Bcr (breakpoint cluster region) protein binds to PDZ-domains of scaffold protein PDZK1 and vesicle coat protein Mint3

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Summary

The breakpoint cluster region protein (Bcr) is a large soluble oligomeric multidomain protein best known because of its involvement in chronic myelogenous leukemia (CML). A chromosomal translocation between its gene and that of the c-abl kinase ('Philadelphia chromosome') plays a major causative role in that malignancy. Thus most attention has been paid to the role of the protein in hemopoietic cells. However, Bcr is also expressed in other cell types including epithelia. Bcr is generally considered to be a cytoplasmic protein but in addition to its kinase and GTPase exchange and activating domains it contains potentially membrane-interacting pleckstrin homology and C2 domains as well as a PDZ-binding C terminus mediating an interaction with a PDZ-domain protein at intercellular junctions of epithelial cells. We have examined the ability of Bcr to interact with other epithelial PDZ proteins and found specific binding to both the apical PDZK1 protein and the Golgi-localized Mint3. The former is important in the organization of several apical functions and the latter in vesicular trafficking in the secretory pathway. Hence these findings extend the interactions and likely signaling impact of Bcr in epithelia from the cytosol to at least these two membrane compartments.

Key words: Breakpoint cluster region protein, PDZ-binding, Bcr localization, Apical Bcr, Golgi Bcr, Membrane Bcr

Introduction

Although the primary cellular function of the large multidomain Bcr protein is unknown it has been the focus of much attention because the Bcr-Abl fusion protein formed by genomic translocation is believed to be causative in chronic myelogenous leukemia (CML) (Clark et al., 1988; Faderl et al., 1999; Fainstein et al., 1987; Konopka et al., 1984) whereas overexpression of Bcr itself inhibits cell proliferation (Braselmann and McCormick, 1995). Bcr appears suitable to participate in cell signaling since it has serine/threonine protein kinase, guanine nucleotide exchange factor (GEF) and GTPase-activating domains (Boguski and McCormick, 1993; Maru and Witte, 1991). While Bcr is widely distributed throughout the cytoplasm of cells it contains both pleckstrin homology (PH) and Ca^{2+}-dependent phospholipid binding C2 domains capable of mediating its binding to membranes. In addition Bcr has a consensus PSD-95/discs large/ZO-1 (PDZ)-domain-binding C-terminal sequence which has been shown to mediate its binding to the AF-6 junctional PDZ-domain protein of epithelial cells (Radziwill et al., 2003). This investigation also revealed that a ternary complex of Bcr, AF-6 and Ras at junctional sites of epithelial cell membranes can down-regulate Ras-mediated signaling and cell proliferation. PDZ-mediated binding of Bcr to erbin was also detected recently (Boisguerin et al., 2004). We have been interested in the role of other membrane-associated PDZ-proteins in the localization and regulation of epithelial ion channels including CFTR (Gentzsch et al., 2003). Prominent among this class of PDZ-domain proteins is PDZK1, first described by Kocher et al. (Kocher et al., 1998) at the apical border of epithelial cells as a partner of the MAP17 protein that is overexpressed in carcinomas. A growing number of important apical membrane proteins bind PDZK1 via their C-termini, including MRP2 (Kocher et al., 1999), CFTR (Wang et al., 2000), ClC-3B (Gentzsch et al., 2003), type IIa Na/Pi cotransporter (Gisler et al., 2001; Gisler et al., 2003) and scavenger receptor B (Silver, 2002).

In a search for additional PDZK1-binding ligands in human lung-derived epithelial cells (Calu-3) (Haws et al., 1994) we have found that Bcr binds specifically to its first PDZ domain. Although this association could be confirmed by coimmunoprecipitation of the two proteins, immunofluorescence staining detected only a very small proportion of cellular Bcr at the apical surface. However, much of the staining appeared to be on intracellular membranous structures rather than diffusely distributed in the cytoplasm. Screening for possible binding to other PDZ-domain proteins detected Bcr association with the Golgi localized Mint3 protein (Munc18-interacting protein) involved in protein processing and vesicular trafficking in the distal secretory pathway. This

interaction may help to position Bcr for the effective exertion of its GEF and GTPase-activating protein (GAP) activities on the Rho GTPases that regulate vesicular trafficking (Symons and Rusk, 2003).

Materials and Methods

Plasmids

Plasmids encoding glutathione S-transferase (GST) fusion proteins of the PDZK1, EBP50 and Golgi-associated PDZ and coiled-coil motif-containing protein (GOPC) PDZ domains in the pGEX-5X3 vector and FLAG-tagged PDZK1-M2 in pcDNA3 have been described before (Gentzsch et al., 2003). The pLEF/Bcr plasmid expressing GST-Bcr (Mishra et al., 2003) was a generous gift from Dr Nora Heisterkamp, Laboratory Medicine, Children's Hospital Los Angeles Research Institute, Los Angeles, CA. Bcr/pcDNA3.1 was generated by subcloning the Bcr fragment from pLEF/Bcr into the pcDNA3.1 vector (Invitrogen). Site-directed mutagenesis in the Bcr C terminus generated the Bcr-delTEV/pcDNA3.1 and Bcr-V/A/pcDNA3.1 plasmids. The Mint3/pCMV5 plasmid was a generous gift from Dr Thomas Südhof at the Howard Hughes Medical Institute, University of Texas.

Cell culture and transfections

BHK-21, Calu3, HT29 cell lines (ATCC) were cultured at 37°C in 5% CO_2. The BHK-21 cells were transiently transfected using calcium phosphate, polyethylenimine (PEI) or LipofectAMINE PLUS (Invitrogen).

Antibodies

A mouse monoclonal antibody, G6 (Santa Cruz) was employed to detect Bcr and the polyclonal antibodies anti-Bcr C20 and anti-Bcr N20 were used to direct the C and N terminus of Bcr, respectively (Santa Cruz). The mAb anti-FLAG M2 (Sigma) was used to detect FLAG-tagged PDZK1 and Mint3 was detected with an anti-Mint3 mAb (BD) or a purified rabbit antiserum (Affinity BioReagents, Golden, CO).

Immunoprecipitation, and pull-down experiments

Immunoprecipitations and pull down experiments were performed as described previously (Gentzsch et al., 2003). Briefly, binding was performed in 1% Nonidet P-40 lysis buffer: 1% Nonidet P-40, 150 mM NaCl, 50 mM Tris-HCl pH 7.4, 20 mM $NaMoO_4$ containing a protease inhibitor cocktail, overnight at 4°C. The beads were washed four times in 0.09% Nonidet P-40 buffer, pH 7.4 and bound proteins were eluted for 30 minutes with SDS-PAGE sample buffer containing dithiothreitol reducing agent.

In-gel trypsin digestion and mass spectrometry

Gels to be analyzed by tryptic mapping were stained with Simply Blue SafeStain (Invitrogen) and the bands to be analyzed cut out from the gel. Gel pieces were shaken three times for 40 minutes each in 25 mM NH_4HCO_3 and 50% acetonitrile and dried by vacuum centrifugation (Hetovac, Heto-Holten, Denmark). Sequence grade trypsin (Promega), diluted to 10 μg/ml in 25 mM NH_4HCO_3, was added to the samples followed by an incubation for 16 hours at 37°C. Peptides were eluted from the gel pieces by shaking in 5% TFA with 75% acetonitrile for 30 minutes, the eluate collected and the gel pieces washed again with ultrasonication for 20 minutes. The two fractions were pooled, dried by vacuum centrifugation, and dissolved in formic acid (0.1%) and purified by $ZipTip_{C18}$ (Millipore) according to the manufacturer's instructions. Peptides were analyzed by MALDI-TOF mass spectrometry (MALDI-HT, Micromass, Manchester, UK) and the database search was performed using Profound (http://prowl.rockefeller.edu/).

Confocal immunofluorescence

BHK-21 and HEK293T cells were grown on collagen-coated chamber slides (Becton Dickinson). Calu-3 cells were grown in a liquid air interface on Transwell Clear inserts (Costar) and frozen sections were prepared. Cells were fixed in 4% paraformaldehyde for 10 minutes, washed with PBS, permeabilized in 0.1% saponin in PBS and blocked with 1% BSA and 5% normal goat serum in PBS. Primary antibodies were added for at least 1 hour in the same buffer. Bcr was detected with polyclonal rabbit antibodies N-20 (Santa Cruz) in BHK-21 and HEK293 cells or C-20 (Santa Cruz) in Calu-3 cells. CFTR, Mint3, β-COP, clathrin and PMCA-ATPase were detected by anti-CFTR mAb 596 (JR Riordan, Mayo Clinic, Scottsdale, AZ), anti-Mint3 mAb 32 (BD Transduction Laboratories), anti-β-COP mAb maD (Sigma), anti-clathrin heavy chain mAb (BD Transduction Laboratories) and anti-PMCA-ATPase mAb 5F10 (Affinity Bioreagents). Secondary antibodies were either goat anti-mouse or goat anti-rabbit IgG conjugated to Alexa 488 or Alexa 568 (Molecular Probes). Cells were examined on a LSM510 confocal microscope from Zeiss.

PDZ domain array

The PDZ domain arrays were analyzed using a TranSignal™ PDZ Domain Array (Panomics, Redwood City, CA). Detergent lysate from BHK-21 cells recombinantly expressing the full length Bcr was incubated with the array membrane and bound Bcr was detected with the anti-Bcr C-20 antibody (Santa Cruz).

Results

Bcr as a PDZK1 binding protein in epithelial cells

Calu-3 lung epithelial cells were used to test directly for proteins binding to PDZK1. Each of its PDZ domains were individually, or in pairs, fused to GST and used as bait with which to fish in NP-40 lysates of these cells. Fig. 1A shows a prominent Coomassie Blue-staining band of approximately 160 kDa that bound to baits PDZ1 and PDZ1+2 but not to the other PDZ domains of PDZK1. Peptide fingerprinting by MALD1-TOF mass spectrometry following excision of the band and tryptic digestion identified the protein as Bcr with very high significance (95^{th} percentile). The peptides with precisely matching masses were distributed across the protein sequence (Fig. 1B). To confirm Bcr expression in these cells and three other epithelial cell lines (HT29, T84 and Caco-2) lysates were subjected to western blots after SDS-PAGE and then probed with an antibody to Bcr. Specific single bands of similar mobility were detected in all (Fig. 1C). To further verify the specificity of the PDZK1-Bcr interaction similar western blotting was performed on the pull-downs with each of the PDZ domains of PDZK1 and those of two other PDZ-domain proteins, EBP-50 (Reczek et al., 1997) and GOPC (Yao et al., 2002). Fig. 2A confirms that Bcr binds to only PDZ1 of PDZK1 and not to domains 2, 3 and 4. Bcr did not bind either domain of EBP-50 or the single PDZ domain of GOPC, again demonstrating the high selectivity of the interaction (Fig. 2B). Confirmation of the GST fusion bands containing the EBP-50 and GOPC domains is shown in Fig. 2C.

To determine whether the intact PDZK1 protein and Bcr could be readily detected in association in cells, they were

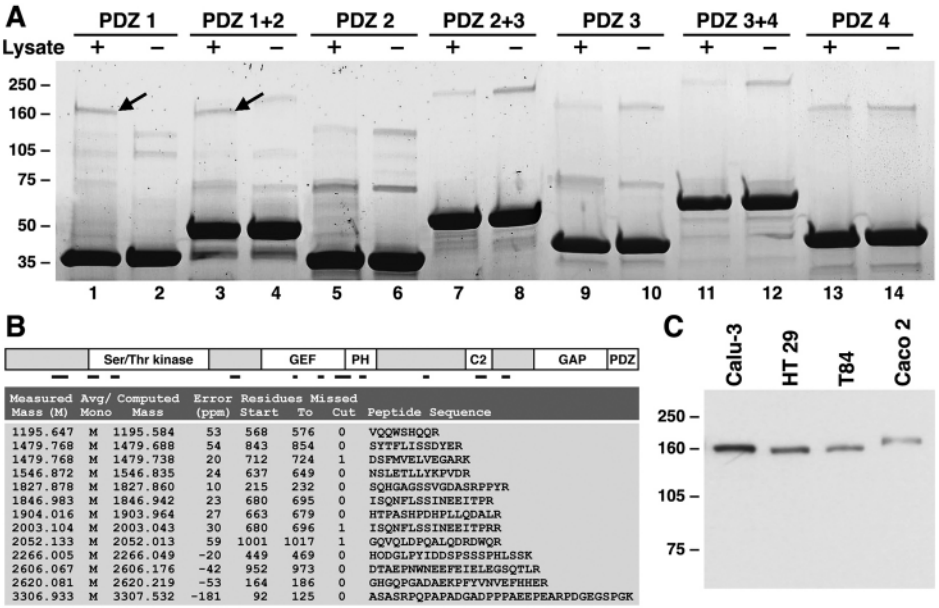

Fig. 1. Bcr is bound by the first PDZ domain of PDZK1. (A) GST fusion proteins with each of the four PDZ domains of PDZK1 immobilized on glutathione-Sepharose beads and incubated with lysates from Calu-3 cells. Bound products were eluted, separated by SDS-PAGE and visualized with Coomassie Blue staining. (B) The peptide masses of the trypsinized major band pulled down with PDZ 1 (marked with arrows in A) identified by MALDI-TOF mass spectrometry. The peptides identified were distributed over the entire Bcr protein. (C) Bcr in several human epithelial cell lines. Lysates from human epithelial cells were separated by SDS-PAGE and Bcr was detected by western blotting using the C20 anti-Bcr antibody.

transiently coexpressed in BHK-21 cells. Immunoprecipitates of PDZK1 with antibodies to the Flag M2 epitope, with which it was fused, contained Bcr only when both proteins were expressed together and not when Bcr alone was expressed (Fig. 2D). The interaction between Bcr and the complete PDZK1 protein was also demonstrated in reciprocal fashion using coexpression of a GST-Bcr fusion and the Flag-tagged PDZK1 (Fig. 2E). The latter was present in the pull-down with glutathione-Sepharose beads.

PDZ-domain proteins most frequently recognize and bind short specific carboxyl-terminal sequences in target proteins (Hung and Sheng, 2002). Bcr has a consensus sequence at its C terminus for binding to class I PDZ domains. This sequence was modified by substitution of the extreme C-terminal hydrophobic residue (V1271A) or deletion of the final three residues (del-TEV). The first alteration reduced interaction with PDZK1 to a very low level and the second completely abolished it (Fig. 2F). Since PDZK1 is localized at the apical surfaces of epithelial

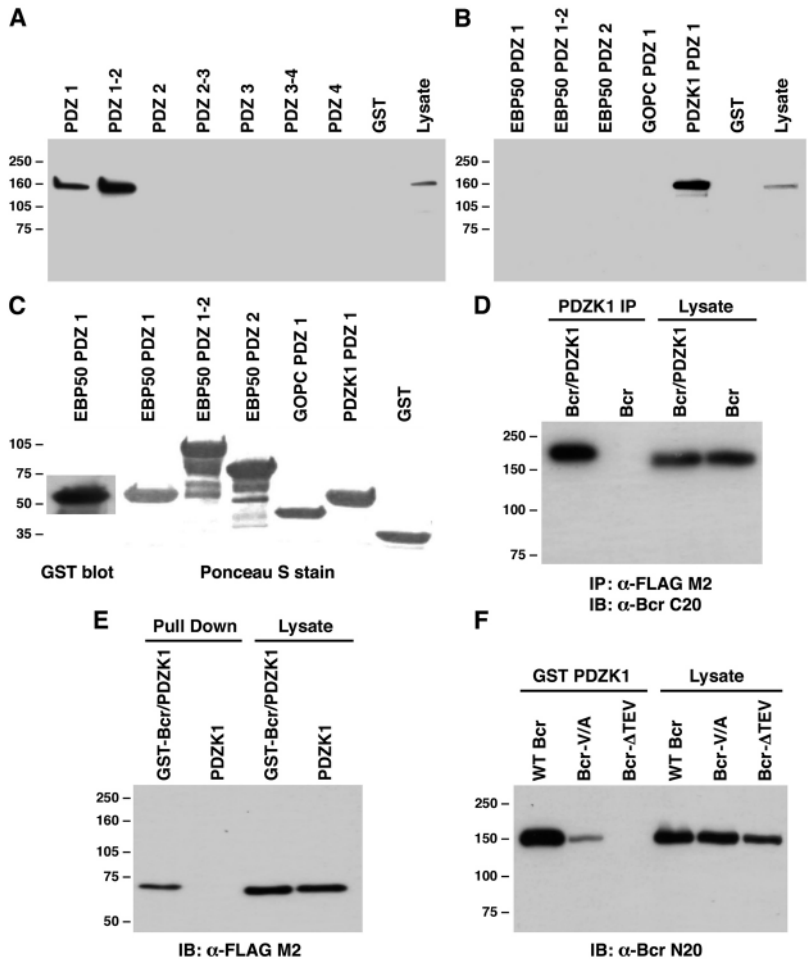

Fig. 2. Confirmation and specificity of the PDZK1-Bcr interaction. GST-PDZ domain fusion proteins from PDZK1 (A) as well as EBP50 and GOPC (B) were bound to glutathione-Sepharose beads, incubated with Calu-3 cell lysate, and the bound products separated by SDS-PAGE followed by western blotting using the anti-Bcr C20 antibody. (C) Confirmation of expression of GST fusions with PDZ domains of EBP50 and GOPC. (D) Bcr was immunoprecipitated from lysates prepared from BHK-21 cells transiently expressing Bcr and FLAG-tagged PDZK1, separated by SDS-PAGE followed by western blotting. (E) FLAG-tagged PDZK1-M2 was transiently expressed in BHK-21 cells with or without Bcr fused to GST. Lysates prepared from these cells were incubated with glutathione-Sepharose beads and the material pulled down was separated by SDS-PAGE. PDZK1 was detected by western blotting with the mAb anti-FLAG M2. (F) Bcr, Bcr-V1271A and Bcr-delTEV were transiently expressed in BHK 21 cells and lysates prepared from these cells were incubated with PDZK1 PDZ 1-GST bound to glutathione-Sepharose beads. The bound material was separated by SDS-PAGE and Bcr was visualized by western blotting using the anti-Bcr N20 antibody.

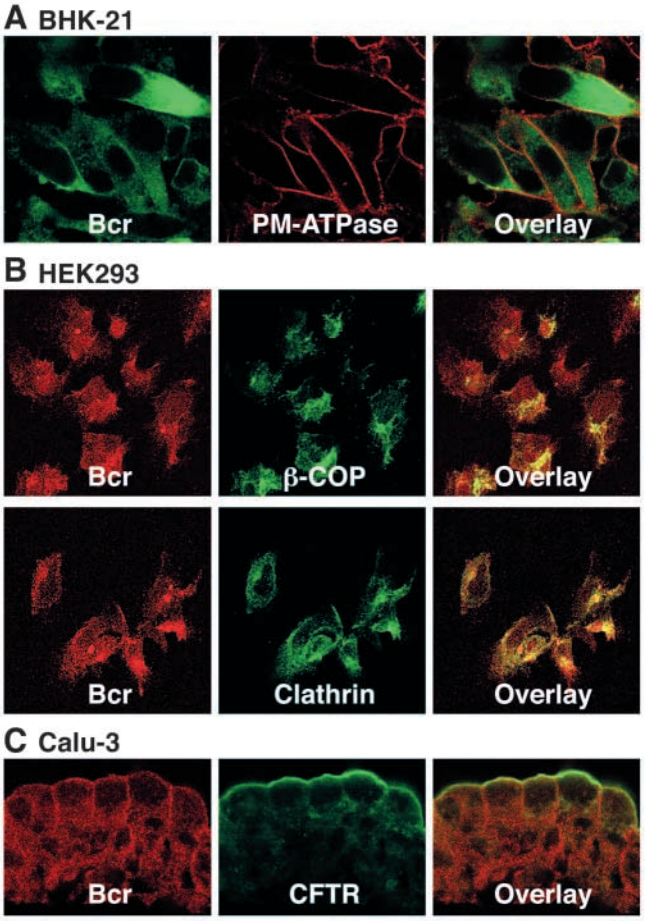

Fig. 3. Cellular localization of recombinantly and endogenously expressed BCR. (A) BHK-21 cells transiently expressing Bcr were immunostained with rabbit anti-Bcr and mouse anti-plasma membrane calcium ATPase (PMCA-ATPase) antibodies as described in Materials and methods and viewed by confocal microscopy. (B) Endogenous Bcr, β-COP and clathrin were visualized in HEK293 cells by immunofluorescence microscopy using rabbit anti-Bcr and mouse anti-β-COP or mouse anti-clathrin heavy chain antibodies. (C) Endogenous Bcr and CFTR were detected by immunofluorescence staining in polarized Calu-3 cells, grown at an air-liquid interface using rabbit anti-Bcr and mouse anti-CFTR antibodies.

cells in which the interaction with Bcr was detected and near the plasma membrane of non-polarized cells we employed immunofluorescence microscopy to see if Bcr was present in these locations. In both cell types most Bcr staining appeared to be in association with membranous structures rather than uniformly distributed throughout the cytoplasm as expected if it were entirely soluble in the cytosol. When heterologously expressed in non-polarized BHK cells Bcr was present throughout the cells including the surface where there was some colocalization with the plasma membrane Ca^{2+}-ATPase (Fig. 3A). Considerable accumulation at intracellular membranes was also apparent in Calu-3 cells where the endogenous expression was at a much lower level and there was a weak staining at the apical surface where CFTR was concentrated (Fig. 3C). To gain some information about the identity of the intracellular membranes with which Bcr is

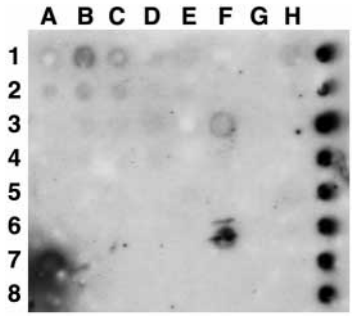

Fig. 4. Bcr binds the first PDZ domain of Mint3. Twenty-eight different PDZ domain-containing proteins were screened for Bcr binding using a TransSignal PDZ Domain Array. Detergent lysates from Bcr-transfected BKH-21 cells were added to the membrane and the bound Bcr visualized with the anti-Bcr C20 antibody. The X and Y coordinates of the array are indicated by letters and numerals, respectively. Mint2 PDZ1 (A1), Mint3 PDZ1 (B1), Mint3 PDZ2 (C1), Mint1 PDZ2 (E1), GIPC (F3), ZO-2 (F6), RGS12 (A7).

associated, several different organelle markers were used and staining patterns compared. As can be seen in Fig. 3B, there is considerable colocalization of endogenous Bcr with both β-COP and clathrin indicating association with coated vesicles of the endocytic pathway and the transGolgi network.

Bcr binding to Mint3

While small amounts of cellular Bcr apparently interact with the plasma membrane PDZ-domain proteins PDZK1 and AF-6 (Radziwill et al., 2003) larger amounts occur in association with intracellular membranes. Therefore we wondered if Bcr might also bind other PDZ-domain proteins so we screened 28 different ones using a TransSignal™ PDZ Domain Array (Panomics). Of the 28, Bcr bound strongly to the first PDZ-domain of Mint3 (spot B1; Fig. 4). Although not obvious in this blot very weak Mint1 signals were also detected in a duplicate blot. Other potential positives included the Golgi-associated GAIP-interacting protein C terminus (GIPC; spot F3), the ZO-2 epithelial junctional complex protein (spot F6) and a regulator of G-protein signaling, regulator of G-protein signaling 12 (RGS12; spot A7). The ZO-2 and RGS12 interactions seem reasonable in view of the association of Bcr with the other junctional PDZ protein, AF-6 (Radziwill et al., 2003) and its G-protein modulating GEF and GAP activities. However, we have first focused on the novel Mint3 binding.

Although the result of the array screen was reproducible it was necessary to gain additional evidence of Bcr binding to Mint3. To do this the GST-Bcr fusion and Mint3 were coexpressed in BHK cells. As indicated in Fig. 5A, immunoblots of the glutathione-Sepharose pull-downs detected the presence of a strong Mint3 signal. This appears to confirm that Bcr is a legitimate binding partner of Mint3 in cells. That this binding is PDZ-mediated was confirmed by the fact that it was ablated by deletion of the three C-terminal amino acids of Bcr (Fig. 5B). In addition to these GST-Bcr pulldown experiments, coimmunoprecipitation of Bcr and Mint3 could also be detected. Fig. 5C shows the presence of Mint3 in a Bcr immunoprecipitate. While this increases the number of confirmed PDZ-domain proteins binding to Bcr to

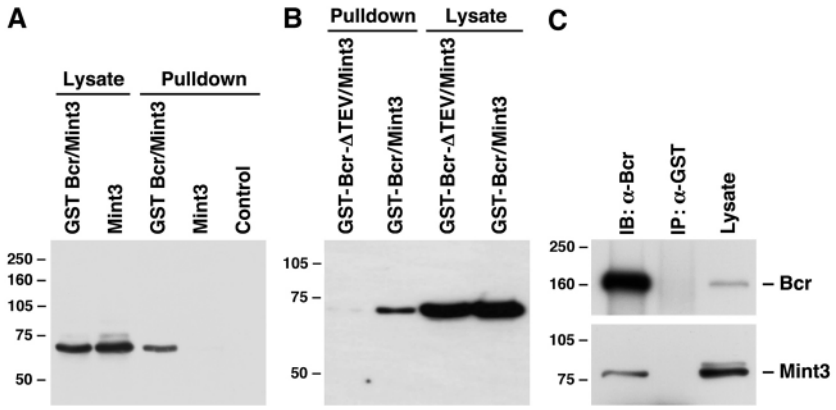

Fig. 5. Confirmation of the Bcr-Mint3 interaction. (A) Mint3 and Bcr-GST were transiently expressed in BHK-21 cells. Lysates prepared from these cells were incubated with glutathione-Sepharose beads and bound products were separated by SDS-PAGE. Mint3 was detected by western blotting using the rabbit anti-Mint3 antibody. The control is from untransfected parental BHK cells. (B) Similar experiment employing Bcr with deletion of 3 C-terminal amino acids. (C) Mint3 is detected in an immunoprecipitate with a Bcr antibody in lysate from parental HEK293 cells. Controls using an irrelevant antibody or immunoblotting with only secondary antibody, were negative for this band.

at least four [AF-6 (Radziwill et al., 2003), erbin (Boisguerin et al., 2004), PDZK1 and Mint3], there does seem to be considerable selectivity of the Bcr C-terminal ligand among PDZ-domain proteins, since EBP-50, GOPC and more than 20 other proteins represented on the array are not recognized. Selectivity among the different Mint proteins is particularly striking since both of their PDZ domains are quite highly conserved (Okamoto and Sudhof, 1998) and some protein ligands are known to bind to all three isoforms (Hill et al., 2003). The facts that in the array screen domain 2 of Mint3 (spot C1) was much weaker than domain 1 (spot B1), as was domain 1 of Mint2 (spot A1) and that domain 2 of Mint1 (spot E1) was completely negative indicates a high degree of specificity. It seems reasonable that Bcr present in hemopoietic, epithelial and other cell types binds the ubiquitously expressed Mint3 rather than the neuronal-specific Mints 1 and 2.

Mint3 has been described as residing at the Golgi and is seen in association with other intracellular membranous structures (Hill et al., 2003). We observed localization of the endogenously expressed protein by immunofluorescence in Calu-3 and HEK293 cells and when heterologously expressed in BKH cells (Fig. 6). In the polarized Calu-3 cells there was staining of a quite uniform population of vesicles throughout the cytoplasm, some of which were also stained for Bcr. In 293 cells the endogenous Mint3 appears on intracellular vesicles of different sizes to which some Bcr also colocalizes. A similar localization is seen in BHK cells exogenously expressing the protein and again a portion of this overlaps with Bcr when it is coexpressed in these cells. Thus Mint3-associated Bcr could account for at least a portion of the Bcr observed at intracellular membranes.

Discussion

Over the past decade it has been increasingly realized that cellular function depends not only on the structural and catalytic properties of individual proteins but also on spatially and temporally regulated interactions between them (Pawson and Nash, 2003). These interactions may be direct or mediated by adaptor proteins containing domains that recognize and bind specific ligands formed by polypeptide sequence segments with or without posttranslational modifications (Scott and Pawson, 2000). One prominent class of adaptors are the PDZ-domain proteins that contain variable numbers of approximately 90 amino acid segments that bind specific short sequence motifs usually at the C termini of proteins (Hung and Sheng, 2002). Many of these ligands are the cytoplasmic tails of transmembrane proteins which become juxtaposed at areas of membrane specialization such as synapses (Chen et al., 1998) and epithelial junctional complexes (Radziwill et al., 2003). Several PDZ domain-connected complexes contribute to apical-basal polarity of epithelial cells (Bilder et al., 2003). Regulatory complexes may be assembled by PDZ-domain-mediated associations of soluble cytoplasmic proteins with

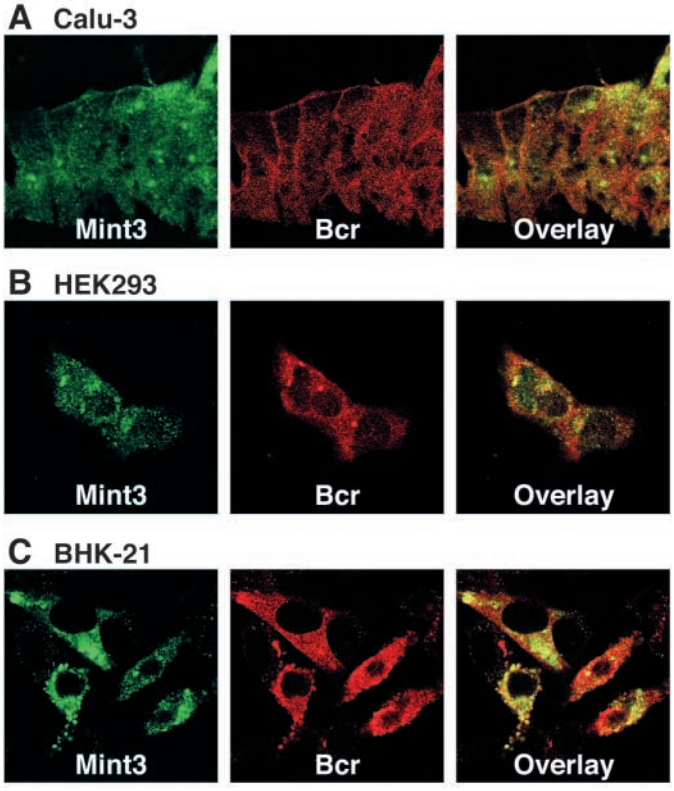

Fig. 6. Cellular localization of endogenous and recombinantly expressed Mint3. Endogenous Mint3 and Bcr were visualized by confocal immunofluorescence microscopy in polarized Calu-3 layers (A) and HEK293 cells (B) as described in Materials and Methods. (C) BHK-21 cells were transiently transfected with Bcr/pcDNA3.1 and Mint3/pCMV5 using LipofectAMINE Plus and proteins were detected 30 hours after transfection.

integral membrane proteins. One example is the localization of soluble PDZK1 with the epithelial apical membrane by its binding to the C terminus of the integral membrane protein, MAP17 (Pribanic et al., 2003). The remaining unoccupied PDZ domains of PDZK1 are available to interact with several known additional integral membrane proteins of functional importance, such as MRP2 (Kocher et al., 1999), the type IIa Na/Pi cotransporter (Gisler et al., 2003) and CFTR (Wang et al., 2000).

We have now found that the multifunctional cytoplasmic Bcr protein also interacts specifically with the first PDZ domain of PDZK1. This finding is of interest with respect to the recent report of Radziwill et al. (Radziwill et al., 2003) that the C terminus of Bcr binds another PDZ domain protein, AF-6 at the specialized junctional region of the epithelial cell surface. As this interaction appears to affect Ras regulated signaling it is possible that there may be important functional consequences of Bcr docking at additional apical sites in epithelia via PDZK1. For example it will be of interest to learn if any of the known integral apical membrane protein ligands of PDZK1 are substrates for the kinase activity of Bcr or Bcr-binding kinases such as CKII (casein kinase II) (Mishra et al., 2003). CFTR may be of particular interest in this regard. It may also be possible that the GEF and GAP domains of Bcr act on apically localized G proteins. In this respect it may be significant that Bcr also bound the RGS12 regulator of G-protein signaling in the array screen (Fig. 4).

Despite the potential functional importance of the localization of some Bcr at the surface membrane of epithelial cells both our immunofluorescence observations and those of Radziwill et al. (Radziwill et al., 2003) indicate this is a small part of the total Bcr pool in these cells. Most of the protein appears associated with intracellular membranous compartments. Therefore it was notable when we screened for other PDZ domain proteins that the Golgi-associated Mint3 was found. A larger proportion of cellular Bcr was observed colocalized with Mint3 than with PDZK1 and this association is especially intriguing in terms of known functions of the two proteins. Both the GEF and GAP domains of Bcr act on Rho family G-proteins which are important in the control of vesicular trafficking in cells (Ridley, 2001). The Mint proteins also function in vesicular membrane traffic (Ho et al., 2003; Kaech et al., 1998; Okamoto and Sudhof, 1997; Sastre et al., 1998). They are products of three different genes and play important roles in protein processing and vesicular trafficking in the distal secretory pathway (Biederer et al., 2002; Hill et al., 2003; Ho et al., 2003; Lau et al., 2000; Okamoto and Sudhof, 1997; Okamoto and Sudhof, 1998). All three contain single PTB and PDZ domains in their C-terminal halves while the N-terminal halves of Mints 1 and 2 bind the Munc18 fusion protein. The N-terminal half of Mint3 is distinct from the other two and does not bind Munc18. Mints 1 and 2 have been intensively studied in neurons because of binding of their PTB domains to a phosphotyrosine motif in ameloid precursor protein (APP) and the binding of their PDZ domains to presenilin-1 (Lau et al., 2000). While Mints 1 and 2 are neuronal-specific Mint3 is ubiquitously expressed in tissues. Mint3 influences vesicular trafficking in all cell types and has recently been proposed to act as an Arf GTPase-dependent vesicle coat protein and thereby contribute to the trafficking of some proteins to the plasma membrane (Hill et al., 2003). A great deal of further work is required to test the speculation that Mint3 may recruit some Bcr molecules to Golgi vesicles where it could influence nucleotide exchange and hydrolysis by Rho GTPases and thereby their effect on vesicular trafficking. It is interesting that Bcr also appeared to bind one other Golgi-localized PDZ domain protein involved in intracellular trafficking of proteins and vesicles, GIPC (Fig. 4) but not another, GOPC (Fig. 2).

The multiple domains of Bcr with different biochemical signatures suggest regulatory and signaling roles in the cell. One such role could be inflammatory regulation as suggested by observations in Bcr-null mice and its potential for MAPK-mediated NF-kB activation (Korus et al., 2002; Voncken et al., 1995). However, the primary function of Bcr remains largely unknown despite the extensive studies of the Bcr-ABL fusion found in chronic myelogenous leukemia (CML). As the PDZ-mediated interactions are absent in this fusion protein, the effects of cell localization have not been taken into account. The potential for Bcr to be a cytoplasmic protein as well as localized to apical and intracellular membranes via interactions with PDZK1 and AF-6, and with vesicular membranes via Mint3, suggest shuttling between different locations as an important part of its functions. Further studies in this direction should shed new light on the primary function of Bcr.

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