described (*2*), except that cells were washed with H_2O instead of phosphate-buffered saline (PBS) [10 mM sodium phosphate (pH 7.4) and 150 mM NaCl] before lysis, and immunoprecipitates were washed with PBS containing 0.1% NP-40. Immunoprecipitation was done with 25 μl of either protein A–Sepharose coupled (*29*) to antibodies to HA (*23*) or protein G–Sepharose coupled (*29*) to antibodies to Py (*15*).

31. Polyclonal serum to PHO85 was derived from rabbits immunized with purified glutathione-S-transferase (GST)–PHO85 protein. The plasmid producing GST-PHO85 consists of PHO85 DNA coding for amino acids 4 to 306 (*27*) cloned into pGEX-2T (Pharmacia). Antibodies to PHO85 were affinity-purified from serum with a GST-PHO85 column made by coupling the purified protein to AffiGel-10 (Bio-Rad).
32. To purify Py_2-PHO81, we grew 4 liters of the strain Y57 *pho81*Δ carrying the GPD-Py_2-PHO81 expression vector (*9*) to a value of A_{600} of ~0.9 in synthetic medium containing a high concentration of phosphate. Cells were harvested and resuspended in 50 ml of ice-cold Py_2 buffer [20 mM tris Cl (pH 8.0), 100 mM NaCl, 10% glycerol, 1 mM EGTA, 1 mM $MgCl_2$, 1 mM dithiothreitol (DTT), 1 mM PMSF, 2 mM benzamidine-HCl, 80 mM β-glycerophosphate, 10 mM NaF, and 10 nM calyculin A (LC Laboratories, Woburn, MA)]. All subsequent steps except the elution were done at 4°C. Acid-washed glass beads were added, and the cells were lysed in a bead beater (Biospec Products, Bartlesville, OK) by beating for seven 30-s pulses with 1-min interludes on ice. Beads were pelleted by spinning the lysate for 5 min at 2000 rpm in a Sorvall RC-3B rotor. Debris was removed by spinning the supernatant in a Beckman Ti70 rotor for 90 min at 60,000 rpm. The supernatant contained ~40 mg of protein in ~40 ml. *N*-Octylglucoside (Boehringer Mannheim) was added to the supernatant to a final concentration of 0.1%, and PMSF was added to a final concentration of 1 mM; the solution was spun again for 10 min in an SS34 rotor at 10,000 rpm. The supernatant was combined with 750 μl of a 1:1 slurry of protein G–Sepharose coupled (*29*) to antibodies to Py (*15*) and mixed on a rotating wheel for 60 min. The resin was washed twice with 15 ml of Py_2-buffer containing 0.1% *N*-octylglucoside, then twice with 15 ml of Py_2-buffer containing 0.5% NP-40 and 1 mM DTT. Finally, the resin was washed once with 10 ml of PBS with 0.1% *N*-octylglucoside and then eluted by incubation for 10 min at room temperature with 250 μl of PBS containing 0.1% *N*-octylglucoside and 100 μg/ml Py peptide (EYMPME) (*24*). The eluted protein was approximately 10 to 20 μg of highly purified Py_2-PHO81.
33. Unless noted, all strains are isogenic derivatives of Y57 (*MAT***a** *ura3-52 trp1-Δ63 leu2 his3-Δ1 prb1-1122 pep4-3 prc1-407*), which is protease-deficient but wild-type with respect to the *PHO* genes (*2*). Disruption strains were constructed by one-step gene replacement techniques [R. Rothstein; *Methods Enzymol.* **194**, 281 (1991)].
34. Five-milliliter cultures of either Y57 *pho81*Δ or Y57 *pho80*Δ harboring the pACHA$_3$80 vector (*3*) were grown overnight to saturation in synthetic medium lacking uracil but with a high phosphate concentration. The cells were harvested, resuspended in an equal volume of H_2O, and then inoculated into 100 ml of selective medium that had been depleted of inorganic phosphate [K. F. O'Connell and R. E. Baker, *Genetics* **132**, 63 (1992)] and supplemented with either KH_2PO_4 (1.5 mg/ml, high phosphate concentration) or KCl (1.5 mg/ml, low phosphate concentration). These cultures were grown for approximately 15 hours to a value of A_{600} of 0.5 to 1.0. Liquid whole-cell phosphatase assays were performed (*22*) to compare the levels of acid-phosphatase activity in these cultures. The relative amounts of such activity normalized to A_{600} were pACHA$_3$80/*pho80*Δ (low phosphate concentration), 105; pACHA$_3$80/*pho80*Δ (high phosphate concentration), 31; pACHA$_3$80/*pho81*Δ (low phosphate concentration), 7; and pACHA$_3$80/*pho81*Δ (high phosphate concentration), 17. We believe that the relatively modest induction that we observed in low phosphate concentrations in the *pho80*Δ strain carrying the complementing pACHA$_3$80 plasmid is due at least in part to the presence of the *PHO3* constitutive acid phosphatase gene [W. Bajwa *et al.*, *Nucleic Acids Res.* **12**, 7721 (1984)]. Lysates were prepared from these cultures (*30*) and processed as described above.
35. After transfer to nitrocellulose, the blot was probed with either affinity-purified antibodies to PHO81 (*16*) or affinity-purified antibodies to PHO85 (*31*) in TBST [10 mM tris-Cl (pH 8.0), 150 mM NaCl, 0.05% Tween-20, and 0.25 mM EDTA] containing 3% nonfat milk. Peroxidase coupled to donkey antibody to rabbit immunoglobulin G (Amersham) was used as the secondary antibody.
36. After immunoprecipitation (*30*) and a kinase assay (*2*) were done, the beads were sedimented for 2 min in a microfuge, and 20 μl of the supernatant was transferred to a second immunoprecipitate. This mixture was then incubated for an additional 5 min at room temperature before the reactions were stopped with SDS sample buffer.
37. Silver-stained SDS-polyacrylamide gels of HA-PHO80 (*2*) immunoprecipitation reactions done in parallel indicated that each reaction contained approximately the same amount of both HA-PHO80 and PHO85 (*12*). This gel also indicated that PHO81 is present in the HA-PHO81 immunoprecipitation and in the HA-PHO80 immunoprecipitations from strains overexpressing PHO81.
38. HA-PHO81 consists of amino acids 36 to 1179 of PHO81 preceded by a single copy of the HA epitope (*23*). The NH_2-terminal sequence of the construct reads MGYPYDVPDYAIEGRHTIP-PHO81 (*24*). The expression of this construct is under the control of the *GPD* promoter on a CEN-ARS plasmid (*25*).
39. HA-PHO80 was immunoprecipitated (*2*) from a whole-cell extract [M. Woontner *et al.*, *Mol. Cell Biol.* **11**, 4555 (1991)] made from a *pho4*Δ*pho80*Δ strain overexpressing HA-PHO80 or from the same cells lacking the overexpressed HA-PHO80. The immunoprecipitates were washed with kinase buffer [20 mM tris Cl (pH 7.5) and 10 mM $MgCl_2$] and incubated for 10 min at room temperature with either purified PHO81 ankyrin repeats (*28*), equivalent fractions from bacteria not expressing the PHO81 ankyrin repeats, or purified Py_2-PHO81 (*32*). A kinase assay was then done with PHO4 as the substrate (*2*).
40. We thank Y. Oshima and A. Toh-e for providing strains and plasmids; A. Kaffman, D. Rio, M. Peter, I. Herskowitz, D. Morgan, F. H. Espinoza, and the University of California, San Francisco, Cell Cycle Club for helpful discussions; F. H. Espinoza for the PHO85-HA construct; B. Andrews for the GST-PHO85 vector; P. O'Farrell, I. Herskowitz, B. O'Neill, and M. Lenburg for comments on the manuscript; D. Rio, B. O'Neill, and K. Lopardo for the antibody to polyoma medium T antigen and peptide; and W. Lau for construction of the T7 His-PHO81 ankyrin repeats construct. Supported in part by the Lucille P. Markey Charitable Trust.

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Interaction of the Protein Kinase Raf-1 with 14-3-3 Proteins

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Members of a family of highly conserved proteins, termed 14-3-3 proteins, were found by several experimental approaches to associate with Raf-1, a central component of a key signal transduction pathway. Optimal complex formation required the amino-terminal regulatory domain of Raf-1. The association of 14-3-3 proteins and Raf-1 was not substantially affected by the activation state of Raf.

Raf-1, a mitogen-stimulated serine-threonine protein kinase, functions in the control of cell growth, transformation, and differentiation (*1*). Binding of ligands to tyrosine kinase receptors at the cell surface leads to an increase in the amount of the active [guanosine triphosphate (GTP)–bound] form of Ras (*2*). Activated Ras interacts directly with the NH_2-terminal regulatory domain of Raf-1 (*3*), resulting in the recruitment of Raf-1 to the plasma membrane (*4, 5*). There, Raf-1 is activated by an unknown mechanism that is independent of Ras. Because Raf-1 exists as a large (300- to 500-kD) complex (*5, 6*), identification and analysis of proteins that interact with Raf-1 are crucial for understanding how it is activated in the plasma membrane.

To search for proteins that directly participate in Raf function, we immunoprecipitated Raf-1 from ^{35}S-methionine–labeled NIH 3T3 cells stably expressing human Raf-1 in amounts about five times greater than the amount of endogenous Raf-1. When Raf immune complexes were analyzed on two-dimensional isoelectric focusing–SDS-polyacrylamide gels, four major protein spots (of 27, 29, 50, and 90 kD) were found that were absent in preimmune controls (Fig. 1, A and B) (*7*). The 90-kD and 50-kD proteins are the molecular chaperones hsp90 and p50 (*6*). The migration of the p27 and p29 proteins on these gels was similar to that of 14-3-3 proteins, which had been identified previously as polyoma-

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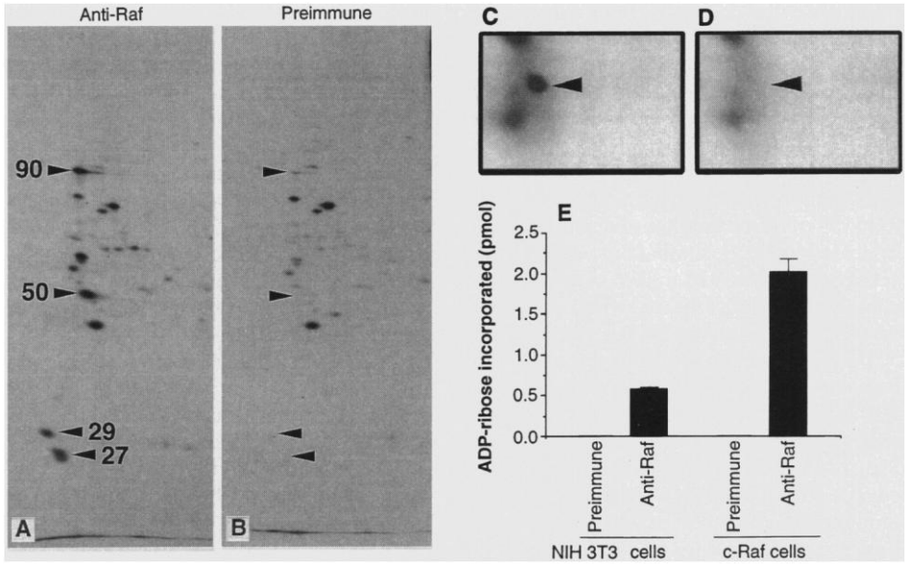

Fig. 1. Characterization of the 27- and 29-kD Raf-associated proteins. (**A** and **B**) To examine Raf-associated proteins in mammalian cells, lysates of ^{35}S-labeled cells overexpressing c-Raf (*25*) were immunoprecipitated with (A) rabbit antiserum to Raf (*26*) or (B) preimmune serum from the same rabbit and the immunoprecipitates analyzed on 2D gels (*27*). The position and size (in kilodaltons) of the major Raf-associated proteins are indicated. The 50- and 90-kD proteins have been described previously (*6*). The 27- and 29-kD proteins migrate in positions corresponding to those of 14-3-3 proteins. Raf-1 migrates in a streak located in the right half of our two-dimensional gels, which is not shown. (**C** and **D**) Raf immunoprecipitates from cells overexpressing c-Raf were probed for the presence of 14-3-3 proteins. Immunoprecipitates were prepared with rabbit antiserum to Raf (C) or with preimmune serum (D), analyzed on two-dimensional gels, and immunoblotted with rabbit polyclonal antiserum specific for a peptide found in several subspecies of the 14-3-3 proteins (*8*). Arrowheads in (C) and (D) indicate the position of migration of an immunoreactive spot corresponding to a protein of 27 kD. (**E**) Raf immunocomplexes (2.5 μl) prepared from NIH 3T3 cells or cells overexpressing c-Raf and extensively washed were tested for their ability to activate the ADP-ribosylating activity of exoenzyme S from *P. aeruginosa* (*13*).

virus middle tumor antigen (MT)–associated proteins (*8*). Consistent with this assignment, the 27-kD form in Raf immune complexes immunoblotted with a polyclonal antibody that recognizes several 27-kD isoforms of 14-3-3 protein (*8*) (Fig. 1, C and D). The 14-3-3 proteins are a family of highly conserved dimeric molecules. In yeast, 14-3-3 proteins have been implicated in cell cycle control (*9*). A variety of biochemical activities have been reported for members of this protein family (*10*), including one that provides a sensitive assay for these proteins. 14-3-3 proteins serve as obligatory activators of *Pseudomonas aeruginosa* exoenzyme S, an adenosine 5′-diphosphate (ADP)–ribosyltransferase with a substrate preference for Ras and selected small GTP-binding proteins (*11*). This property, denoted factor activating exoenzyme S (FAS) activity, is common to 14-3-3 proteins from various sources (*12*). When we examined Raf-1 immunoprecipitates from normal NIH 3T3 cells and from the NIH 3T3 cells overproducing Raf-1, FAS activity was present in Raf-1 immunoprecipitates from both cell types but was three to five times greater in the Raf-overproducing line (Fig. 1E) (*13*).

To further examine the interaction of Raf-1 with 14-3-3 proteins, we turned to the baculovirus expression system (*14*, *15*). The earlier discovery that polyomavirus MT interacts with insect 14-3-3 proteins suggested that mammalian Raf-1 might also interact with the insect proteins. Insect cells were infected with baculoviruses expressing mammalian Raf-1 or various truncated forms of Raf-1. Raf immunoprecipitates from the Raf-1–expressing cells, but not control cells, contained large amounts

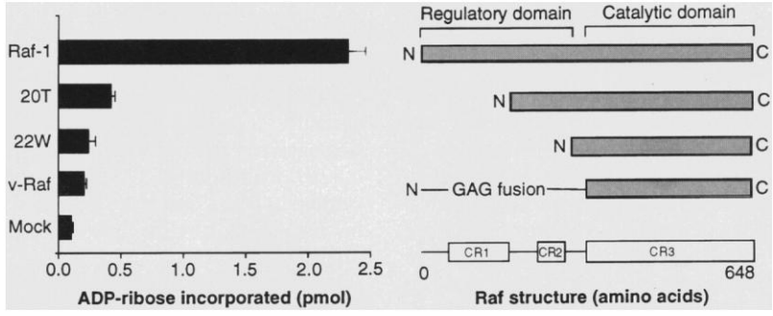

Fig. 2. Association of FAS activity with Raf mutants. Anti-Raf immunoprecipitates were prepared from wild-type (mock), or recombinant baculovirus–infected insect cells expressing Raf-1, Raf-20T, Raf-22W, and v-Raf as described (*14*, *15*). Immunoprecipitates (1.25 μl) were assayed for ability to activate the ADP-ribosyltransferase activity of exoenzyme S (*13*). Normalization was done by protein immunoblotting with an antibody that recognizes an epitope shared by all Raf-1 alleles tested. The right panel indicates the corresponding structure of the Raf proteins expressed in insect cells.

of FAS activity, indicating that Raf-1 is associated with 14-3-3 proteins in insect cells (Fig. 2) (*16*). Structural features of Raf-1 required for the interaction with 14-3-3 proteins were examined. Raf-1, a 648–amino acid polypeptide, consists of an NH_2-terminal regulatory domain and a COOH-terminal kinase domain. The NH_2-terminal domain is further subdivided into conserved region 1 (CR1), a region that contains a zinc finger motif and a Ras binding region, and conserved region 2 (CR2), a serine-rich region. The deletion mutant Raf-20T lacks CR1, whereas Raf-22W lacks the entire regulatory NH_2-terminal domain (*17*). Raf-20T immunoprecipitates contained 15 to 20% of the FAS activity from cells expressing wild-type Raf-1, whereas Raf-22W immunecomplexes contained only ~8 to 10% of the activity associated with wild-type Raf-1. v-Raf, a virus-generated mutant in which the NH_2-terminal regulatory region is replaced by viral sequences, showed about the same amount of 14-3-3 binding activity as Raf-22W. In the above experiments, the amount of 14-3-3 proteins measured by blotting paralleled the amount of FAS activity in the various Raf immunoprecipitates (*18*). In a reciprocal experiment, an immobilized full-length 14-3-3 ζ/glutathione-S-transferase (GST) fusion protein (but not GST alone) was found to bind Raf-1 from baculovirus-infected insect cells. The truncated forms of Raf (Raf-22W, Raf-20T, and v-Raf) in cell lysates also bound to GST–14-3-3 ζ, albeit with decreased efficiency (*18*).

The influence of the activation state of Raf-1 on the binding of 14-3-3 proteins was also tested. In the baculovirus system, two signals must synergize for the efficient activation of Raf-1 autophosphorylation and substrate-directed kinase activities. One signal is supplied by Ras and presumably serves

to localize Raf-1 to the membrane. The second signal can be supplied by $pp60^{c\text{-}Src}$ and is independent of Ras (*14*, *19*). Either signal alone gives a modest activation of Raf-1. The association of 14-3-3 proteins with Raf-1 in various activation states was measured by monitoring of the amount of FAS activity in Raf-1 immunoprecipitates from different recombinant baculoviruses as indicated in Fig. 3. Similar amounts of FAS activity were detected in Raf-1 immunoprecipitates from cells infected with only Raf-1 or with the catalytically inactive mutant 301 protein or from cells infected with both Raf and Ras. Slightly larger amounts of Raf-associated FAS activity were consistently detected in cells infected with Raf, Ras, and v-Src, in which Raf-1 was activated 10- to 100-fold. No FAS activity was immunoprecipitated with antisera to Src or Ras.

We tested the ability of purified 14-3-3 proteins to modulate the kinase activity of Raf-1. Raf-1 immunoprecipitates from Sf9 cells were tested for kinase activity in the presence or absence of 14-3-3 ζ protein purified from *Escherichia coli* (Table 1) (*20*). Five preparations of 14-3-3 ζ were tested. Addition of one preparation of 14-3-3 ζ increased the autophosphorylation and MAP kinase/ERK–kinase-1 (MEK-1) kinase activity of Raf-1 two- to threefold; however, four other preparations had no stimulatory activity. Addition of 14-3-3 ζ had the same effect on Raf-1 isolated from insect cells co-infected with Raf-1 and Ras viruses. All batches of 14-3-3 ζ had comparable FAS activity (Table 1). We have no explanation for the variation in 14-3-3 protein preparations. Trivial explanations for the activation by the first preparation, such as the possibility of a contaminating MEK kinase activity or buffer effects, have been ruled out.

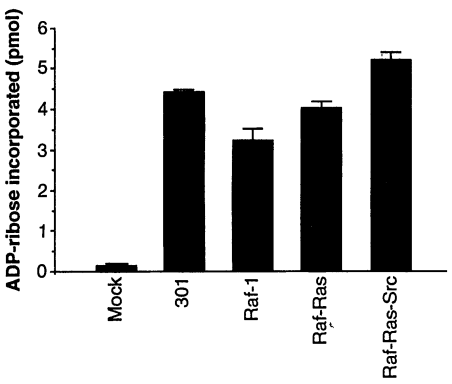

Fig. 3. Effect of the activation state of Raf-1 on the binding of 14-3-3 protein. Anti-Raf immunoprecipitates were prepared from Sf9 cells singly infected with recombinant baculoviruses encoding Raf-1 alone or kinase-inactive Raf-1 mutant 301, or cells doubly infected with baculoviruses encoding Raf-1 and Ras, or cells triply infected with all three baculoviruses encoding Raf-1, Ras, and $pp60^{v\text{-}Src}$ Extensively washed immunoprecipitates were tested for their ability to activate exoenzyme S (*13*). Data were normalized on the basis of the amount of Raf revealed by protein immunoblotting.

Taken together, our results indicate that 14-3-3 proteins interact with Raf at multiple sites and suggest that the primary interaction sites are located in the NH_2-terminal domain. The CR1 region of Raf-1 contains zinc finger motifs as do two other proteins known to bind 14-3-3–polyomavirus MT and c-Bcr (*21*). Furthermore, the CR2 domain of Raf-1 also has similarity to the serine-threonine–rich region in the first exon of c-Bcr (*21*). However, the kinase domain of Raf-1 alone clearly has some 14-3-3–binding ability. This binding pattern distinguishes 14-3-3 proteins from hsp90 and p50, which bind strongly to the COOH-terminal catalytic domain of the Raf-1 protein (*22*). Further work is needed to determine the precise structural features required for the interaction. For full-length Raf-1, the amount of FAS activity in Raf-1 immunoprecipitates was roughly proportional to the amount of Raf-1 expression in the cell. The 14-3-3 proteins do not interact with all protein kinases. No interaction was detected with protein kinase C (PKC) α (*18*), even though PKCs are structurally similar to Raf-1 and have been reported to be regulated in vitro by 14-3-3 proteins (*10*).

It is premature at this point to propose a precise role of the 14-3-3 proteins in the Ras-Raf signaling pathway. Our data suggest that 14-3-3 ζ does not substantially activate Raf-1 in vitro but do not rule out the possibility that another isoform of 14-3-3 protein might activate (or inhibit) Raf-1 or that 14-3-3 protein must be modified in some manner in which it is not modified in *E. coli*. Interpretation of either an activation or inhibition by 14-3-3 protein would not be straightforward, because the interaction between 14-3-3 protein and Raf-1 appears to be constitutive. 14-3-3 protein bound to both unactivated Raf-1 (from single infection) and a catalytically inactive mutant of Raf-1 (Fig. 3). If Raf-1 is regulated by 14-3-3 proteins in cells, we are currently at a loss to suggest a mechanism to turn a constitutive interaction into a regulated activation. Alternatively, it is possible that 14-3-3 protein may function downstream of Raf-1.

Table 1. Effect of 14-3-3 ζ on Raf-1 kinase activity. Five preparations were purified individually as described (*11*). Preparation 4 was further purified by a mono-Q ion exchange column on fast protein liquid chromatography. Preparation 5 contains a hexahistidine tag. Raf activation was calculated by phosphorylation amounts of MEK-1 in the presence or absence of 14-3-3 proteins normalized for Raf concentration. FAS activity was assayed basically as described (*13*), except that the reactions were incubated for 30 min and the final concentration of FAS used was 0.25 μg/ml.

Preparation of 14-3-3	Raf activation (fold)	FAS activity (pmol)
1	2.60	3.8
2	0.82	4.4
3	0.86	4.2
4	0.89	4.5
5	0.80	4.4

The ADP-ribosylation cofactor activity of 14-3-3 proteins is conserved in evolution; that is, yeast 14-3-3 substitutes for mammalian 14-3-3 in ADP-ribosylation catalyzed by exoenzyme S (*12*). The ADP-ribosylation cofactor activity we are measuring may well be a surrogate for an important physiological role of the 14-3-3 proteins. At the moment, we would only remark that, by means of its association with Raf-1, 14-3-3 protein is complexed in vivo with a protein (Raf-1) immediately proximal to its preferred target of ADP-ribosylation by exoenzyme S in vitro (Ras).

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with dithiothreitol (DTT) (1 mM), phenylmethylsulfonyl fluoride (1 mM), aprotinin (0.15 unit/ml), and sodium orthovanadate (1 mM). Lysates were stored at −80°C. Immunoprecipitations were done as described (*23*), except that $LiCl_2$ washes were omitted.
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20. The anti-Raf immunoprecipitates were prepared as described (*16*) and washed one more time with kinase buffer [25 mM Hepes (pH 7.4), 1 mM DTT, 10 mM $MgCl_2$, and 10 mM $MnCl_2$] before assay. For kinase reactions, washed immunoprecipitates were incubated in 40 μl of kinase buffer containing 15 μM nonradioactive adenosine triphosphate (ATP), 10 μCi (370 kilobecquerels) of [γ-^{32}P]ATP (3000 Ci/mmol), and 0.2 μg of 5′-*p*-fluorosulfonyl-bezoyladenosine–treated MEK-1 at room temperature for 30 min in the presence or absence of purified 14-3-3 protein or 14-3-3 protein that had been boiled in kinase buffer for 4 min. The assays were terminated by addition of Laemmli sample buffer and resolved by SDS–polyacrylamide gel electrophoresis. The phosphorylation of MEK-1 was quantitated by Phosphorimager, Molecular Dynamics, Sunnyvale, CA, and the amount of Raf-1 protein in each lane was quantitated by a protein immunoblot and Fluroimager, Molecular Dynamics, Sunnyvale, CA.
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Association of the Protein Kinases c-Bcr and Bcr-Abl with Proteins of the 14-3-3 Family

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In this study, a protein that interacts with sequences encoded by the first exon of the protein kinase Bcr was cloned. The Bcr-associated protein 1 (Bap-1) is a member of the 14-3-3 family of proteins. Bap-1 interacts with full-length c-Bcr and with the chimeric Bcr-Abl tyrosine kinase of Philadelphia chromosome (Ph^1)–positive human leukemias. Bap-1 is a substrate for the Bcr serine-threonine kinase and is also phosphorylated on tyrosine by Bcr-Abl but not by c-Abl. Bap-1 may function in the regulation of c-Bcr and may contribute to the transforming activity of Bcr-Abl in vivo. 14-3-3 proteins are essential for cell proliferation and have a role in determining the timing of mitosis in yeast. Through direct binding to sequences present in Bcr and in other proteins implicated in signaling, the mammalian 14-3-3 proteins may link specific signaling protein components to mitogenic and cell-cycle control pathways.

The product of the *bcr* gene is a 160-kD protein with multiple functional and structural domains. Among the functional domains ascribed to c-Bcr are a serine-threonine kinase encoded by the NH_2-terminal first exon sequences (*1*) and a COOH-terminal domain-encoded guanosine triphosphatase (GTPase)–activating function for the Rac GTP-binding protein (*2*). Several structural domains have been identified in c-Bcr, including an oligomerization domain (*3*), a region that binds Src homology 2 (SH2) domains in a phosphotyrosine-independent manner (*4*), a region of sequence similarity to guanine nucleotide exchange factors for the Rho family of GTP-binding proteins (*5*), a calcium-dependent lipid binding (Calb) domain, and a pleckstrin homology domain (*6*). Though the presence of these distinct biochemical and structural properties in c-Bcr suggests that this protein may function as a point of cross-talk among multiple intracellular signaling pathways, little is known about its biological mechanism of action.

The *bcr* gene was first discovered because of its involvement in Ph^1-positive leukemias. Ph^1 is produced by a reciprocal translocation event between chromosomes 9 and 22. The translocation fuses the *bcr* gene upstream of the second exon of the *c-abl* proto-oncogene (*7*). Two alternative Bcr-Abl chimeric proteins are produced, P210 and P185, which are associated with chronic myelogenous and acute lymphocytic leukemias, respectively (*8*). Sequences within the first exon of Bcr are essential for the transforming activity of Bcr-Abl (*9*). A tyrosine (Tyr^{177}) within the Bcr first exon becomes phosphorylated in the activated Bcr-Abl oncoproteins and serves as a binding site for the SH2 domain of the Grb2 adaptor protein (*10*). A mutant of Bcr-Abl that lacks Tyr^{177} is defective in transformation (*10*). The first exon sequences of Bcr have the potential to interact with cellular proteins in phosphotyrosine-dependent and phosphotyrosine-independent manners. Together, these interactions may contribute to the activation of the tyrosine kinase and the transforming activities of Abl in the Bcr-Abl chimera (*4*, *10*).

To understand the mechanism or mechanisms whereby the first exon of Bcr activates the transforming activity of Abl in the Bcr-Abl chimera and to gain insight into the normal functions of c-Bcr, we sought to identify cellular proteins that bind directly to this region of Bcr in a phosphotyrosine-independent manner. A λEXlox mouse embryo library was screened with amino acid sequences encoded by the first exon of Bcr (amino acids 1 to 413). The Bcr sequences were fused downstream of glutathione-S-transferase (GST) and the GST-Bcr (1–413) fusion was then cloned into a baculovirus vector for expression in insect cells. Sf9 insect cells were infected with the purified recombinant GST-Bcr (1–413) baculovirus. After cell lysis, the fusion protein was purified on glutathione Sepharose beads. The GST-Bcr (1–413) protein was labeled with ^{32}P by its intrinsic kinase activity (*1*). A single phosphorylated protein band was detected after gel electrophoresis and autoradiography. The phosphorylated protein was recognized by antibodies to Bcr. The labeled GST-Bcr protein was used as a probe to screen a complementary DNA (cDNA) library from a 16-day mouse embryo (*11*). Seven independent phage clones were isolated. There were two sets of identical clones, of five and two clones, respectively, that overlapped in sequence. Northern (RNA) blot analysis revealed the presence of two closely migrating transcripts of about 1.9 and 2.2 kb in all murine and human tissues examined (*12*). Analysis of the DNA sequence corresponding to the longest insert (1.9 kb) revealed that the cDNA encoded a member of a large family of proteins. The protein was named Bcr-associated protein 1 (Bap-1) and is identical in sequence (except for a single aspartic acid to glutamic acid substitution) to a member of the 14-3-3 family of proteins isolated from human T cells (*13*). The 14-

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