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Abr and Bcr are multifunctional regulators of the Rho GTP-binding protein family

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ABSTRACT **Philadelphia chromosome-positive leukemias result from the fusion of the *BCR* and *ABL* genes, which generates a functional chimeric molecule. The Abr protein is very similar to Bcr but lacks a structural domain which may influence its biological regulatory capabilities. Both Abr and Bcr have a GTPase-activating protein (GAP) domain similar to those found in other proteins that stimulate GTP hydrolysis by members of the Rho family of GTP-binding proteins, as well as a region of homology with the guanine nucleotide dissociation-stimulating domain of the *DBL* oncogene product. We purified as recombinant fusion proteins the GAP- and Dbl-homology domains of both Abr and Bcr. The Dbl-homology domains of Bcr and Abr were active in stimulating GTP binding to CDC42Hs, RhoA, Rac1, and Rac2 (rank order, CDC42Hs > RhoA > Rac1 = Rac2) but were inactive toward Rap1A and Ha-Ras. Both Bcr and Abr acted as GAPs for Rac1, Rac2, and CDC42Hs but were inactive toward RhoA, Rap1A, and Ha-Ras. Each individual domain bound in a noncompetitive manner to GTP-binding protein substrates. These data suggest the multifunctional Bcr and Abr proteins might interact simultaneously and/or sequentially with members of the Rho family to regulate and coordinate cellular signaling.**

BCR was originally identified as a gene involved in the Philadelphia translocation, a chromosome abnormality present in a well-defined group of leukemia patients. As a result of this translocation, part of the *BCR* gene fuses to *ABL*, the gene encoding the c-Abl protein-tyrosine kinase, producing Bcr/Abl fusion proteins responsible for the development of chronic myelogenous and acute lymphoblastic leukemias (1–5).

The normal biological functions of the intact Bcr protein remain largely unknown. Structurally, p160 Bcr consists of three defined functional domains. The amino terminus contains an intrinsic kinase activity which phosphorylates Bcr on serine and/or threonine residues (6). The central part of the protein has homology to the guanine nucleotide dissociation-stimulating (GDS) region of the protooncogene product Dbl, which catalyzes guanine nucleotide exchange on Rho and CDC42Hs (7, 8). To date however, Bcr itself has not been shown to exert such activity. The carboxyl terminus of Bcr is homologous to the catalytic domain of GTPase-activating proteins (GAPs) which stimulate GTP hydrolysis by members of the Rho family (9, 10). Bcr thus has a unique structure, potentially having two opposing regulatory activities toward small GTP-binding proteins (GDS and GAP) combined within one molecule.

Interestingly, a number of other potentially bifunctional regulators of small GTP-binding proteins have been identified. RasGRF has GDS domains for both Ras and Rho family members (11), whereas p190 has a Rho/Rac GAP domain and binds to p120 RasGAP (12, 13). The Bcr-related protein Abr contains both Dbl- and GAP-homology domains analogous to those present in Bcr, suggesting that it too may function as a bidirectional modulator of Rho family function. Abr has a high degree of sequence homology across its entire length with Bcr but lacks sequences homologous to the first exon of Bcr, which encodes the intrinsic kinase activity (14, 15).

Members of the Rho family of GTP-binding proteins are involved in regulation of cytoskeletal organization in organisms as diverse as yeast and mammals (16). In addition to its cytoskeletal effects (17), Rac2 has been shown to be a regulatory component of the human phagocyte NADPH oxidase (18, 19). We have recently generated Bcr-null mutant mice (20) in which we observed enhanced phagocyte oxidant generation correlated with an increased percentage of membrane-associated Rac2 protein. Bcr thus appears to play a prominent regulatory role for Rac function in leukocytes, perhaps relevant to its involvement in leukemias affecting this type of cell.

The normal biological function of the closely related Abr protein remains unknown. To begin to establish the biochemical activities of Abr, we have measured and compared the regulatory activities of Abr and Bcr toward members of the Rho GTP-binding protein family. We show that both Abr and Bcr are specific GAPs for Rac and CDC42Hs and that the Dbl-homology domains on each protein are able to stimulate guanine nucleotide binding to Rac, CDC42Hs, and Rho. The data presented suggest that sequential and/or simultaneous interactions with regulatory domains in Abr and Bcr might up- and downregulate Rho family function during cell activation.

MATERIALS AND METHODS

Glutathione *S*-Transferase (GST) Fusion-Protein Constructs. The *ABR* and *BCR* subdomains were inserted in-frame with the GST gene in the pGEX-3X bacterial expression vector (Pharmacia). A segment of the *BCR* cDNA encoding aa 508–790 was isolated by digestion with *Nhe* I/*Bgl* II and made blunt-ended. This candidate GDS domain, homologous with the GDS domain of Dbl, was inserted into pBluescript SK (Stratagene) digested with *Sma* I, and clones containing a *Bam*HI site on the 5′ end were identified. The insert was cloned as a (*Bam*HI–*Nco* I)–(*Nco* I–*Eco*RI) fragment into pGEX-3X digested with *Bam*HI/*Eco*RI. The comparable segment of *ABR* encoded aa 58–337 and was inserted as a (*Bgl* II–*Bst*EII)–(*Bst*EII–*Sst* I)–(*Sst* I–*Eco*RI) fragment into pGEX-3X digested with *Bam*HI/*Eco*RI. The *Eco*RI site from the insert was introduced by subcloning into pBluescript SK.

ABR and *BCR* GAP domains encompassed a common *Pvu* II site at the 5′ end (aa 550, nt *1675* in *ABR*) and terminated at a stop codon at the carboxyl terminus. The *BCR* segment (aa 1005–1271) was inserted as a (*Pvu* II–*Bst*EII)–(*Bst*EII–*Eco*RI) fragment into pGEX-3X digested with *Sma* I/*Eco*RI. A 1.3-kb

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Abbreviations: GAP, GTPase-activating protein; GDS, guanine nucleotide dissociation-stimulating; GTPγS, guanosine 5′-[γ-thio]-triphosphate.

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*Hin*dIII–*Xho* I fragment of *ABR* was first subcloned into pBluescript SK digested with *Hin*dIII/*Sal* I to provide a 3′ *Eco*RI site. An 0.8-kb *Pvu* II–*Eco*RI fragment (aa 551–813) from this subclone was ligated into pGEX-3X digested with *Sma* I/*Eco*RI.

Isolation of Bacterial Fusion Proteins. *Escherichia coli* XL1-Blue (Stratagene) was transformed with the pGEX-3X plasmids described above. Cultures were induced with 1 mM isopropyl β-D-thiogalactopyranoside for 3 hr at 37°C. The bacteria were pelleted, suspended in ice-cold buffer A [50 mM Tris·HCl, pH 7.5/150 mM NaCl/1 mM EDTA/1 mM phenylmethanesulfonyl fluoride/1% (vol/vol) Triton X-100], and lysed with sonication. The lysates were centrifuged at 10,000 × *g* for 15 min and the supernatants incubated with glutathione-agarose beads for 30 min at 20°C. The unbound materials were removed by centrifugation, the beads were washed three times with buffer A, and the fusion proteins were released by incubation with 50 mM Tris·HCl, pH 8.0/10 mM glutathione. The eluted proteins were concentrated in Centricon-120 concentrators (Amicon) and dialyzed against 50 mM Tris·HCl, pH 7.5/1 mM EDTA/1 mM dithiothreitol. The purified fusion proteins were ≈90% homogeneous as determined by Coomassie Blue staining after SDS/PAGE.

Expression and Purification of GTP-Binding Proteins. Rac1, Rac2, RhoA, and CDC42 were expressed in *E. coli* and purified as described (21, 22). Posttranslationally processed GTP-binding proteins were expressed in a baculovirus/Sf9 insect cell system and purified (23).

Guanine Nucleotide Exchange Assay. The activity of the Abr or Bcr Dbl-homology domain to stimulate the incorporation of guanosine 5′-[γ-[^{35}S]thio]triphosphate ([^{35}S]GTPγS) into various small GTP-binding proteins was determined by incubating 18 pmol of each GTP-binding protein with 6 pmol of purified GST/Dbl-homology domain in 300 μl of 27 mM Tris·HCl, pH 8.0/4.5 mM Hepes, pH 8.0/1 mM dithiothreitol/5.1 mM EDTA/10.2 mM $MgCl_2$/2.0 μM [^{35}S]GTPγS (2.5×10^4 cpm/pmol) at 30°C. At the indicated time, 35-μl aliquots were removed and added to 2 ml of ice-cold stop solution (25 mM Tris·HCl, pH 8.0/100 mM NaCl/30 mM $MgCl_2$/2 mM dithiothreitol/0.01% bovine serum albumin). Binding of [^{35}S]GTPγS was determined by vacuum filtration over BA-85 nitrocellulose filters (Schleicher & Schuell) and liquid scintillation counting (21). Results are expressed as percent of maximal binding, which is defined as the maximal binding activity obtained at ≈50 nM free Mg^{2+} (a concentration that is optimal for these small GTP-binding proteins) and which is indicative of the total active protein available based on 1:1 [^{35}S]GTPγS–protein binding (22).

Dissociation of nucleotides in the presence or absence of Abr or Bcr domains was measured in the presence of 164 μM unlabeled GDP as described (21), with a 3:1 ratio of GTP-binding protein to Dbl-homology domain, as above.

GAP Assay. GAP assays were performed (23) with 9 pmol of [γ-^{32}P]GTP-labeled GTP-binding protein per 35-μl sample in the presence of an equimolar amount GST/Abr or GST/Bcr GAP domain, or as indicated.

Protein Binding Assays. The GTPγS-bound form of each GTP-binding protein was prepared by incubating protein at ≈1 μM with 25 mM Tris·HCl, pH 7.5/2.34 mM EDTA/1 mM dithiothreitol/0.16 mM $MgCl_2$/0.005% bovine serum albumin/20 μM GTPγS for 10 min at 30°C. After addition of $MgCl_2$ to 20 mM, the GST/Abr or GST/Bcr GAP domain was added at a ratio of 1:1 in a total volume of 400 μl and then incubated at room temperature for 5 min. One hundred microliters of glutathione-agarose resin preequilibrated with an equal volume of 25 mM Tris·HCl, pH 7.5/1 mM EDTA/5 mM $MgCl_2$ was added to the reaction mixture and incubated with gentle agitation for 3 hr at 4°C. The resin was then pelleted in a microcentrifuge and washed five times with 1 ml of the equilibration buffer. Bound proteins were eluted in Laemmli sample buffer for analysis by Western blotting. The Rac antibody R785, RhoA antibody 26C4, and CDC42 antibodies used have been described (22, 24). Binding of GST/Dbl-homology domains was assayed in a similar fashion, except that the GTP-binding proteins were in the GDP-bound form and were present in a 5-fold excess over the Dbl domain during the incubation.

RESULTS

Stimulation of Guanine Nucleotide Binding by Abr and Bcr. We examined the activity of the Abr and Bcr Dbl-homology domains to stimulate the incorporation of [^{35}S]GTPγS into various small GTP-binding proteins in the presence of millimolar free Mg^{2+}. The putative GDS domain from Abr was able to stimulate the binding of GTPγS to CDC42Hs, RhoA, and Rac1 (Fig. 1). Similar results were obtained with the Bcr Dbl-homology domain (data not shown), and the activity of both proteins was less than the action of Dbl itself when assessed with CDC42Hs in this assay (Fig. 1). GST controls had no effect on guanine nucleotide binding. Both the Abr and Bcr proteins were somewhat more effective in stimulating binding to CDC42Hs when compared with Rac and Rho. However, each protein was totally inactive in enhancing guanine nucleotide binding to Ha-Ras (Fig. 1*D*) or Rap1A (data not shown) under the same conditions. These data indicate that the Abr and Bcr Dbl-homology domains interact specifically with members of the Rho family.

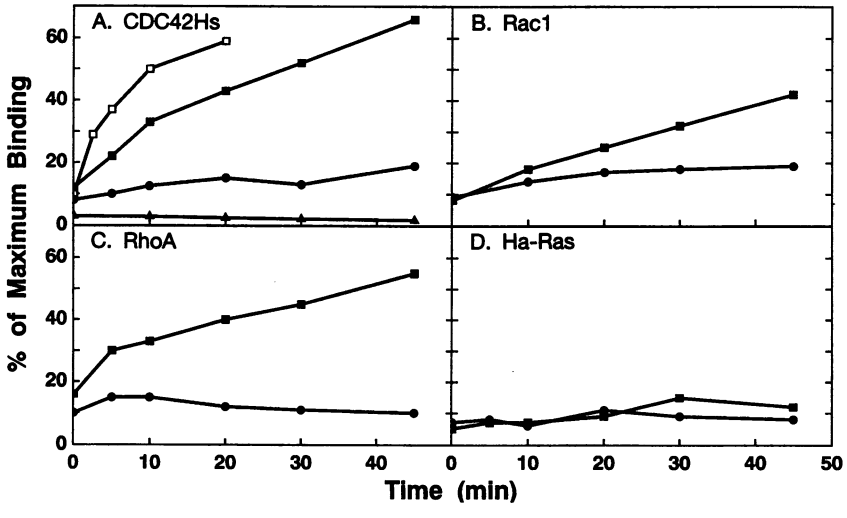

FIG. 1. The Abr Dbl domain stimulates GTPγS binding to Rho family GTP-binding proteins. Binding of [^{35}S]GTPγS to the indicated GTP-binding proteins was determined in the absence (●) or presence (■) of the Abr Dbl domain. Controls in *A* show the absence of nucleotide binding to the purified Abr Dbl domain alone (▲) and binding catalyzed by the Dbl protein itself (□). Binding to Rac2 (data not shown) in the presence of Abr Dbl was similar to the binding to Rac1. Results shown are representative of at least four experiments.

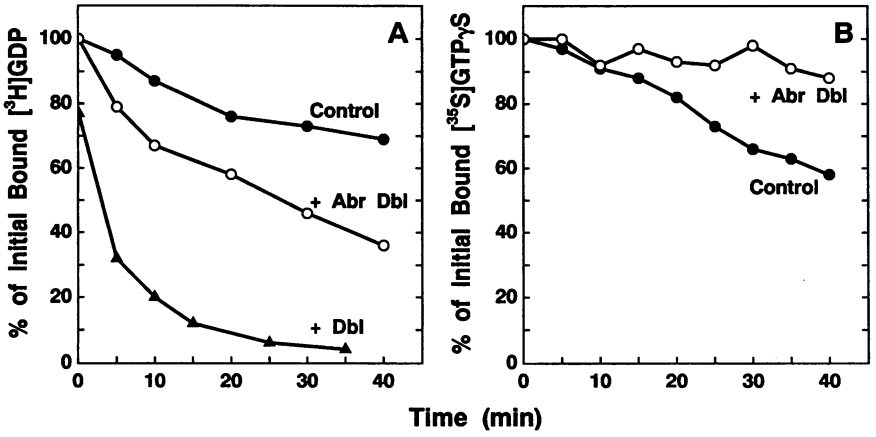

FIG. 2. Abr Dbl domain enhances [^{3}H]GDP dissociation but inhibits [^{35}S]GTPγS dissociation. (*A*) Dissociation of GDP from CDC42Hs was determined in the absence (●) or presence (○) of GST/Abr Dbl or the intact Dbl protein (▲) at a 1:3 ratio to CDC42Hs. Data shown are the average of two experiments. (*B*) Dissociation of [^{35}S]GTPγS from CDC42Hs was determined in the absence (●) or presence (○) of a 1:3 to 1:1 molar ratio of GST/Abr Dbl. Results shown in each panel are representative of at least four experiments.

We examined the activity of the Abr Dbl domain in a GDP-release assay with CDC42Hs and observed that it stimulated [^{3}H]GDP dissociation (Fig. 2*A*), although Dbl itself had a greater effect in this assay. We also observed that the Abr Dbl domain markedly inhibited the rate of [^{35}S]GTPγS dissociation from CDC42Hs (Fig. 2*B*). This effect was not mimicked by an unrelated protein(s), and did not appear to be a result of stabilizing the protein from denaturation (data not shown).

The ability of small GTP-binding protein substrates to bind directly to the Abr Dbl-homology domain was assessed. Rac1 and Rho (Fig. 3*A*), as well as Rac2 and CDC42 (data not shown), bound to the Abr Dbl-homology domain, consistent with the domain's ability to stimulate nucleotide binding on these proteins. Using this mode of analysis, we did not observe significant differences in the binding of Abr Dbl domain to these proteins in the nucleotide-free- vs. GDP- vs. GTP(γS)-bound form, although binding to GDP- and nucleotide-free forms generally appeared slightly better.

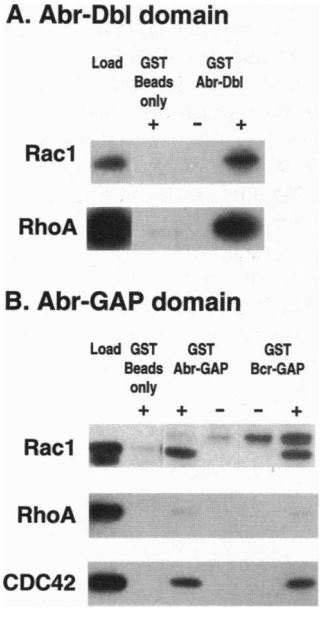

FIG. 3. Direct binding of Abr and Bcr GAP domains and Abr Dbl domain to small GTP-binding protein substrates. Absence (−) or presence (+) of GTP-binding protein in the reaction mixture is indicated. "GST Beads only" lane serves as the control, as essentially no binding of added GTP-binding protein was detectable. "Load" lane represents an equivalent portion of the total GTP-binding protein which was loaded onto the beads, and serves to indicate the relative protein loads in each case. Results shown are Western blots with antibody against the indicated GTP-binding protein and are representative of two or more similar experiments. The band appearing above Rac protein in the Rac blot of *B* was a nonspecific crossreactant present in the GST/Bcr GAP fusion protein preparation, as indicated by its presence in the absence of added Rac (see − lane).

GAP Activity of Abr and Bcr. We next compared the activity and specificity of the GAP domain of Abr with that of Bcr. Both Abr and Bcr stimulated GTP hydrolysis by Rac1, Rac2, and CDC42Hs (Fig. 4). Stimulation of GTP hydrolysis was observed even at concentrations of each GAP domain that were 10 times lower than the concentration of GTP-binding protein (data not shown). In contrast, Abr and Bcr did not stimulate GTP hydrolysis by Rho (Fig. 4) or by Ha-Ras or Rap1A (data not shown), even at concentrations 2–3 times greater than that of the GTP-binding protein.

We assessed binding of substrates to GST fusion proteins containing the Abr or Bcr GAP domain. Rac1 (Fig. 3*B*), as well as CDC42HS and Rac2 (data not shown), but not RhoA (Fig. 3*B*), specifically bound to the GAP domain from both Abr and Bcr. Thus, the inability of Abr and Bcr to catalyze GTP hydrolysis on Rho appears to be due to the inability of Rho to physically interact with this domain.

Interactions of the Abr GAP and Dbl-Homology Domains. Since both Abr and Bcr contain separate protein domains capable of binding to and modulating the activity of Rho family proteins, we wondered whether these domains would compete with each other for binding to small GTP-binding protein substrates. Fig. 5*A* shows the results of an experiment in which various amounts of Abr GAP were added to GTPγS binding assays in the presence of the Abr Dbl-homology domain. The ability of the Dbl domain to stimulate [^{35}S]GTPγS binding was not antagonized by the presence of a molar excess of the GAP domain. The reciprocal experiment (Fig. 5*B*), in which GAP assays were performed in the presence of various amounts of Abr Dbl domain, also showed that there was no competition between these domains for interaction with the small GTP-binding protein.

DISCUSSION

The Bcr protein was originally shown by Diekmann *et al.* (9) to stimulate GTP hydrolysis by Rac through a domain which appears to be present in all Rho family GAPs. The presence of this motif is not sufficient to establish Rho GAP activity, however, since some proteins containing the Bcr GAP-homology domain do not stimulate GTP hydrolysis *in vitro* (i.e., the p85 subunit of phosphatidylinositol 3-kinase; ref. 25). Bcr and Abr also contain a Dbl-homology domain which might be capable of regulating GTP/GDP exchange on small GTP-binding proteins. While this domain in the Dbl oncoprotein was demonstrated to stimulate the binding of GTPγS to CDC42Hs and Rho in guanine nucleotide exchange assays (7, 8), a number of other proteins containing a similar motif have not demonstrated such activity in *in vitro* assays, and the

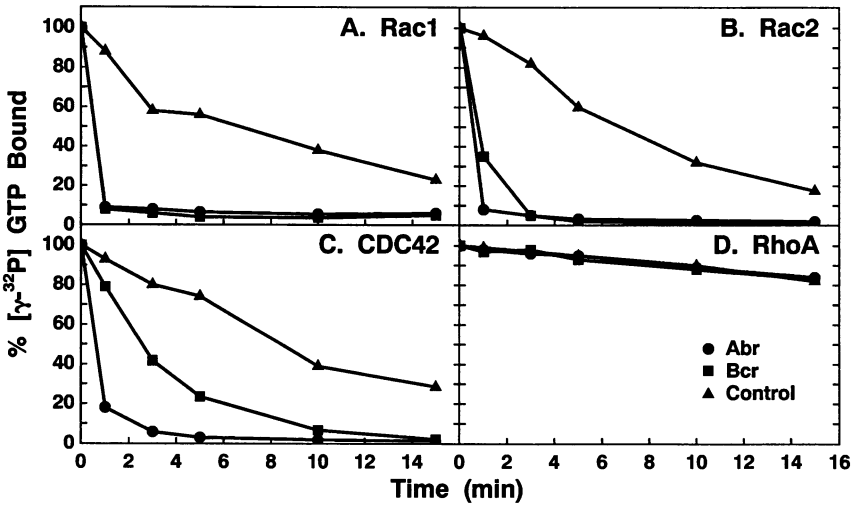

FIG. 4. Time course of GTP hydrolysis by Rho family proteins. GTP hydrolysis was determined in the absence (▲) or presence of equimolar Abr GAP (●) or Bcr GAP (■) domain. Results shown are representative of at least four experiments.

equivalent domain in Vav appears to have activity toward Ras instead of Rho family proteins (26).

We have shown here that both the GAP domain and the Dbl domain of Abr are specifically active toward members of the Rho family. As a GAP, Abr is active on Rac1 = Rac2 > CDC42Hs but does not stimulate GTP hydrolysis on RhoA, Ras, or Rap1A (Fig. 3), as also reported by Tan *et al.* (27). The lack of activity toward Rho is somewhat surprising in view of its high degree of sequence homology with Rac and CDC42Hs but is consistent with the substrate specificity of the GAP domain of Bcr itself (9, 28). GAP activity of Abr was observed at substoichiometric concentrations, suggesting that Abr GAP acted catalytically.

The Abr Dbl-homology domain is active as a stimulator of guanine nucleotide binding to Rho family proteins at physiological Mg^{2+} concentrations. The enhancement of GTP binding by Abr was observed with all members of the Rho family, although activity was best toward CDC42Hs and RhoA and relatively weak toward Rac (Fig. 1). The increase in GTP binding did not appear to be due solely to the ability of the Abr Dbl-homology domain to stimulate GDP release, since this activity was weak compared with that of Dbl itself. Rather, the Abr and Bcr Dbl domains also acted as GTPγS-dissociation inhibitors, decreasing the release of bound [^{35}S]GTPγS from small GTP-binding protein substrates. This activity has not been reported previously, but a similar activity of the Dbl domain of Vav toward Ras has been observed (J.-H. Han, W. Wie, and D. Broek, personal communication). In full-length Vav, this GTP-dissociation inhibitory activity is stimulated by Vav phosphorylation, suggesting that this is a regulatable function of the Dbl domains in proteins such as Vav, Bcr, and Abr. The binding of GTPγS to small GTP-binding proteins in the presence of millimolar Mg^{2+} rarely approaches equilibrium with time (see controls in Fig. 1). This is not due solely to protein degradation and has not been explained adequately. It is possible that this lack of nucleotide binding may be an intrinsic property of the small GTP-binding proteins, reflecting the need for an additional conformational change induced by Dbl-like domains to effectively and stably bind GTP.

The two separate GAP and Dbl-related regulatory domains exhibit distinct functional specificities toward small GTP-binding proteins, and the basis for this specificity appears to reside in the selectivity of these domains for physically binding to their respective targets (Fig. 3). Reciprocal competition experiments using the Abr GAP and Dbl domains (Fig. 5) indicate that these domains do not interfere with each other in functional assays for GTP hydrolysis or stimulated GTP binding. These data suggest two possible explanations. The first is that these domains on Abr (and Bcr) bind to separate, nonoverlapping sites on their GTP-binding protein targets. One could also envision simultaneous interaction of Abr or Bcr with two distinct and potentially different members of the Rho family. One of these target proteins would be inactivated by the GAP domain, while the other would be activated by the Dbl-homology domain, perhaps under the control of exogenously controlled phosphorylation events (J.-H. Han, W. Wie, and D. Broek, personal communication). Potentially, the coordinated turning on and off of competing cell signals would

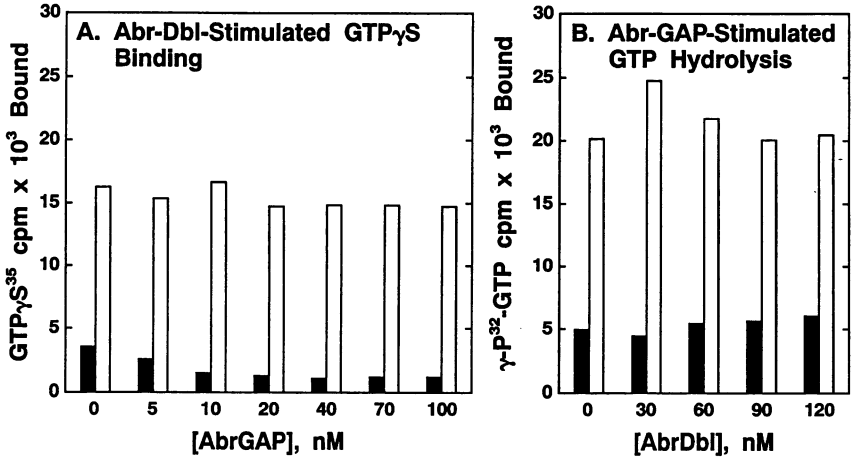

FIG. 5. Competition analysis of Abr GAP and Dbl domains. (*A*) GTPγS binding to 50 nM CDC42Hs alone (solid bars) or stimulated by 20 nM Abr Dbl domain (open bars) was determined in the presence of the indicated concentrations of Abr GAP domain. (*B*) GTP hydrolysis by 60 nM CDC42Hs alone (open bars) or stimulated by 60 nM Abr GAP domain (solid bars) was determined in the presence of the indicated concentrations of Abr Dbl domain. Results are representative of four experiments.

be possible. Such regulation would most likely be desirable for cytoskeletal events involved in cell migration, mitosis, or neuronal development. Indeed, there appears to be a hierarchy of interactions involving the Rho GTPases: Ras and CDC42 activation leads to activation of Rac (ruffling), which in turn stimulates the activity of Rho (stress fibers) (17, 29).

It is conceivable that such binary binding may be sterically prevented in full-length Abr and Bcr. A second possibility, then, is that binding to these functional domains is determined by the GTP vs. GDP state of the target. Sequential binding of substrates suggests a mechanism in which the initial binding of the GDP-bound substrate to the Abr or Bcr Dbl domain could enhance the formation of the GTP-bound protein, producing an active form of the GTP-binding protein bound to Abr or Bcr. Alternatively, binding to this domain could occur subsequent to an interaction with another guanine nucleotide exchange protein (J.-H. Han, W. Wie, and D. Broek, personal communication). The resulting complex, containing a stabilized GTP-bound small GTP-binding protein, could relay downstream signals. The GTP-bound protein would eventually, and perhaps in a regulated fashion, be "handed over" to the adjacent GAP domain, which would stimulate hydrolysis of the bound GTP and inactivate the protein.

In Philadelphia chromosome-positive leukemias, an abnormal fusion protein is found which consists of a variable part of the Bcr protein and a constant segment of the Abl protein. In chronic myelogenous leukemia, the Philadelphia translocation results in the production of p210 Bcr/Abl, in which the Bcr serine/threonine kinase and Dbl-homology domains are fused to Abl. In contrast, in ≈50% of cases of Philadelphia-positive acute lymphoblastic leukemia, a smaller Bcr/Abl fusion protein, p190, is found which differs only from p210 in that it lacks the Dbl-homology domain of Bcr. The presence of this domain thus appears to influence the oncogenic activity of Bcr/Abl: the p190 Bcr protein is associated with a more acute type of leukemia than p210. It is possible that the Dbl domain in p210 is still able to interact with small GTP-binding protein substrates and that this mitigates the oncogenicity of p210 Bcr/Abl in comparison with p190. How the regulatory activities of Bcr and Abr described in this report are related to their normal and abnormal cellular activities will require further investigation.

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1. Heisterkamp, N., Stephenson, J. R., Groffen, J., Hansen, P. F., de Klein, A., Bartram, C. R. & Grosveld, G. (1983) *Nature (London)* **306,** 239–242.
2. Groffen, J., Stephenson, J. R., Heisterkamp, N., de Klein, A., Bartram, C. R. & Grosveld, G. (1984) *Cell* **36,** 93–99.
3. Shtivelman, E., Lifshitz, B., Gale, R. P. & Canaani, E. (1985) *Nature (London)* **315,** 550–554.
4. Heisterkamp, N., Stam, K., Groffen, J., de Klein, A. & Grosveld, G. (1985) *Nature (London)* **315,** 758–761.
5. Hermans, A., Heisterkamp, N., von Lindern, M., van Baal, S., Meyer, D., van der Plan, D., Wiedermann, L., Groffen, J., Bootsma, D. & Grosveld, G. (1987) *Cell* **51,** 33–40.
6. Maru, Y. & Witte, O. N. (1991) *Cell* **67,** 459–468.
7. Hart, M. J., Eva, A., Evans, T., Aronson, S. A. & Cerione, R. A. (1991) *Nature (London)* **354,** 311–315.
8. Hart, M. J., Eva, A., Zangrilli, D., Aaronson, S. A., Evan, T., Cerione, R. A. & Zheng, Y. (1994) *J. Biol. Chem.* **269,** 62–65.
9. Diekmann, D., Brill, S., Garret, M. D., Totty, N., Hsuan, J., Monthes, C., Hall, C., Lim, L. & Hall, A. (1992) *Nature (London)* **351,** 400–402.
10. Fry, M. J. (1992) *Curr. Biol.* **2 (2),** 78–80.
11. Shou, C., Farnsworth, C. L., Neel, B. G. & Feig, L. A. (1992) *Nature (London)* **358,** 351–354.
12. Settleman, J., Narasimhan, V., Foster, L. C. & Weinberg, R. A. (1992) *Cell* **69,** 539–549.
13. Settleman, J., Albright, C. F., Foster, L. C. & Weinberg, R. A. (1992) *Nature (London)* **359,** 153–154.
14. Heisterkamp, N., Morris, C. & Groffen, J. (1989) *Nucleic Acids Res.* **17,** 8821–8831.
15. Heisterkamp, N., Kaartinen, V., van Soest, S., Bokoch, G. M. & Groffen, J. (1993) *J. Biol. Chem.* **268,** 16903–16906.
16. Hall, A. (1994) *Annu. Rev. Cell Biol.* **10,** 31–54.
17. Ridley, A. J., Paterson, H. F., Johnston, C. L., Diekmann, D. & Hall, A. (1992) *Cell* **70,** 401–410.
18. Knaus, U. G., Heyworth, P. G., Evans, T., Curnutte, J. T. & Bokoch, G. M. (1991) *Science* **254,** 1512–1515.
19. Bokoch, G. M. (1994) *Curr. Opin. Cell Biol.* **6,** 212–218.
20. Voncken, J. W., van Schaick, H., Kaartinen, V., Deemer, K., Coates, T., Landing, B., Pattengale, P., Dorseuil, O., Bokoch, G. M., Groffen, J. & Heisterkamp, N. (1995) *Cell* **80,** 719–728.
21. Chuang, T. H., Xu, X., Quilliam, L. A. & Bokoch, G. M. (1994) *Biochem. J.* **303,** 761–767.
22. Knaus, U. G., Heyworth, P. G., Kinsella, B. T., Curnutte, J. T. & Bokoch, G. M. (1992) *J. Biol. Chem.* **267,** 23575–23582.
23. Xu, X., Barry, D. C., Settleman, J., Schwartz, M. A. & Bokoch, G. M. (1994) *J. Biol. Chem.* **269,** 23569–23574.
24. Bokoch, G. M., Bohl, B. P. & Chuang, T. H. (1994) *J. Biol. Chem.* **269,** 31674–31679.
25. Zheng, Y., Bagrodia, S. & Cerione, R. A. (1994) *J. Biol. Chem.* **269,** 18727–18730.
26. Gulbins, E., Coggeshall, K. M., Baier, G., Katsav, S., Burn, P. & Altman, A. (1993) *Science* **260,** 822–824.
27. Tan, E.-C., Leung, T., Manser, E. & Lim, L. (1993) *J. Biol. Chem.* **268,** 27291–27298.
28. Ridley, A. J., Self, A. J., Kasmi, F., Patterson, H. F., Hall, A., Marshall, C. J. & Ellis, C. (1993) *EMBO J.* **12,** 5151–5160.
29. Nobes, C. D. & Hall, A. (1995) *Cell* **81,** 53–62.