Identification and Characterization of a Novel BASH N Terminus-associated Protein, BNAS2*

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A B cell-specific adaptor protein, BASH (also known as BLNK or SLP-65), is crucial for B cell receptor (BCR) signaling. BASH binds to various signaling intermediates, such as Btk, PLCγ2, Vav, and Grb2, through its well defined motifs. Although functional significance of such interactions has been documented, BASH-mediated signal transduction mechanism is not fully understood. Using the yeast two-hybrid system, we have identified a novel protein that binds to a conserved N-terminal domain of BASH, which we named BNAS2 (BASH N terminus associated protein 2). From its deduced amino acid sequence, BNAS2 is presumed to contain four transmembrane domains, which are included in a central MARVEL domain, and to localize to endoplasmic reticulum. BNAS2 was co-precipitated with BASH as well as Btk and ERK2 from a lysate of mouse B cell line. In the transfected cells, the exogenous BNAS2 was localized in a mesh-like structure in the cytoplasm resembling that of endoplasmic reticulum (ER) and nuclear membrane. BASH was co-localized with BNAS2 in a manner dependent on its N-terminal domain. RT-PCR analysis indicated that BNAS2 mRNA is expressed ubiquitously except for plasma cells. In chicken B cell line DT40, overexpression of BNAS2 resulted in an enhancement of PLCγ2 activation. Thus BNAS2 may serve as a scaffold for signaling proteins such as BASH, Btk, and ERK at the ER and nuclear membrane and may facilitate ERK activation by signaling from cell-surface receptors.

The membrane immunoglobulin (Ig) and Igs/β heterodimers compose the B cell antigen receptor (BCR). BCR engagement evokes a signal that eventually causes various outcomes of B cells, such as activation, proliferation, differentiation, or apoptosis, depending on the developmental stage and circumstances of the cells. Cross-linking of BCR induces phosphorylation of tyrosine residues in the immunoreceptor tyrosine-based activation motifs (ITAM) of Igα and Igβ. When the ITAM of Igα is phosphorylated, the tyrosine kinase Syk is recruited and activated. The other tyrosine kinases belonging to the Src family (Lyn, Fyn, and Blk), as well as Btk, are also phosphorylated and activated through BCR engagement. These kinases phosphorylate and activate signaling intermediates like phospholipase C (PLC)γ, Vav, and phosphatidylinositol 3-kinase, and the following signaling cascades such as RAS-Raf-MEK-ERK pathway, and those leading to the activation of transcription factors, NF-AT, AP-1, or NF-κB (1–3).

BASH (also known as BLNK or SLP-65) is a B cell-specific member of SLP-76 adapter protein family. The primary structure of BASH reveals a string of basic, acidic, proline-rich, and SH3 domains from N to C terminus. As a result of BCR cross-linking in B cells, BASH is rapidly tyrosine-phosphorylated by Syk and bound to the SH2 domains of PLCγ2, Btk, Vav, and Nck, or to the SH3 domain of Grb2 (4–9). These molecules interacting with BASH serve as linkers and enzymes for the downstream effecter targets of the signal pathways. BASH mediates PLCγ2 activation by Btk (8), and thus is required for elevation of intracellular calcium level and activation of ERK, JNK, and p38 in response to BCR stimulation (4, 7). BASH is also necessary for BCR-induced activation of NF-κB (10) and HPK1 (11, 12). It has recently been shown that BASH and Grb2 cooperate to localize Vav into membrane rafts and thus to activate Rac1 during BCR engagement (13). BASH-deficient mice manifest a phenotype exhibiting BCR/pre-B cell receptor (pre-BCR) signaling deficiencies such as partial blocking of early B cell development, severe reduction of peripheral mature B and peritoneal B-1 cells, defective activation, and proliferation of B cells upon BCR ligation in vitro, low serum Ig levels, and impaired T-independent immune response (14–18). A congenital immunodeficiency patient lacking B cells in the peripheral lymphoid organs has been reported to have a mutation in the BASH gene (19). Furthermore, acute pre-B cell leukemia development due to BASH deficiency in mice and humans has been recently demonstrated (18, 20, 21). Thus BASH plays a pivotal role in pre-BCR/BCR signal transduction that is essential for B cell development, function, and homeostasis.

Previously, proteins with four transmembrane domains (tetra-span transmembrane protein) have been identified, including CD9, CD37, CD53, CD63, CD81, CD82, and PETA-3 (22–29), and some of which are known to be a component of a cell

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§ The abbreviations used are: BCR, B cell antigen receptor; PLC, phospholipase C; MEK, mitogen-activated kinase; ERK, extracellular signal-regulated protein kinase; NF-AT, nuclear factor of activated T cells; NF-κB, nuclear factor-κB; SH2, Src homology 2; Btk, Bruton's tyrosine kinase; CD, clusters of differentiation; GST, glutathione S-transferase; PMs, phospholipid bilayer; C, cytoplasm; FITC, fluorescein isothiocyanate; BNAS, BASH N terminus-associated protein 2; MAP, mitogen-activated protein; ER, endoplasmic reticulum; RT, reverse transcriptase.

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The N-terminal basic domain of BASH is evolutionarily conserved and necessary for signaling function. However, proteins that interact with the basic domain have not yet been identified. We have set out to clone such proteins by yeast two-hybrid system using chicken BASH N-terminal domain as a bait, and with a CDNA library from chicken B cell DT40. Here we describe the identification of a novel tetra-span transmembrane protein, which we have named BASH N terminus associated protein 2 (BNAS2), and the characterization of this molecule.

**EXPERIMENTAL PROCEDURES**

**Yeast Two-hybrid Screening**—Handling of yeast strain LA40, screening procedure, and isolation of plasmids were carried out as described in the Hybrid Hunter User Manual (Invitrogen). Complementary DNAs of forward and reverse primers (5'-GGG AAT TCA CCT CCA AGT CTA-3', 5'-GGG AAT TCA CCT CCA AGT CTA-3'), 1 µg of pCAT7-mBNAS2, corrected to the total amount of DNA (20 ng/g of each plasmid, pHybLex/Zeo-cBASH(1-62) as a template and 2.5 units of PfuTurbo DNA polymerase (Toyobo). The amplified DNA was digested with EcoRI and XhoI and inserted into pHybLex/Zeo (Invitrogen). pGRST-α1B (Clontech) as a bait were introduced into a reporter strain containing a pOPI3CAT (Stratagene) as a template. The amplified fragments were streaked on agar plates (SD/-H-W-U) and cultured at 30 °C over 1 week. Candidate positive colonies were restreaked on agar plates of the same type for further purification and propagation (second and third screenings). Sequencing of the positive clones was carried out using primers for pG4-5 (Invitrogen).

**Plasmid Constructions**—**pHybLex/Zeo-cBASH(1-62)**: A fragment of chicken BASH cDNA encoding amino acids 1-62 amino acid was amplified by PCR with 100 pmol of forward and reverse primers (5'-GGG AAT TCG GAT CCA TGA AGC TGA ACA AAC-3' and 5'-GGC TCG AGT CAT GGT TTT TTT TTT AGC CGA TCC-3', respectively), 1 µg of pCAT7-cBASH as a template and 2.5 units of Phusion DNA polymerase (Takara). The amplified DNA was digested with EcoRI and XhoI and inserted into pHybLex/Zeo (Invitrogen). pGRST-α1B (Clontech) as a template and 2.5 units of Phusion DNA polymerase (Takara). The transformed yeasts were streaked on agar plates (SD/-H-W-U-Zeo) and cultured at 30 °C over 1 week. Candidate positive colonies were restreaked on agar plates of the same type for further purification and propagation (second and third screenings). Sequencing of the positive clones was carried out using primers for pG4-5 (Invitrogen).

**Transfections and Luciferase Assay**—For co-immunoprecipitation assay, expression vectors, pCFPFLAG-mBNAS2, and pTA7-mBASH (1 µg each), were mixed with TransIT-LT1 reagents (Mirus). These mixtures were co-transfected into 2 × 10^5 COS-7 cells. Two days after transfection, the cells were harvested. For confocal microscopy, 0.5 µg of each vector, pCA-mBNAS2, pECFP-cBASH, and pECFP-cBASHH2, and/or pPEYF-mBNAS2, was transfected into 2 × 10^5 COS-7 cells as indicated. For the GST pull-down assay, 25 µg of pCAT7-mBNAS2 were transfected into 2 × 10^5 BASH-deficient DT40 cells by electroporation. One day after transfection, the cells were washed, and not normal, anti-chicken IgM antibody (M4, 1 µg/ml). For the Elk-luciferase assay (Trans-reporting system; Stratagene), 20 µg of empty vector (pCATT-neo) or various dose of BASH expression vectors (pCATT-cBASH or pCATT-mBNAS2), was cotransfected with the total amount of DNA (20 µg) with pCATT-neo, were mixed with 1 µg of pCF2-EIlk1, 10 µg of pFR-Luc, and 1 µg of pBIV-β-gal. The mixtures were transfected into 5 × 10^5 DT40 cells by electroporation. In the case of the NF-κB-luciferase assay, 20 µg of pCATT-neo or BASH expression vectors described above were mixed with 10 µg of pBtkB-luc (38) and 1 µg of pBIV-β-gal. 24 h after transfection, the cells were stimulated for 6 h with M4 antibody (1 µg/ml), or with a combination of ionomycin (1 µM) and phorbol 12-myristate 13-acetate (PMA, 100 ng/ml), and luciferase activities in the lysates were accessed as described previously (39).

**Antibodies**—Antiserum against mouse BNAS2 protein was produced by immunizing rabbit with a synthetic peptide corresponding to N-terminal residue of mouse BNAS2 (MWWPDAEPEPDPEGGG-3'), respectively. 1 µg of pCATT-cBASH as a template and 2.5 units of Phusion DNA polymerase (Takara). The EcoRI-HinII fragment was ligated with a HinII-BamHI fragment of eBSH from pCATT-cBASH and an EcoRI-BamHI fragment of pUC19 (named pBASHE-E-B). An EcoRI-Sall fragment from the pBASHE-B was inserted into pEFP-C. pEYFP-mBNAS2: the mBNAS2 cDNA (an EcoRI and Sall fragment) from pCATT-mBNAS2 was inserted into pEYFP-C (Clontech). pBSV-gal Rous sarcoma virus (RSV-LTR promoter was amplified by PCR with specific primers (5'-CCG CTC TAG TAT CTG CTC CCT GCT TGC-3' and 5'-CAT GCC ATG CAT CTG GTG ACC AAT GTG-3') and with poly3CAT (Stratagene) as a template. The amplified fragment was digested with Xhol and NcoI and then ligated with an Xhol-NcoI fragment from pACT-βgal (30).

**Preparation of pCFPs**—After PCR, the PCR products from various cell lines and CDNA synthesis were described previously (37). The CDNAs thus obtained (0.5 µl) were mixed in 50 µl of final reaction volume with 50 pmol of each of the m2D11-1-6 and m2D11-6-A primers, and 0.625 units of PfuTurbo DNA polymerase. The primers of β-actin for the internal control were β-actin 5'-TAC AAT GAT CGT CGT GTG GC-3' and β-actin 3'-ATA GCT CTT CTC GAG GGA GG-3'. PCR conditions were as follows: first denaturation for 5 min at 94 °C followed by 30 cycles of denaturation for 1 min at 94 °C, 2 min annealing at 65 °C, 1 min extension at 72 °C. Finally, 12.5 µl of PCR products were electrophoresed on 3% agarose gel and stained with ethidium bromide. The size marker (100-bp ladder, Invitrogen) was also electrophoresed.

**Culture**—DT40 cells were cultured and maintained as described previously (7). COS-7 cells were cultured in Dulbecco's modified Eagle's medium containing 10% of fetal bovine serum.

**Antiserum against mouse BNAS2 protein was produced by immunizing rabbit with a synthetic peptide corresponding to N-terminal residue of mouse BNAS2 (MWWPDAEPEPDPE-3') conjugated with keyhole limpet hemocyanin. The serum was purified with affinity column carrying the same peptide. Mouse monoclonal anti-T7 tag (Novagen) and anti-FLAG (M2, Sigma-Aldrich), goat anti-β-GT (Amersham Biosciences), goat anti-Btk (C-20; Santa Cruz Biotechnology), rabbit anti-ERK2 (C-14; Santa Cruz Biotechnology), and rabbit anti-MERK1/2 (Cell Signaling) antibodies were purchased, respectively. Rabbit antiserum against BASH was previously described (16).

**Immunoprecipitation, Pull-down Assay, and Western Blotting**—**Immunoprecipitation and Western blot analysis** were performed as previously described (11). For the GST pull-down assay, GST and GST-BASH(1-62) proteins were expressed in Escherichia coli BL21(DE3) (Invitrogen) containing pGEX-5x-1 and pGST-BASH(1-62), respectively. These proteins were purified with a glutathione-Sepharose 4B matrix (Amersham Biosciences) (11). Fifty nanograms of purified proteins were bound to glutathione-Sepharose 4B again, and cell lysates were added. After washing extensively, the proteins bound to the Sepharose were analyzed by Western blotting.

**Confluent Microscopy**—Confluent microscopy was carried out using TCS SP2 microscope (Leica). For COIF, excitation and emission wavelengths were 485 and 500 nm, respectively. For YFP, excitation and emission wavelengths were 514 and 560-670 nm, respectively. MB-2BAS2 proteins expressed from pCA-mBNAS2 in COS-7 cells were detected with rabbit anti-BNAS2 antibody and fluorescein isothiocyanate-conjugated goat anti-rabbit IgG (Jackson ImmunoResearch).

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D. Kitamura, N. Okamoto, Y. Ebina, and Y. Imamura, unpublished data.
BNAS2, a Novel BASH-associated Scaffold Protein

RESULTS

Cloning of BASH N Terminus-associated Protein (BNAS) 2 by Yeast Two-hybrid System—Although BASH is known to associate with various signaling proteins through its phosphotyrosine motifs, proline-rich region, and the SH2 domain as described above, proteins binding to a conserved N-terminal domain remain undefined. Since this domain is crucial for BASH function in BCR signal transduction, we sought to identify proteins that bind to this domain. We employed a yeast two-hybrid screening method using the chicken BASH 1–62 amino acids (basic) domain as a bait. After several rounds of screening, we finally isolated 8 positive clones from 6.5 × 10⁶ colony-forming units of a cDNA library derived from DT40 cells, which represents 5.2 × 10⁶ independent colonies. Binding of these clones was confirmed by back transformation of each into the reporter yeast strain containing the bait construct. We sequenced the inserts of the 8 positive clones and focused on one of them. This clone was about 2.5-kbp long and presumed to contain a full coding sequence by comparison to human/mouse orthologs as described below, and also to chicken EST clones in a database. Following a full-length sequencing, the coding sequence for BNAS2 proteins was determined, the amino acid sequences of which are shown in Table 1. BNAS2 is expressed in all hematopoietic and non-hematopoietic cell lines. As can be seen in Fig. 2, BNAS2 is expressed in T, macrophage, mast, erythroid and non-hematopoietic cell lines. In a B cell lineage, BNAS2 is expressed in pre-B and mature B cell lines, but not in plasma cell lines (J558L and P3U1). This observation suggested that the expression of BNAS2 is essentially ubiquitous but may be down-regulated as B cells terminally differentiate into antibody-secreting cells.

Expression Profile of BNAS2—In order to investigate the expression pattern of BNAS2, RT-PCR was carried out using RNA from various hematopoietic and non-hematopoietic cell lines. As can be seen in Fig. 2, BNAS2 is expressed in T, macrophage, mast, erythroid and non-hematopoietic cell lines. In a B cell lineage, BNAS2 is expressed in pre-B and mature B cell lines, but not in plasma cell lines (J558L and P3U1). This observation suggested that the expression of BNAS2 is essentially ubiquitous but may be down-regulated as B cells terminally differentiate into antibody-secreting cells.

Binding of BNAS2 to BASH in Vitro—Binding of BNAS2 and the N-terminal domain of BASH was evident in yeast two-hybrid assay as described above. Here we tested whether full-length BASH is capable of binding to BNAS2. The expression vectors encoding T7-tagged mouse BASH (T7-mBASH) and FLAG-tagged mouse BNAS2 (FLAG-mBNAS2) were co-transfected into COS-7 cells, which were immunoprecipitated with anti-T7 antibody, and Western blotting was carried out using anti-FLAG antibody. As shown in Fig. 3, BNAS2 was actually co-precipitated with BASH. As a control, irrelevant mouse IgG of the same subclass as the anti-T7 antibody did not precipitate BNAS2 (lane IgG). Conversely, when the FLAG-mBNAS2 was immunoprecipitated, the T7-mBASH was also co-precipitated (Fig. 3B). Therefore, full-length BASH protein was shown to bind to BNAS2. To analyze a direct interaction between BASH and BNAS2 proteins, we next carried out a GST pull-down assay. We demonstrated that the GST-cBASH(1–62) fusion protein, but not the mere GST protein, could precipitate T7-cBNAS2, which was expressed in BASH-deficient DT40 cells regardless of BCR cross-linking (Fig. 3C), indicating that BASH (and more specifically the 1–62 domain) and BNAS2 directly bind to each other. We next examined a binding of endogenously expressed

![Diagram](image.png)
BNAS2 and BASH proteins by immunoprecipitation assay. Lysate from a mouse B cell line, BAL17, was mixed with anti-BNAS2 antibody, and the immunoprecipitated proteins were analyzed by Western blotting. As shown in Fig. 3D, cell lysate was immunoprecipitated with anti-FLAG antibody (top). The filter was re-probed with anti-FLAG antibody (bottom). Cystosomas were subjected to immunoprecipitation with anti-FLAG antibody (α-FLAG) or control IgG, and probed with anti-T7 antibody (top), then re-probed with anti-FLAG antibody (bottom). C, GST pull-down assay. GST or GST-cBASH(1–62) proteins immobilized to glutathione-Sepharose 4B were mixed with lysates from cells transfected with pCAT7-cBNAS2 and stimulated (+), or not (−), with anti-IgM antibody. Bound proteins and cell lysate were analyzed by 15% SDS-PAGE and the following Western blotting with anti-T7 antibody (top). The filter was re-probed with anti-GST antibody (bottom). D, lysates of BAL17 cells stimulated (+), or not (−), with anti-mouse IgM antibody, were immunoprecipitated with anti-BNAS2 antibody. The precipitates and the lysates were subjected to Western blotting. The filter was sequentially probed with the indicated antibodies. Shown is a representative of three times repeated experiments with essentially the same results.

Co-localization of BASH and BNAS2 in a Cell—To examine the localization of BNAS2 in a cell; expression vector encoding mouse BNAS2 was transfected into COS-7 cells. Then the cells were fixed and stained with antibody against BNAS2, then analyzed by confocal microscopy (Fig. 4). mBNAS2 located in cytoplasm unevenly; it was localized in a mesh network-like compartment reminiscent of ER morphology, with brightest staining in the perinuclear region. In some instances, strong accumulation of mBNAS2 at a distinctive juxtanuclear compartment was observed.

To examine the localization of BNAS2 and BASH in living cells, the expression vectors encoding fluorescent protein-fused BNAS2 (pEYFP-mBNAS2) and BASH (pECFP-cBASH), respectively, were transfected into COS-7 cells, and the cells were inspected by confocal microscopy. As can be seen in Fig. 5A, CFP-cBASH located diffusely in the cyto-
plasm, with gradual concentration from the periphery toward the nuclei. CFP-cBASHΔ62, in which a BNAS2-binding domain (N-terminal 62 amino acids) had been removed, displayed a similar cytoplasmic but more diffuse pattern (Fig. 5B). In contrast, YFP-mBNAS2 was localized in a mesh network-like structure in the perinuclear region including nuclear membrane, and often accumulated in undefined cytoplasmic compartments, which might be a property of this fusion protein (Fig. 5C). CFP or YFP alone located diffusely throughout the cell (data not shown). When CFP-cBASH and YFP-mBNAS2 were co-expressed in COS-7 cells, both proteins were co-localized in cytoplasmic compartments where YFP-mBNAS2 alone was characteristically localized (Fig. 5D–F). On the other hand, cBASHΔ62 located diffusely in the cytoplasm regardless of the presence of YFP-mBNAS2 (Fig. 5B, G, H, and I). The localization of YFP-mBNAS2 was not markedly affected by the presence of cBASH proteins. These results indicate that BASH indeed binds to BNAS2 through its N-terminal domain in living cells, and suggests that localization of BASH is determined mainly by its binding partners, whereas that of BNAS2 is self-determined.

Enhancement of BCR-mediated Activation of Transcription Factor Elk1, but Not NF-κB, by BNAS2—In order to postulate a function of BNAS2 in BCR signaling, we examined the effect of overexpression of BNAS2 on BCR-mediated activation of transcription factors Elk1 and NF-κB. Elk1 is known to be phosphorylated and activated by ERK, therefore used as readout for ERK activation. Chicken or mouse BNAS2 was transiently expressed in DT40 cells together with Elk1-driven luciferase reporter system, then the cells were stimulated with anti-IgM antibody, or with a combination of PMA and ionomycin, respectively. As shown in Fig. 6A, both chicken and mouse BNAS2 expression led to dose-dependent enhancement of anti-IgM-mediated activation of Elk1. In the similar experimental conditions, BCR-mediated activation of an AP-1/NF-AT-dependent reporter gene was also increased upon overexpression of the BNAS2 proteins (data not shown). In contrast, BNAS2 expression did not affect the anti-IgM-mediated transcriptional activation of NF-κB (Fig. 6B). These results suggest that BNAS2 plays a positive regulatory role in the BCR signaling pathways leading to ERK activation, possibly through interaction with the BASH as well as Btk and ERK.

DISCUSSION

Here we report the isolation and characterization of BNAS2, a novel, presumably tetra-span transmembrane protein, which binds to an N-terminal basic domain of BASH, as well as ERK2 and Btk. BNAS2 was localized in an ER-like structure in the cytoplasm and nuclear membrane. BNAS2 overexpression resulted in an enhancement of BCR ligation-induced activation of
Elk1, a transcription factor known to be phosphorylated and activated by ERK.

The amino acid sequence of BNAS2 is well conserved among chicken, human and mouse, especially in the putative transmembrane regions. This underscores possible characteristics of this protein as a transmembrane protein, which is consistent with the apparent intracellular localization of BNAS2 to ER and the nuclear membrane. This localization of BNAS2 is different from that of other tetra-span transmembrane proteins, such as CD9, CD37, CD53, CD63, CD81, CD82, PETA-3, occludin, and claudin, all of which are known to be plasma membrane proteins. In addition, the four transmembrane regions in BNAS2 are involved in the MARVEL domain, a recently proposed domain conserved in proteins of the myelin and lympho-
cyte (MAL), physins, gyrins, and occludin families, some of which are associated with specialized cholesterol-rich membrane microdomains (41). Therefore, BNAS2 may represent a novel type of tetra-span membrane proteins.

Our data indicate that BNAS2 binds to the N-terminal domain (1–62 amino acids) of cBASH, which does not contain a tyrosine residue, in yeast cells (two hybrid assay) as well as in vitro (GST pull-down assay). In the latter assay, BNAS2 in the lysates from both anti-IgM treated and untreated DT40 B cells binds equally to BASH protein. Therefore, tyrosine phosphorylation of both proteins is unlikely to be necessary for this binding. The N-terminal domain of BASH has no homology to known protein interacting motifs, and thus the mode of its interaction to BNAS2 remains unknown. The function of the conserved N-terminal basic domain of BASH has not yet been reported, but our preliminary data have suggested an essential role for this domain in BCR signaling leading to the activation of a promoter consisting of AP-1/ NF-AT binding sites. Our present data indicated that BNAS2 strongly binds to ERK2 in addition to BASH (Fig. 3D) and may function to positively regulate ERK activation in response to BCR ligation (Fig. 6). BASH was shown to be necessary, if not essential, for ERK activation upon BCR ligation in DT40 B cell line (7). Taken together, BNAS2 may serve to link BASH and ERK functionally in the BCR signaling pathway.

Implication of Ras-ERK pathway in BCR signal transduction was first documented by the fact that Ras is co-captured with surface immunoglobulin molecules upon BCR cross-linking of mouse splenic B lymphocytes (43). Since then, many reports have demonstrated the BCR-mediated activation of Ras-ERK pathway and its correlation with cell-cycle entry and activation of B cells (44–49). It has also been shown in vivo that active form of Ras or Raf restores to some extent the pre-BCR-dependent pre-B cell development which is impeded in RAG- or μH chain-deficient mice (50–52), or conversely dominant-inhibitory form of Ras inhibits the pre-B cell development (53).

Despite clear activation of Ras-ERK pathway by BCR ligation in B cells, the mechanism for this activation and signal transmission to the nucleus remains unclear. It was reported that, upon stimulation, MAPK dissociates from MAPKK, which behaves as a cytoplasmic anchor of MAPK (54). MEK-phosphorylated ERK would thus be detached from MEK, translocated into the nuclei, and then would activate nuclear targets such as transcription factor Elk1 (55–57). Our present data imply a possible formation of BASH/BNAS2/Btk/ERK2 complex at nuclear membrane and perinuclear ER.

Taking these into account, we propose a possible model in which BNAS2 serves as a scaffold for BASH, Btk, ERK, and other proteins on the ER and nuclear membrane. Such scaffold proteins for MAPKs and upstream kinases have been identified: for example, MEK Partner 1 (MP1) for MEK1 and ERK1 (58), and JIP1/JIP2 for multiple components of the c-Jun N-terminal kinase (JNK) signaling pathway (59, 60). Although our data indicate that the binding of these molecules to BNAS2 is constitutive, BNAS2-bound complex may be reorganized upon BCR stimulation, which might result in full activation of ERK or its liberation from MEK. Furthermore, BNAS2 might also serve to transport the active ERK to the nuclear membrane and thus facilitate its nuclear translocation. Noteless to mention, many other models for the function of BNAS2 should be possible at present, and further characterization of this molecule will verify these possibilities.

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