling partners via 14-3-3 in vivo.

Acknowledgments—We thank Y. Maru (University of Tokyo), O. N. Witte (Howard Hughes Medical Institute, UCLA), and D. E. H. Afar (UCLA) for reagents and for helpful discussions.
Heisterkamp, N. (1995) Cell 80, 719–728
11. Sawyers, C. L. (1992) Cancer Surv. 15, 37–51
12. McWhirter, J. R., Galasso, D. L., and Wang, J. Y. J. (1993) Mol. Cell. Biol. 13, 7587–7595
13. Pendergast, A. M., Muller, A. J., Havlik, M. H., Maru, Y., and Witte, O. N. (1991) Cell 66, 161–171
14. Pendergast, A. M., Quilliam, L. A., Cripe, L. D., Bassing, C. H., Dai, Z., Li, N., Batzer, A., Rabun, K. M., Der, C. J., Schlessinger, J., and Gishizky, M. L. (1993) Cell 75, 175–185
15. Puil, L., Liu, J., Gish, G., Mhamalu, G., Bostell, D., Pelicci, P. G., Arlinghaus, R., and Pawson, T. (1994) EMBO J. 13, 764–773
16. Goga, A., McLaughlin, J., Afar, D. E. H., Saffran, D. C., and Witte, O. N. (1995) Cell 82, 981–988
17. Sawyers, C. L., McLaughlin, J., and Witte, O. N. (1995) J. Exp. Med. 181, 307–313
18. Lu, D., Liu, J., Campbell, M., Guo, J. Q., Heisterkamp, N., Groffen, J., Canaani, E., and Arlinghaus, R. (1993) Blood 82, 1257–1263
19. Liu, J., Campbell, M., Guo, J. Q., Lu, D., Xian, Y. M., Anderson, B. S., and Arlinghaus, R. B. (1995) Oncogene 8, 101–109
20. Liu, J., Wu, Y., Ma, G. Z., Lu, D., Haataja, L., Heisterkamp, N., Groffen, J., and Arlinghaus, R. B. (1996) Mol. Cell. Biol. 16, 998–1005
21. Maru, Y., Peters, K. L., Afar, D. E. H., Shibuya, M., Witte, O. N., and Smithgall, T. E. (1995) Mol. Cell. Biol. 15, 835–842
22. Braselmann, S., and McCormick, F. (1995) EMBO J. 14, 4839–4848
23. Briggs, S. D., Bryant, S. S., Jeve, R., Sanderson, S. D., and Smithgall, T. E. (1995) J. Biol. Chem. 270, 14718–14724
24. Hjermstad, S., Peters, K. L., Briggs, S. D., Glazer, R. I., and Smithgall, T. E. (1995) Oncogene 8, 2283–2292
25. Hjermstad, S. J., Briggs, S. D., and Smithgall, T. E. (1993) Biochemistry 32, 10519–10525
26. Rogers, J. A., Read, R. D., Li, J., Peters, K. L., and Smithgall, T. E. (1996) J. Biol. Chem. 271, 17519–17525
27. Ladbury, J. E., Lemmon, M. A., Zhou, M., Green, J., Botfield, M. C., and Schlessinger, J. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 3199–3203
28. MacDonald, I., Levy, J., and Pawson, T. (1985) Mol. Cell. Biol. 5, 2543–2551
29. Greer, P. A., Meckling-Hansen, K., and Pawson, T. (1988) Mol. Cell. Biol. 8, 578–587
30. Dai, Z., and Pendergast, A. M. (1996) Genes Dev. 9, 2569–2582
31. Muslin, A. J., Tanner, J. W., Allen, P. M., and Shaw, A. S. (1996) Cell 84, 889–897