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Molecular Cloning of IBP, a SWAP-70 Homologous GEF, Which is Highly Expressed in the Immune System

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ABSTRACT: Rho GTPases play a fundamental role in a variety of biological processes ranging from the reorganization of the actin cytoskeleton to the regulation of cell proliferation. The activation of Rho GTPases is regulated by guanine nucleotide exchange factors (GEFs) belonging to the Dbl family of proteins. The hallmark of this large family of GEFs is the presence of a tandem DH-PH module in which a pleckstrin-homology (PH) domain is located at the C-terminus of a Dbl-homology (DH) domain. Recent studies have demonstrated that SWAP-70 constitutes a novel class of Rac-GEF, in which the PH domain is located at the N-terminus, rather than the C terminus, of the DH domain. Here we report the molecular cloning of human IBP (IRF-4 binding protein), a new member of this novel family of GEFs. The IBP gene maps to human chromosome 6p21.31 centromeric to the MHC locus. Isolation of the murine IBP cDNA reveals a very high degree of homology with the human IBP cDNA suggesting that IBP is evolutionarily conserved. The 5′ portion of the murine IBP cDNA is furthermore identical to the Def-6 cDNA fragment, which was identified in the course of a search for genes differentially expressed in the murine hematopoietic system. IBP is broadly expressed in the immune system and can be detected in both T and B cell compartments in contrast to SWAP-70 whose expression is primarily restricted to B cells. Taken together these findings indicate that IBP is a novel type of GEF, which participates in the activation of Rho GTPases in lymphoid tissues. *Human Immunology 64, 389–401 (2003).* © American Society for Histocompatibility and Immunogenetics, 2003. Published by Elsevier Science Inc.

KEYWORDS: SWAP-70; GEF; Rho GTPases

INTRODUCTION

The Rho subfamily of small GTPases, which include RhoA, Rac1 and Cdc42, control a wide range of biological processes, from cell survival and proliferation to motility and invasion [1–3]. Like all small GTPases, Rho GTPases function as molecular switches that cycle between an inactive GDP-bound form and an active GTP-bound form. Guanine nucleotide exchange factors (GEFs) promote the formation of the GTP-bound state, and therefore these proteins represent one of the major classes of regulators that control the activation state of Rho GTPases [4–6]. About 50 different GEFs for Rho GTPases have been identified to date in eukaryotes. These GEFs usually contain a sequence of ~200 amino acids termed the Dbl-homology (DH) domain, responsible for catalyzing the GDP/GTP exchange reactions, followed by a C-terminal pleckstrin-homology (PH) domain necessary for proper intracellular localization and function. The expression of many Rho- GEF proteins is confined to specific cell-types, suggesting that the activity of Rho GTPases can be controlled in a tissue-restricted manner, possibly contributing to the unique biologic properties of different cells.

Recent studies have revealed that SWAP-70 is a unique type of Rac-GEF in which the DH domain is flanked at its N-terminus, rather than at its C-terminus, by a PH domain [7]. Interestingly, the DH domain of SWAP-70 exhibits only a very low degree of homology to that of other known Rho-GEFs like Vav1 and Tiam1 further suggesting that this class of GEFs may activate a unique subset of Rho GTPases-mediated functions. SWAP-70 expression is predominantly confined to acti-

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   1                                                                AGTGTCAGAGCCGCCCCCAGCCGGGCGGGCGCCTCAGCC
  40  ATGGCCCTGCGCAAGGAACTGCTCAAGTCCATCTGGTACGCCTTTACCGCGCTGGACGTGGAGAAGAGTGGCAAAGTCTCCAAGTCCCAG
   1  M  A  L  R  K  E  L  L  K  S  I  W  Y  A  F  T  A  L  D  V  E  K  S  G  K  V  S  K  S  Q

 130  CTCAAGGTGCTGTCCCACAACCTGTACACGGTCCTGCACATCCCCCATGACCCCGTGGCCCTGGAGGAACACTTCCGAGATGATGATGAC
  31  L  K  V  L  S  H  N  L  Y  T  V  L  H  I  P  H  D  P  V  A  L  E  E  H  F  R  D  D  D  D

 220  GGCCCTGTGTCCAGCCAGGGATACATGCCCTACCTCAACAAGTACATCCTGGACAAGGTGGAGGAGGGGGCTTTTGTTAAAGAGCACTTT
  61  G  P  V  S  S  Q  G  Y  M  P  Y  L  N  K  Y  I  L  D  K  V  E  E  G  A  F  V  K  E  H  F

 310  GATGAGCTGTGCTGGACGCTGACGGCCAAGAAGAACTATCGGGCAGATAGCAACGGGAACAGTATGCTCTCCAATCAGGATGCCTTCCGC
  91  D  E  L  C  W  T  L  T  A  K  K  N  Y  R  A  D  S  N  G  N  S  M  L  S  N  Q  D  A  F  R

 400  CTCTGGTGCCTCTTCAACTTCCTGTCTGAGGACAAGTACCCTCTGATCATGGTTCCTGATGAGGTGGAATACCTGCTGAAAAAGGTACTC
 121  L  W  C  L  F  N  F  L  S  E  D  K  Y  P  L  I  M  V  P  D  E  V  E  Y  L  L  K  K  V  L

 490  AGCAGCATGAGCTTGGAGGTGAGCTTGGGTGAGCTGGAGGAGCTTCTGGCCCAGGAGGCCCAGGTGGCCCAGACCACCGGGGGGCTCAGC
 151  S  S  M  S  L  E  V  S  L  G  E  L  E  E  L  L  A  Q  E  A  Q  V  A  Q  T  T  G  G  L  S

 580  GTCTGGCAGTTCCTGGAGCTCTTCAATTCGGGCCGCTGCCTGCGGGGCGTGGGCCGGGACACCCTCAGCATGGCCATCCACGAGGTCTAC
 181  V  W  Q  F  L  E  L  F  N  S  G  R  C  L  R  G  V  G  R  D  T  L  S  M  A  I  H  E  V  Y

 670  CAGGAGCTCATCCAAGATGTCCTGAAGCAGGGCTACCTGTGGAAGCGAGGGCACCTGAGAAGGAACTGGGCCGAACGCTGGTTCCAGCTG
 211  Q  E  L  I  Q  D  V  L  K  Q  G  Y  L  W  K  R  G  H  L  R  R  N  W  A  E  R  W  F  Q  L

 760  CAGCCCAGCTGCCTCTGCTACTTTGGGAGTGAAGAGTGCAAAGAGAAAAGGGGCATTATCCCGCTGGATGCACACTGCTGCGTGGAGGTG
 241  Q  P  S  C  L  C  Y  F  G  S  E  E  C  K  E  K  R  G  I  I  P  L  D  A  H  C  C  V  E  V

                                                  *
 850  CTGCCAGACCGCGACGGAAAGCGCTGCATGTTCTGTGTGAAGACAGCCACCCGCACGTATGAGATGAGCGCCTCAGACACGCGCCAGCGC
 271  L  P  D  R  D  G  K  R  C  M  F  C  V  K  T  A  T  R  T  Y  E  M  S  A  S  D  T  R  Q  R

 940  CAGGAGTGGACAGCTGCCATCCAGATGGCGATCCGGCTGCAGGCCGAGGGGAAGACGTCCCTACACAAGGACCTGAAGCAGAAACGGCGC
 301  Q  E  W  T  A  A  I  Q  M  A  I  R  L  Q  A  E  G  K  T  S  L  H  K  D  L  K  Q  K  R  R

1030  GAGCAGCGGGAGCAGCGGGAGCGGCGCCGGGCGGCCAAGGAAGAGGAGCTGCTGCGGCTGCAGCAGCTGCAGGAGGAGAAGGAGCGGAAG
 331  E  Q  R  E  Q  R  E  R  R  R  A  A  K  E  E  E  L  L  R  L  Q  Q  L  Q  E  E  K  E  R  K

1120  CTGCAGGAGCTGGAGCTGCTGCAGGAGGCGCAGCGGCAGGCCGAGCGGCTGCTGCAGGAGGAGGAGGAACGGCGCCGCAGCCAGCACCGC
 361  L  Q  E  L  E  L  L  Q  E  A  Q  R  Q  A  E  R  L  L  Q  E  E  E  E  R  R  R  S  Q  H  R

1210  GAGCTGCAGCAGGCGCTCGAGGGCCAACTGCGCGAGGCGGAGCAGGCCCGGGCCTCCATGCAGGCTGAGATGGAGCTGAAGGAGGAGGAG
 391  E  L  Q  Q  A  L  E  G  Q  L  R  E  A  E  Q  A  R  A  S  M  Q  A  E  M  E  L  K  E  E  E

1300  GCTGCCCGGCAGCGGCAGCGCATCAAGGAGCTGGAGGAGATGCAGCAGCGGTTGCAGGAGGCCCTGCAACTAGAGGTGAAAGCTCGGCGA
 421  A  A  R  Q  R  Q  R  I  K  E  L  E  E  M  Q  Q  R  L  Q  E  A  L  Q  L  E  V  K  A  R  R

1390  GATGAAGAATCTGTGCGAATCGCTCAGACCAGACTGCTGGAAGAGGAGGAAGAGAAGCTGAAGCAGTTGATGCAGCTGAAGGAGGAGCAG
 451  D  E  E  S  V  R  I  A  Q  T  R  L  L  E  E  E  E  E  K  L  K  Q  L  M  Q  L  K  E  E  Q

1480  GAGCGCTACATCGAACGGGCGCAGCAGGAGAAGGAAGAGCTGCAGCAGGAGATGGCACAGCAGAGCCGCTCCCTGCAGCAGGCCCAGCAG
 481  E  R  Y  I  E  R  A  Q  Q  E  K  E  E  L  Q  Q  E  M  A  Q  Q  S  R  S  L  Q  Q  A  Q  Q

1570  CAGCTGGAGGAGGTGCGGCAGAACCGGCAGAGGGCTGACGAGGATGTGGAGGCTGCCCAGAGAAAACTGCGCCAGGCCAGCACCAACGTG
 511  Q  L  E  E  V  R  Q  N  R  Q  R  A  D  E  D  V  E  A  A  Q  R  K  L  R  Q  A  S  T  N  V

1660  AAACACTGGAATGTCCAGATGAACCGGCTGATGCATCCAATTGAGCCTGGAGATAAGCGTCCGGTCACCAGCAGCTCCTTCTCAGGCTTC
 541  K  H  W  N  V  Q  M  N  R  L  M  H  P  I  E  P  G  D  K  R  P  V  T  S  S  S  F  S  G  F

1750  CAGCCCCCTCTGCTTGCCCACCGTGACTCCTCCCTAAAGCGCCTGACCCGCTGGGGATCCCAGGGCAACAGGACCCCCTCGCCCAACAGC
 571  Q  P  P  L  L  A  H  R  D  S  S  L  K  R  L  T  R  W  G  S  Q  G  N  R  T  P  S  P  N  S

1840  AATGAGCAGCAGAAGTCCCTCAATGGTGGGGATGAGGCTCCTGCCCCGGCTTCCACCCCTCAGGAAGATAAACTGGATCCAGCACCAGAA
 601  N  E  Q  Q  K  S  L  N  G  G  D  E  A  P  A  P  A  S  T  P  Q  E  D  K  L  D  P  A  P  E

1930  AATTAGCCTCTCTTAGCCCCTTGTTCTTCCCAATGTCATATCCACCAGGACCTGGCCACAGCTGGCCTGTGGGTGATCCCAGCTCTTACT
 631  N  •
2020  AGGAGAGGGAGCTGAGGTCCTGGTGCCAGGGGCCCAGGCCCTCCAACCATAAACAGTCCAGGATGGAACCTGGTTCACCCTTCATACCAG
2110  CTCCAAGCCCCAGACCATGGGAGCTGTCTGGGATGTTGATCCTTGAGAACTTGGCCCTGTGCTTTAGACCCAAGGACCCGATTCCTGGGC
2200  TAGGAAAGAGAGAACAAGCAAGCCGGGGCTACCTGCCCCCAGGTGGCCACCAAGTTGTGGAAGCACATTTCTAAATAAAACTGCTCTTA
2290  GAATGAAAAAAAAAAAAAAAAAAAAAAAAAAA
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A

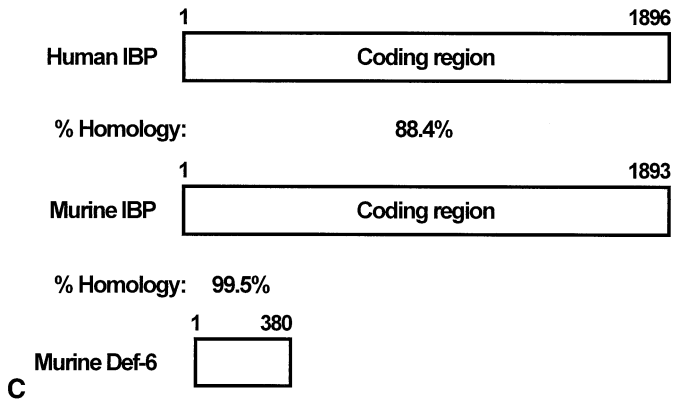

FIGURE 1 Sequence analysis of IBP cDNA and protein. (A) Nucleotide sequence of the full-length human IRF-4 binding protein (IBP) cDNA and its predicted amino acid sequence in single-letter code. Nucleotides and amino acids are numbered on the left. A poly(A) addition signal (AATAAA) is double-underlined. The asterisk indicates the position of a potential polymorphic nucleotide that differs from the corresponding nucleotide in the deposited sequence for the human Def-6 cDNA (GenBank accession # NM_022047). The human IBP nucleotide sequence has been deposited in the GenBank database (accession no. AY241694). (B) The murine IBP cDNA sequence and comparison with the murine Def-6 cDNA fragment (GenBank accession # X96705). Nucleotide numbers are shown on the right. Identical nucleotides are marked by asterisks. Bars indicate absent nucleotides. The murine IBP nucleotide sequence has been deposited in the GenBank database (accession no. AY241695). (C) Diagram summarizing the sequence homologies (in percentage) amongst the human IBP cDNA, murine IBP cDNA and murine Def-6 cDNA fragment. The numbers of the nucleotides are indicated on the top. (D) Organizations of the human and murine IBP genes. Data were generated utilizing the University of California, Santa Cruz (UCSC) genome browser. (E) Alignment of the predicted amino acid sequences of human and murine IBP proteins. Amino acids are numbered on the right. Asterisks indicate identical residues; dots indicate similar residues; and bars indicate skipped amino acids. Figure 1 continues on pages 391 and 392.

```
Murine Def6     ------------------------------------------CTTCACTGCACTGGACGTG   19
Murine IBP      ATGGCCCTGCGCAAGGAGCTGCTCAAGTCTATCTGGTACGCCTTCACTGCACTGGACGTG   60
                                                          ********************

Murine Def6     GAGAAGAGCGGCAAGGTCTCCAAGTCCCAACTCAAGGTGCTGTCCCACAACCTGTACACT   79
Murine IBP      GAGAAGAGCGGCAAGGTCTCCAAGTCCCAACTCAAGGTGCTGTCCCACAACCTGTACACT   120
                ************************************************************

Murine Def6     GTCCTGAACATCCCC-ATGACCCCGTGGCCCTGGAGGAGCACTTCCGG-ATGACGATGAT   137
Murine IBP      GTCCTGAACATCCCCCATGACCCCGTGGCCCTGGAGGAGCACTTCCGGGATGACGATGAT   180
                *************** ********************************** **********

Murine Def6     GGCCCGGTGTCCAGTCAAGGTTACATGCCCTACCTCAACAAGTACATCCTAGACAAGGTG   197
Murine IBP      GGCCCGGTGTCCAGTCAAGGTTACATGCCCTACCTCAACAAGTACATCCTAGACAAGGTG   240
                ************************************************************

Murine Def6     GAGGAGGGAGCTTTCGTTAAGGAGCACTTCGATGAGTTGTGCTGGACCCTGACTGCCAAG   257
Murine IBP      GAGGAGGGAGCTTTCGTTAAGGAGCACTTCGATGAGTTGTGCTGGACCCTGACTGCCAAG   300
                ************************************************************

Murine Def6     AAGAACTACAGGGCAGATGGCATCGGGAGCAGCCCACTCTCTAATCAGGATGCTTTCCGT   317
Murine IBP      AAGAACTACAGGGCAGATGGCATCGGGAGCAGCCCACTCTCTAATCAGGATGCTTTCCGT   360
                ************************************************************

Murine Def6     CTATGGTGCCTCTTCAACTTCCTGTCTGAGGACAAGTACCCGCTGATCATGGTTCCCGAT   377
Murine IBP      CTATGGTGCCTCTTCAACTTCCTGTCTGAGGACAAGTACCCGCTGATCATGGTTCCCGAT   420
                ************************************************************

Murine Def6     GAG-------------------------------------------------------   380
Murine IBP      GAGGTAGAGTATCTGCTGAAGAAGCTCCTTGGCAGCCTGAGTTTGGAGATGGGCCTGGGC   480
                ***

Murine IBP      AAGCTGGAGGAGCTGCTGGCCCAGGACGCCCAGTCAGCCCAGACCGCCGTGGGGCTCAGC   540

Murine IBP      GTCTGGCAGTTTCTGGAACTCTTCAACTCAGGCCGCTGCCTTCGGGGTGTGGGGCGCGAC   600

Murine IBP      TCCCTCAGCATGGCCATCCAAGAAGTCTACCAGGAGCTCATCCAAGACGTCCTGAAGCAG   660

Murine IBP      GGCTATCTGTGGAAGCGAGGGCACCTGCGGAGGAACTGGGCCGAGCGCTGGTTCCAGCTG   720

Murine IBP      CAACCCAGCAGCCTCTGCTACTTTGGGAGTGAGGAATGCAAGGAGAAACGAGGCACCATT   780

Murine IBP      CCCCTGGATGCTCACTGCTGTGTGGAGGTGCTTCCGGACCGCGAAGGAAAGCGCAGCATG   840

Murine IBP      TTTTGTGTGAAGACTGCCAGCCGCACCTATGAGATGAGCGCCTCAGACACCCGCCAGCGC   900

Murine IBP      CAGGAGTGGACGGCCGCCATCCAGACTGCGATCCGGCTGCAGGCGGAGGGGAAGACGTCG   960

Murine IBP      CTGCACAAGGACCTGAAACAGAAGCGGCGGGAGCAGCGGGAACAGCGCGAGCGACGCCGG   1020

Murine IBP      GCAGCCAAGGAGGAGGAGCTGCTGCGACTGCAGCAGCTGCAGGAGGAGAAGGAGAGGAAG   1080

Murine IBP      CTGCAAGAACTGGAGCTGCTGCAGGAGGCTCAGCGGCAGGCCCAGCCGGCTGCTGCAGGAA   1140

Murine IBP      GAGGAGGAGCGCCGCCGTAGCCAGCACAAGGAGCTGCAGCAGGCTCTGGAGGGCCAGCTG   1200

Murine IBP      CGGGAGGCGGAGCAGGCCCGGGCCTCTATGCAGGCTGAGATGGAGCTGAAGAAGGAAGAG   1260

Murine IBP      GCGGCCCGGCAACGGCAGCGCATCGCTGAACTGGAGGAGATGCAGGAGCGGCTGCAGGAA   1320

Murine IBP      GCTCTGCAACTAGAGGTGAAAGCTAGGCGGGATGAGGAGGCCGTGCGCCTCGCCCAGACC   1380

Murine IBP      AGGCTGCTGGAGGAGGAGGAGAAGCTGAAGCAGCTGATGCATCTGAAGGAGGAGCAA     1440

Murine IBP      GAGCGCTACATCGAGCGAGCGCAGCAGGAGAAGCAGGAGCTCCAGCAGGAGATGGCGCTG   1500

Murine IBP      CAGAGCCGTTCCCTGCAGCACGCCCAGCAGCAGCTGGAGGAAGTGCGGCAGAACCGGCAG   1560

Murine IBP      AGGGCAGACGAGGACGTGGAGGCTGCCCAGAGGAAGTTGCGCCAGGCCAGCACCAACGTG   1620

Murine IBP      AAACACTGGAATGTCCAGATGAACAGGCTCATGCATCCGATCGAGCCAGGAGATAAGCGA   1680

Murine IBP      CCCACCACCAGCAGCTCCTTCACGGGCTTCCAGCCCCCTCCGCTTGCCCGCCGGGACTCC   1740

Murine IBP      TCTCTAAAGCGCCTGACCCGCTGGGGTTCCCAAGGTAACAGAACCCTCTCAGTCAATAGC   1800

Murine IBP      AGCGAACAGAAGTCCCTCAATGGTGGAGATGAGACTCCCATCCTAGCTTTGGCCTCTCAG   1860

Murine IBP      GAAGAAAAACTGGATCCAGCACCAGGGAATTAG                              1893
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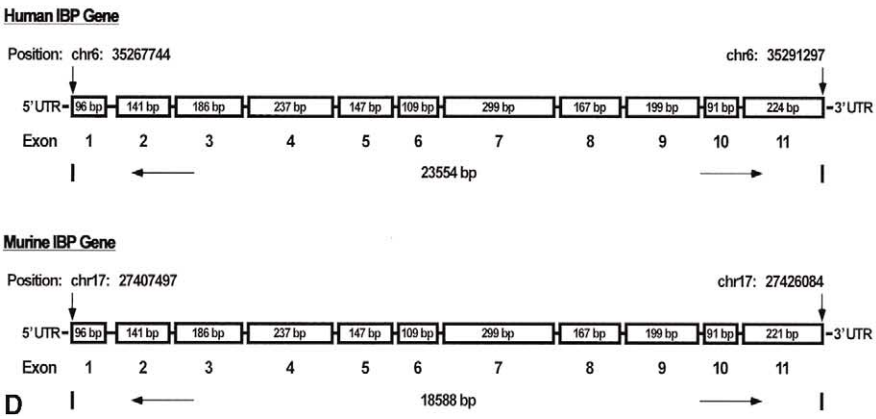
B



D

FIGURE 1 Continued from page 390.

FIGURE 1 Continued from pages 390 and 391.

vated mature B lymphocytes although low levels can also be detected in mast cells and fibroblasts [8, 9]. Consistent with its expression pattern, mice deficient in SWAP-70 primarily display alterations in antibody production and defects in mast cell development [10, 11]. Furthermore, consistent with its Rac-GEF activity, SWAP-70-deficient kidney fibroblasts exhibit impaired membrane ruffling upon growth factor stimulation [7]. The existence of additional members of this unique family of GEFs has been suggested by the fact that, during studies geared to identifying genes differentially expressed in the murine haematopoietic system, a cDNA fragment exhibiting homology to SWAP-70 cDNA was isolated [12]. This murine cDNA fragment was termed Def-6 (differentially expressed in FDCP-Mix), since its expression was shown to be downregulated in the course of the differentiation of a myeloid progenitor cell line (FDCP-Mix A4) toward either myeloid or erythroid lineages.

Here we report the molecular cloning of a human cDNA encoding a novel SWAP-70- homologous protein, which we have termed IBP. In this report, we also describe the expression pattern of human IBP. The 5′ portion of the human IBP cDNA displays a very high degree of sequence homology to murine Def-6. This finding thus suggests that IBP represents the human orthologue of Def-6. Consistent with this notion the gene encoding human IBP is located on chromosome 6p21.31 in a syntenic region to murine chromosome 17 where the murine Def-6 gene is located. The IBP protein exhibits a significant homology with SWAP-70 and, like SWAP-70, it contains a putative EF-hand motif at the N-terminus, and a central PH domain flanked at its C-terminus by a DH domain [13]. A systematic investigation of the tissue distribution of IBP reveals that IBP is expressed in both central and peripheral lymphoid tissues.

MATERIALS AND METHODS

Cell Cultures and Transfections

The Jurkat (human T-cell leukemia) cell line was obtained from American Type Culture Collection (ATCC, Manassas, VA, USA). The human T-cell line HUT78 was obtained from Dr. Seth Lederman, Columbia University (New York, NY). Ramos (obtained from Dr. Lederman), and JY (obtained from Dr. Riccardo Dalla-Favera, Columbia University) are human B-cell lines. All these cell lines were grown in Iscove's modified Dulbecco's medium supplemented with 10% fetal calf serum (Atlanta Biologicals, Inc, Norcross, GA, USA). WI-38 VA (a human embryonic lung fibroblast), WISH (a human epithelial-like amnion tissue derived cell line), FS2 (a human foreskin-derived fibroblast cell line), and

293T (a human embryonic kidney cell line) cells were a kind gift of Dr. Chris Schindler, Columbia University, and were grown in Dulbecco's Modified Eagle's Medium supplemented with 10% FCS.

For expression of recombinant proteins, 293T cells were transfected with expression plasmids by the calcium phosphate/DNA precipitation method. After 24-hour incubation, the transfected cells were harvested for cell extract preparation.

Yeast Two-Hybrid Interaction Assay

Yeast two-hybrid interaction screen was conducted according to previously described protocols [14]. Briefly, the "bait" plasmid (pAS2-1-IRF-4 200-360) consisted of a human IRF4 cDNA fragment encoding amino acids R200-T360. This fragment was subcloned into the pAS2-1 vector (Clontech Laboratories, Inc., Palo Alto, CA, USA) in-frame with the cDNA sequence encoding the DNA-binding domain of GAL4. The pAS2-1-IRF-4 (200–360) plasmid was cotransformed into the yeast reporter strain Y190 together with the "prey" plasmids, which consisted of a human lymph node cDNA library cloned into the pACT2 vector (Clontech). $His^+/Leu^+/Trp^+$ yeast clones were selected, and then screened for β-galactosidase expression. His^+/β-galactosidase$^+$ prey plasmids underwent secondary screens, including retransformation into the Y190 cells with an empty pAS2-1 vector or a pAS2-1-murine p53 plasmid (Clontech) to eliminate false-positives.

DNA and RNA

Full-length human IBP cDNA was obtained from a human lymph node cDNA library (Clontech) by polymerase chain reaction (PCR). The nucleotide sequence of the IBP cDNA was determined by DNA sequencing in an automated cycle sequencer (Perkin Elmer, Norwalk, CT, USA). The 5′ end of the cDNA was determined by 5′ rapid amplification of cDNA ends (5′-RACE) method using the SMART RACE cDNA amplification kit (Clontech). Total RNA was extracted by using the TRIZOL reagent (Invitrogen Life Technologies, Gronigen, The Netherlands). A murine IBP cDNA, containing the complete coding region, was cloned from total RNA derived from C57BL6/J mouse thymus by reverse transcription-PCR, and the nucleotide sequence of the cDNA was determined. The human immune system multiple tissue Northern (MTN) blot II and human multiple tissue expression (MTE) Array 2 blot were purchased from Clontech. The MTN blot was sequentially probed with a [^{32}P]-labeled human IBP cDNA fragment (~1.9-kb full-length coding sequence), and then with a glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA according to the manufacturer's instructions. The MTE blot was probed with either a radiolabeled human IBP cDNA fragment (a ~1.1-kb fragment from the 5′ region of the coding sequence), a human SWAP-70 cDNA, or a ubiquitin cDNA according to the manufacturer's instructions.

DNA Constructs

The IBP expression plasmids (pCEP4-HA-IBP and pIRES2-EGFP-HA-IBP) were constructed by cloning the entire coding region of the human IBP cDNA, fused in-frame with a sequence encoding a hemagglutinin (HA) epitope at its 5′-terminus, into the pCEP4 mammalian cell expression vector (Invitrogen) or pIRES2-EGFP bicistronic mammalian expression vector (Clontech), respectively. An IBP expression plasmid without any epitope tag (pIRES2-EGFP-IBP) was also generated by cloning the full-length coding sequence of the human IBP cDNA into the pIRES2-EGFP expression vector. For the glutathione S-transferase (GST) fusion protein, a GST-IBP (amino acids 410–631) expression plasmid was constructed by cloning the corresponding coding segment of the human IBP cDNA, in-frame, into the pGEX-KG *E. coli* expression vector (Pharmacia, Piscataway, NJ, USA). The in-frame junction in the GST-IBP (410–631) fusion construct was confirmed by DNA sequencing in an automated cycle sequencer (Perkin Elmer).

Protein Purification, Antibodies, Cell Extracts, and Protein Assays

GST fusion proteins were expressed in *E. coli* DH5α and affinity purified on glutathione (GSH)-agarose beads (Sigma Chemical Co., St. Louis, MO, USA), as described previously [15]. The polyclonal anti-IBP antibody was generated by immunizing rabbits with purified GST-IBP (amino acids 410–631) fusion protein (Covance, Inc., Princeton, NJ, USA). This GST fusion protein contains a portion of the human IBP protein, which is least homologous to SWAP-70 and thus avoids crossreactivity of the antibody with SWAP-70. The anti-IBP antibody was utilized at 1:1000 in Western blotting and at 1:200 in the immunofluorescence experiments. The β-actin antibody was purchased from Santa Cruz Biotechnology. Cell extracts were prepared as previously described [16]. The protein components were resolved by 7% SDS-PAGE. The gel was transferred to a nitrocellulose membrane, and then immunoblotted with either an anti-IBP antibody or a β-actin antibody. The bands were visualized by ECL (Amersham, Braunschewig, Germany).

For *in vitro* transcription and translation, the HA epitope-tagged full-length coding sequence of the human IBP cDNA was cloned into the pBluescript vector (Stratagene, La Jolla, CA, USA). The cDNA was transcribed and translated *in vitro* by using the TNT T7

RNA Polymerase Coupled Reticulocyte Lysate System (Promega, Eugene, OR, USA) and [^{35}S]methionine according to the procedures recommended by the manufacturer. The reaction products were resolved on a 7% SDS-polyacrylamide gel, and then blotted onto a nitrocellulose membrane. The proteins were detected by autoradiography as well as by immunoblotting with an anti-IBP antibody.

Immunofluorescence

Double immunofluorescence staining of tonsillar tissues was conducted as previously published [17].

RESULTS

Cloning and Sequence Analysis of Human IBP

The cDNA for human IBP was cloned while performing a yeast two-hybrid interaction analysis aimed at identifying potential partners of the lymphoid-restricted transcription factor IRF-4 [18–22]. For these studies, a human lymph node cDNA library was screened utilizing as a bait a domain of IRF-4 (amino acids 200–360) known to be involved in protein-protein interaction [23]. This analysis identified a novel ~1.4-kb cDNA fragment, which was termed IBP (IRF-4 binding protein). A ~2.3-kb full-length cDNA was subsequently isolated and found to contain a single open reading frame (ORF) of 1893 bp (Figure 1A). The IBP cDNA sequence contained two possible initiator ATG codons for translation at nucleotides 40 and 244 in this ORF. The sequence surrounding the first ATG codon closely conforms to the Kozak consensus sequence for translation initiation [24] and, therefore, this ATG was considered as the translation initiation codon. Conceptual translation of the entire predicted ORF of the IBP cDNA revealed that it can encode a protein of 631 amino acid residues with a predicted molecular mass of ~74 kDa. An additional cDNA was also isolated, which contains only the 3′ portion of the ~2.3-kb transcript. Experiments are now in progress to determine whether this cDNA represents an alternatively spliced isoform of IBP, which might encode a truncated form of the protein.

A search of the National Center for Biotechnology Information sequence database with the IBP cDNA revealed that the 5′ portion of the human IBP cDNA is highly homologous to Def-6, a murine cDNA fragment isolated during the search for murine genes differentially expressed in the hematopoietic system [12]. Utilizing a reverse transcription-PCR based approach we then isolated the cDNA for the murine homologue of IBP from murine thymus cDNAs (Figure 1B). A nucleotide sequence comparison of the entire coding regions of the human and murine IBP cDNAs revealed a very high degree of homology (overall 88%) (Figure 1C). A sequence alignment analysis also indicated that the 5′ portion of the murine IBP cDNA is identical to the Def-6 cDNA fragment except for two nucleotides at positions 136 and 169, which are absent in the Def-6 cDNA fragment (Figures 1B and 1C). The murine IBP/Def-6 gene furthermore maps to chromosome 17, in a region syntenic to human chromosome 6p21.31 where the human IBP/Def-6 gene is located (Fig. 1D) [25]. Taken all together these findings thus indicate that IBP represents the human orthologue of Def-6. Consistent with this notion, during the course of these studies a cDNA sequence corresponding to human Def-6 was deposited in GenBank (accession# NM_022047). This sequence is 100% identical to that of the human IBP cDNA except for one nucleotide at position 899 leading to a change in the codon 287 for Thr (T) to that for Asn (N) (Figure 1A). A search of the SNP databank indicates that this may represent a common polymorphism (NCBI SNP Cluster ID: rs2395617).

A comparison of the predicted amino acid sequences of the human and murine IBP proteins revealed that the two proteins are highly homologous (92% identity and 95% similarity over the entire sequence) (Figure 1E). To further determine the extent of evolutionary conservation of IBP we utilized the human IBP cDNA to perform a TBLASTN search of the GenBank database of expressed sequence tags (ESTs) for species other than human or mouse. This search identified a variety of ESTs with a high degree of sequence homology ($>$ 80% over $>$ 200 residues) to the human IBP cDNA in Chicken, Zebrafish, and Oryzias latipes. Interestingly, however, despite employing different search formats, no sequences homologous to the IBP cDNA were identified in *Saccharomyces cerevisiae, Drosophila melanogaster* or *Caenorhabditis elegans* suggesting that the expression of IBP may be confined to vertebrates.

IBP is Highly Homologous to SWAP70

A comparison of the deduced amino acid sequence of human IBP against multiple protein databases revealed that the IBP protein possesses a significant sequence homology to the recently described SWAP-70 molecule (overall 45% identity and 65% similarity) (Figure 2A) [8]. The two proteins also share a remarkably similar molecular structure, which includes an N-terminal EF-hand motif, a central PH domain and a C-terminal α-helical region (Figure 2B) [13]. Consistent with the presence of a PH domain, IBP can bind phosphoinositides and can be recruited to the plasma membrane (unpublished observations). Interestingly, IBP also contains a potential bipartite basic nuclear localization signal (amino acids K328-R340; KRREQREQRERRR) [26] suggesting that, like SWAP-70, this protein might also be able to translocate to the nucleus upon specific stim-

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Human IBP       M-ALRKELLKSIWYAFTALDVEKSGKVSKSQLKVLSHNLYTVLHIPHDPVALEEHFRDDD   59
Human SWAP70    MGSLKEELLKAIWHAFTALDQDHSGKVSKSQLKVLSHNLCTVLKVPHDPVALEEHFRDDD   60
                * :*::****:**:****** ::**************** ***::*************

Human IBP       DGPVSSQGYMPYLNKYILDKVEEGAFVKEHFDELCWTLTAKKNYRADSNGNSMLSNQDAF   119
Human SWAP70    EGPVSNQGYMPYLNRFILEKVQD-NFDKIEFNRMCWTLCVKKNLTKNP---LLITEEDAF   116
                :****.*********::**:**::  * * .*:.:**** .***    :.     :::::***

Human IBP       RLWCLFNFLSEDKYPLIMVPDEVEYLLKKVLSSMSLEVSLGELEELLAQEAQVAQTTGGL   179
Human SWAP70    KIWVIFNFLSEDKYPLIIVSEEIEYLLKKLTEAMGGGWQQ---EQFEHYKINFDDSKNGL   173
                ::* :************:*.:*:******: .:*.    .     *::    : :. :::..**

Human IBP       SVWQFLELFNSGRCLRGVGRDTLSMAIHEVYQELIQDVLKQGYLWKRGHLRRNWAERWFQ   239
Human SWAP70    SAWELIELIGNGQFSKGMDRQTVSMAINEVFNELILDVLKQGYMMKKGHRRKNWTERWFV   233
                *.*:::**:..*:  :*:.*:*:****:**::*** *******: *:** *:**:****

Human IBP       LQPSCLCYFGSEECKEKRGIIPLDAHCCVEVLPDRDGKRCMFCVKTATRTYEMSASDTRQ   299
Human SWAP70    LKPNIISYYVSEDLKDKKGDILLDENCCVESLPDKDGKKCLFLVKCFDKTFEISASDKKK   293
                *:*. :.*: **: *:*:* * ** :**** ***:***:*:* **   :*:*:****.::

Human IBP       RQEWTAAIQMAIRLQAEGKTSLHKDLKQKRREQREQRERRRAAKEEELLRLQQLQEEKER   359
Human SWAP70    KQEWIQAIHSTIHLLKLGSPPPHKEARQRRKELRK---KQLAEQEELERQMKELQAANES   350
                :***  **: :*:*   *... **: :*:*:* *:    :: * :**   ::::**  :*

Human IBP       KLQELELLQEAQRQAERLLQEEEERRRSQHRELQQALEGQLREAEQARASMQAEMELKEE   419
Human SWAP70    KQQELEAVRKKLEEAASRAAEEEKKRLQTQVELQARFSTELEREKLIRQQMEEQVAQKSS   410
                * **** :::  .:*     ***::* . : ***  :. :*.. :  * .*: ::  *..

Human IBP       EAARQRQRIKELEEMQQRLQEALQLEVKARRDEESVRIAQTRLLEEEEEKLKQLMQLKEE   479
Human SWAP70    ELEQYLQRVRELEDMYLKLQEALEDERQARQDEETVRKLQARLLEEESSKRAELEKWHLE   470
                *  :  **::***:*  :*****: * :**:***:**  *:******..*  :* : : *

Human IBP       QERYIERAQQEKEELQQEMAQQSRSLQQAQQQLEEVRQNRQRADEDVEAAQRKLRQASTN   539
Human SWAP70    QQQAIQTTEAEKQELENQRVLKEQALQEAMEQLEQLELERKQALEQYEEVKKKLEMATNK   530
                *:: *: :: **:**::: . :.::**:* :***::. :*::* *: * .::**. *:.:

Human IBP       VKHWN---VQMNRLMHPIEPGDKRPVTSSSFSGFQPPLLAHRDSSLKRLTRWGSQGNRTP   596
Human SWAP70    TKSWK DKVAHHEGLIRLIEPGSKNPHLITNWG----PAAFTEAELEEREKNWKEKKTTE-   585
                .* *:   .: : *:: ****.*.*   :.:.   *    . .  :* ..* .: .

Human IBP       SPNSNEQQKSLNGGDEAPAPASTPQEDKLDPAPEN                           631
Human SWAP70    ----------------------------------
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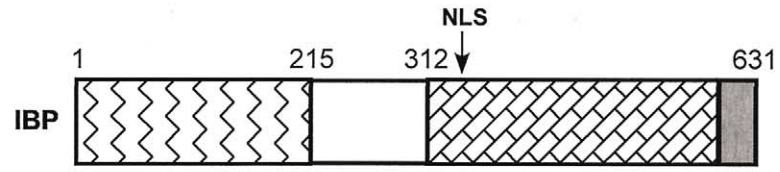
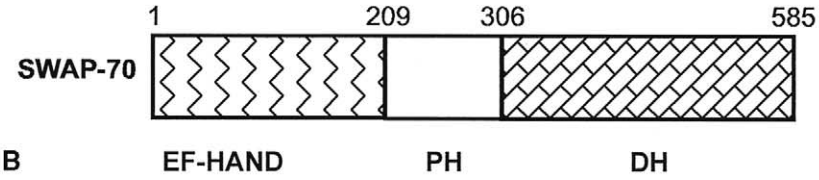
A



%Identity:	55%	56%	34%
%Similarity:	74%	76%	56%



B EF-HAND PH DH

FIGURE 2 Homology of IRF-4 binding protein (IBP) with SWAP-70. (A) Alignment of the human IBP and human SWAP-70 amino acid sequences. Amino acid numbers are shown on the right. Identical amino acids are marked by asterisks. Dots indicate similar residues; and bars indicate gaps introduced for optimal alignment. Comparison was performed utilizing the CLUSTALW program for multiple sequence alignment. (B) Schematic of domain homologies between human IBP and SWAP-70 proteins. The numbers of the amino acids are indicated on the top. The percent of identity and percent of similarity for each domain is indicated. The location of a putative nuclear localization signal (NLS) in IBP is indicated.

ulatory conditions [13]. As described for SWAP-70, the C-terminal portion of IBP contains GEF activity for Rho GTPases (unpublished observations) and displays limited sequence homology to the Dbl-homology (DH) domain of Vav1, Sos1, and Tiam1. Despite exhibiting an overall striking homology with SWAP-70, at its extreme C-terminus IBP contains an additional stretch of ~40 amino acids, which is not found in SWAP-70.

To confirm that the IBP cDNA encodes the predicted

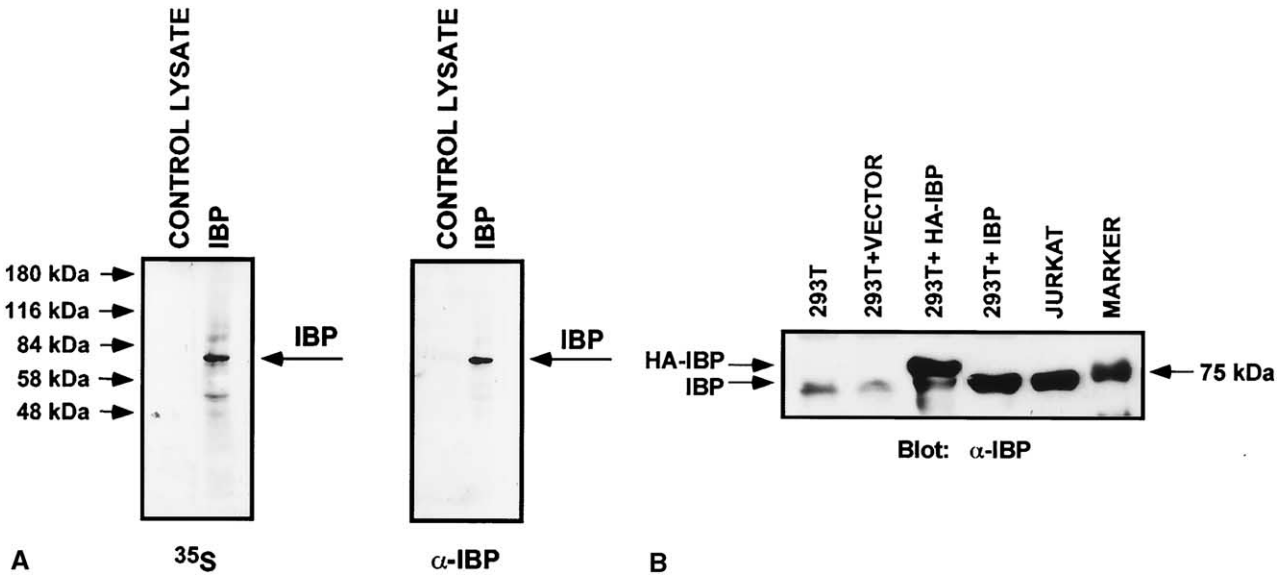


FIGURE 3 Human IRF-4 binding protein (IBP) cDNA encodes a ≈75-kDa protein. (**A**) SDS-PAGE/Western analysis of *in vitro* translated [^{35}S]methionine-labeled recombinant IBP protein. The HA epitope-tagged full-length coding sequence of the human IBP cDNA was transcribed and then translated *in vitro* with reticulocyte lysate in the presence of [^{35}S]methionine. The reaction products were resolved by 7% SDS-PAGE, and then blotted onto a nitrocellulose membrane. The proteins were visualized first by autoradiography (left panel). The blot was subsequently probed with an antiserum raised against human IBP (right panel). The relative mobilities of molecular size markers are shown in kilodaltons on the left. (**B**) Western blot analysis of recombinant human IBP protein expressed in 293T cells. 293T cells were transiently transfected with an empty expression vector (Vector), or with an expression plasmid encoding either an HA epitope-tagged form of full-length IBP (HA-IBP), or an untagged form of full-length IBP (IBP). Whole cell lysates were prepared, electrophoresed on a 7% SDS-polyacrylamide gel, and then analyzed by Western blotting using an anti-IBP antiserum generated against the C-terminal portion of IBP (anti-IBP antibody). Whole cell lysate from Jurkat T cells was also simultaneously analyzed for the identification of endogenous IBP protein. The position of a 75-kDa protein size marker (MARKER) is indicated on the right.

protein, we then proceeded to perform *in vitro* transcription and translation (IVT) analysis of the entire coding region of the human IBP cDNA fused to an HA epitope sequence. The product generated by the IVT of the HA-IBP cDNA had a mobility of ~76 kDa on SDS-PAGE (Figure 3A; left panel), which is in good agreement with the predicted molecular mass (~74 kDa) of the deduced human IBP polypeptide and is, therefore, consistent with the cDNA sequence. Furthermore, the *in vitro* translated product of the IBP cDNA was recognized by an antiserum generated against the IBP protein (Figure 3A; right panel). To further confirm that the human IBP cDNA encodes the expected protein, we subcloned the entire coding region of the IBP cDNA into a mammalian cell expression vector, and transfected it into 293T cells. Whole cell lysates were obtained, the protein components were separated by SDS-PAGE and Western blotting was subsequently performed utilizing the anti-IBP antiserum. As depicted in Figure 3B, this antiserum recognized a single protein band of ~74-76 kDa in the lysates from cells transfected with the cDNAs encoding either an untagged form of IBP or an HA epitope-tagged form of IBP, respectively, but not in the lysates from cells transfected with an empty vector. Furthermore, the mobility of the recombinant untagged human IBP protein produced in 293T cells was similar to that of the endogenous protein recognized by the anti- IBP antibody in the cell lysate from Jurkat, a human leukemic T cell line (Figure 3B). Taken all together these results thus clearly indicate that the deduced ORF of the human IBP cDNA is the correct coding frame, which actually encodes the predicted protein.

Expression Analysis of IBP

To gain insights into the tissue distribution of IBP, we first carried out Northern blot analysis of mRNAs from various human lymphoid tissues (human immune system multiple tissue Northern blot II from Clontech) by utilizing a [^{32}P]-labeled human IBP cDNA fragment (~ 1.9-kb full-length coding sequence) as a probe (Figure 4A). These studies revealed a single ~2.4-kb transcript that was abundantly expressed in all lymphoid tissues except for fetal liver. The generation of an antiserum specific for IBP (Figure 3B) furthermore allowed

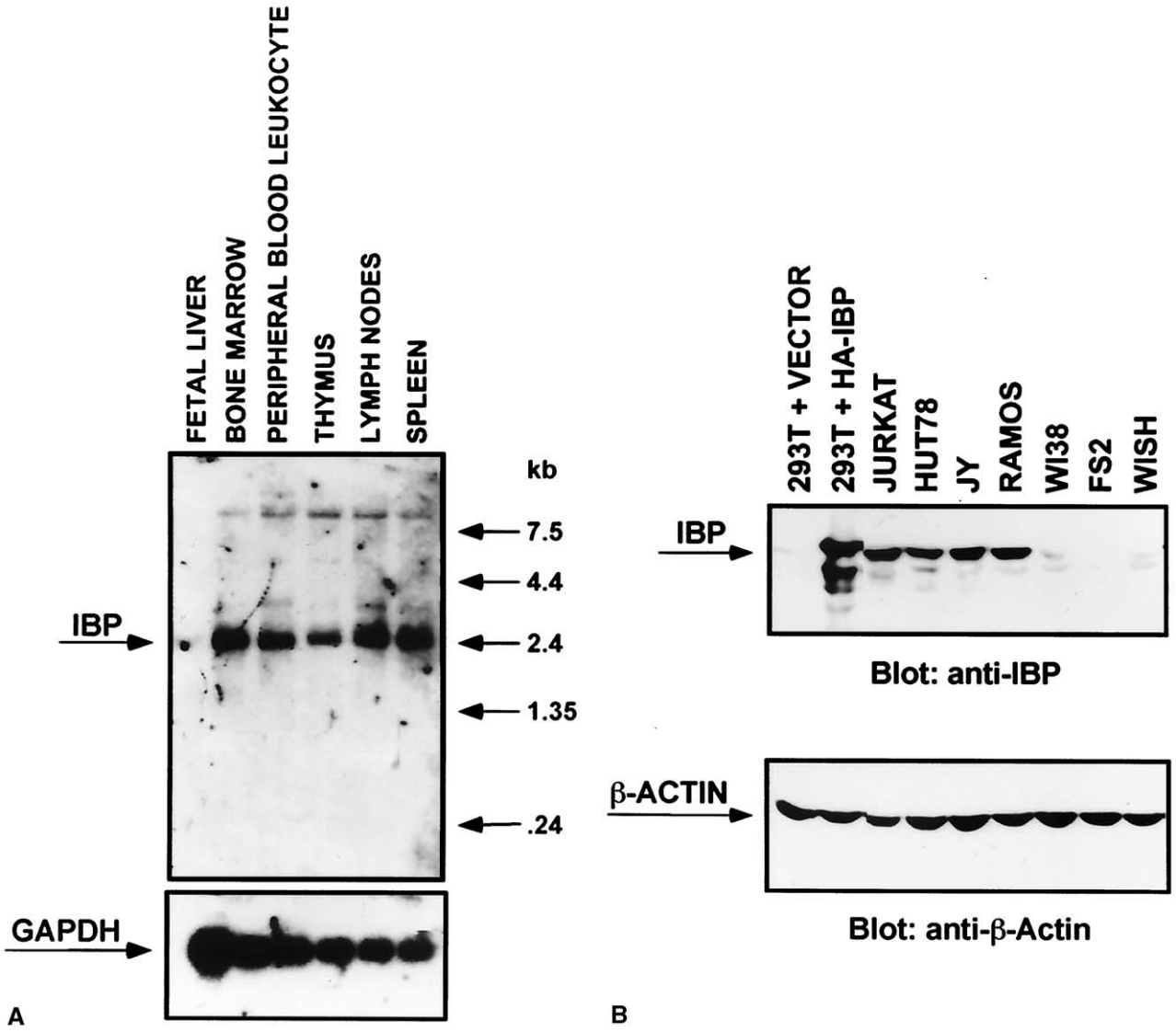


FIGURE 4 Expression pattern of IRF-4 binding protein (IBP) in human lymphoid tissues and human tumor cell lines. (**A**) Northern blot analysis of IBP mRNA distribution in distinct human lymphoid tissues. A human multiple tissue Northern blot (Clontech) containing poly(A)$^+$ RNAs from the indicated lymphoid organs was probed with a [^{32}P]-labeled human IBP cDNA fragment (~ 1.9-kb full-length coding sequence; upper panel). The blot was subsequently stripped and rehybridized with a [^{32}P]-labeled GAPDH cDNA probe (lower panel). The positions of RNA size markers are shown in kilobases on the right. (**B**) Western blot analysis of the endogenous IBP protein distribution in different human tumor cell lines. Whole cell lysates from cell lines of T-cell origin (Jurkat, HUT78), B-cell origin (JY, Ramos), fibroblast origin (WI38, FS2), or derived from human epithelial-like amnion (WISH) were resolved by 7% SDS-PAGE, and then analyzed by Western blotting using an antiserum specific for IBP (anti-IBP antibody; upper panel). Whole cell lysates from 293T cells transfected either with an empty expression vector (Vector), or with an HA epitope-tagged full-length human IBP expression plasmid (HA-IBP) were included as controls. The blot was later stripped and reprobed with an antibody against β-actin to ensure for equal loading (lower panel).

us to start investigating the expression pattern of the endogenous IBP protein. Western blot analysis of the total cell lysates obtained from a panel of human cell lines demonstrated that the IBP protein is expressed in cells of both T (Jurkat and HUT78) as well as B (Ramos and JY) cell origin (Figure 4B). IBP expression was instead undetectable in the lysates from cell lines derived from fibroblasts (WI38, FS2) or amnion cells (WISH).

To determine whether the expression of IBP was solely confined to the immune system, we then probed a membrane containing poly(A)$^+$ RNA samples from 72 distinct human lymphoid and non-lymphoid tissues (hu-

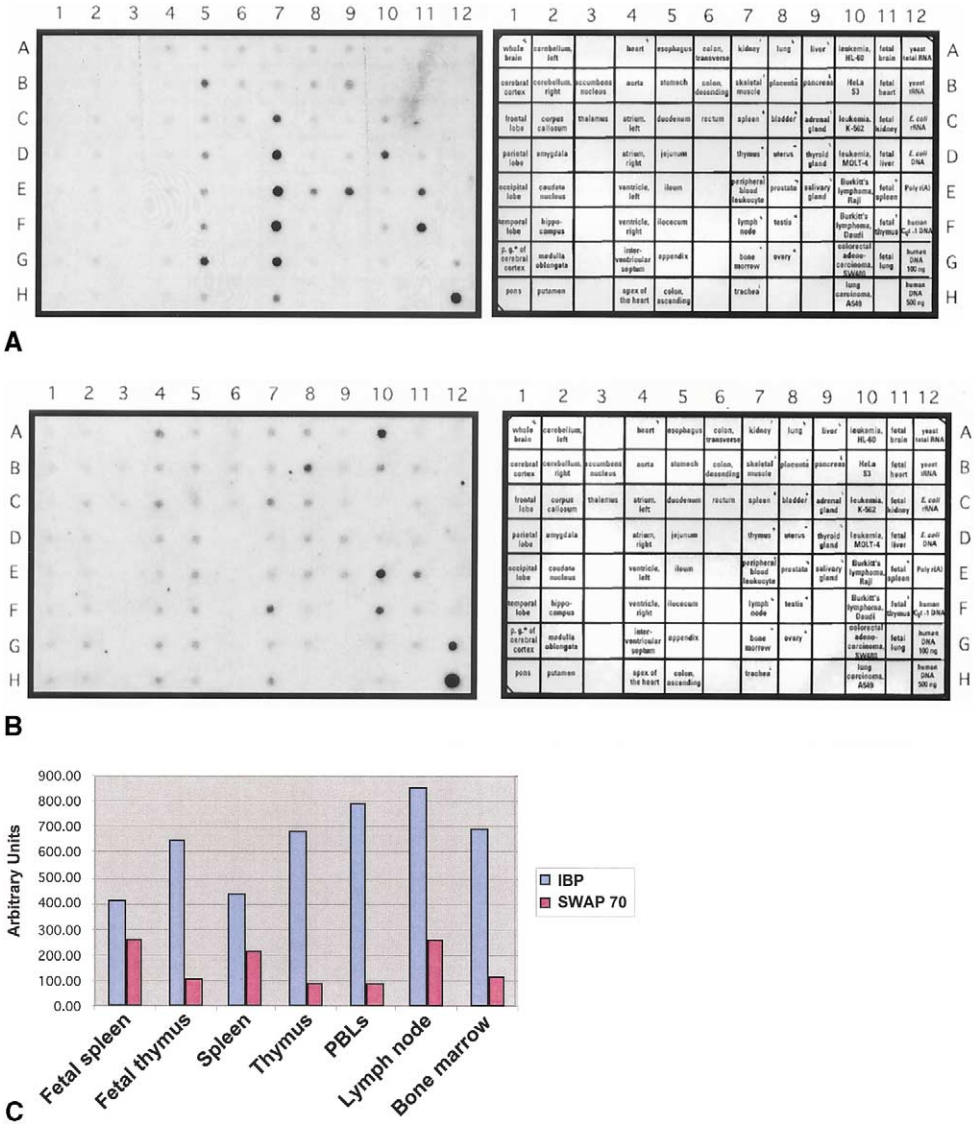


FIGURE 5 Expression pattern of IRF-4 binding protein (IBP) and SWAP-70 mRNA in human tissues. (**A**) Expression pattern of IBP in 72 distinct human tissues was investigated by probing a human multiple tissue expression (mRNA) array blot (Clontech) with a [^{32}P]-labeled human IBP cDNA fragment (a ~1.1-kb fragment from the 5′ region of the coding sequence). (**B**) The array blot was stripped and expression of SWAP-70 in the same tissues was examined by rehybridizing the blot with a [^{32}P]-labeled human SWAP-70 cDNA probe (~1755-bp full-length coding sequence). (**C**) Each probing of the array blot with either the IBP cDNA or the SWAP-70 cDNA was subjected to phosphoimaging quantification analysis, and the relative levels of both IBP transcripts and SWAP-70 transcripts in various lymphoid organs were then compared. The levels of each transcript are illustrated by arbitrary units obtained from the analysis.

man Multiple Tissue Expression Array 2 blot from Clontech) with a [^{32}P]-labeled human IBP cDNA fragment (a ~1.1-kb fragment from the 5′ region of the coding sequence) (Figure 5A). In addition to the tissues where the IBP mRNA had been observed by Northern analysis, these experiments revealed that the IBP transcripts could also be detected, albeit at lower levels, in nonlymphoid organs including the salivary gland, the prostate, and the digestive system. Only very low or no IBP expression could be detected in fetal non-lymphoid tissues, the CNS, or the cardiovascular system. This membrane was subsequently stripped and rehybridized with a [^{32}P]-labeled human SWAP-70 cDNA probe to compare the tissue distribution of these two family members (Figure 5B). Interestingly, this experiment revealed that the expression of IBP in the immune system is broader and more abundant than that of SWAP-70 (Figure 5C). Furthermore, consistent with previous reports [9], SWAP-70 transcripts were virtually undetectable in the thymus or the Molt-4 T cell line suggesting that the T cell compartment predominantly expresses only IBP.

To further investigate the distribution pattern of the IBP protein in normal lymphoid tissues, the polyclonal anti-IBP antibody was employed in immunofluorescence studies of human tonsillar tissue. As illustrated in Figure 6A, most T cells in the interfollicular area as well as within the germinal center stained very strongly for IBP. Interestingly, costaining of the tissue with the anti-IBP antibody as well as an antibody against Pax5 (a B-cell marker) [27] (Figure 6B) revealed that B cells in the corona display moderate IBP expression. Notably, however, no IBP expression was detected in germinal center B cells, which have previously been shown to strongly stain for SWAP-70 [9]. Costaining of these tonsillar

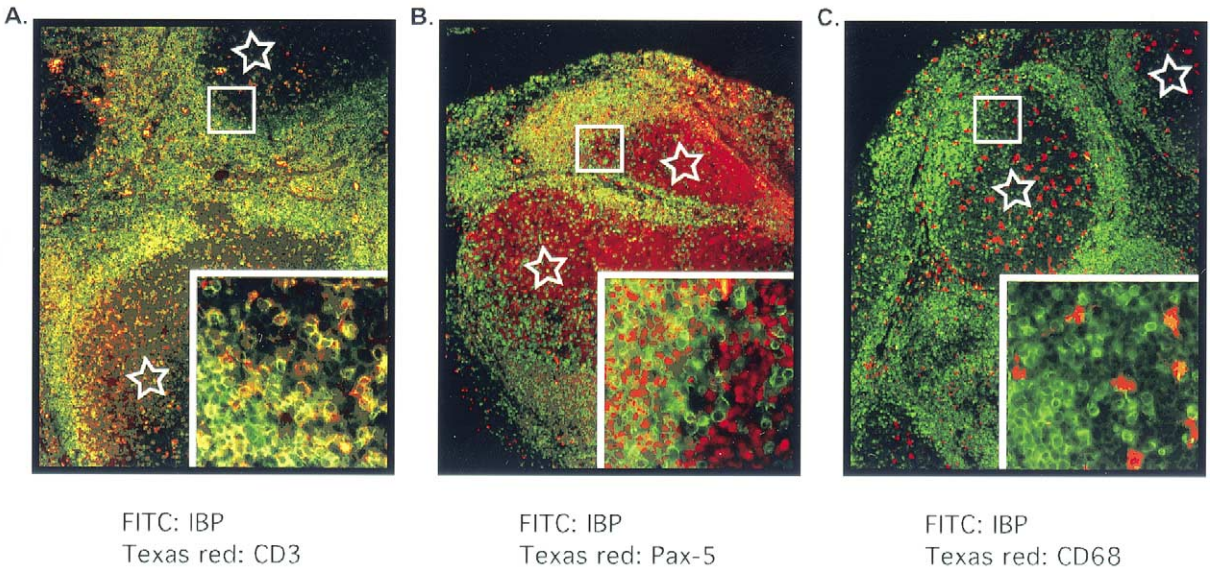


FIGURE 6 Distribution pattern of IRF-4 binding protein (IBP) in human tonsil. (**A**) Costaining of IBP with anti-CD3. Paraffin-embedded tissue sections were stained with a rabbit polyclonal anti-IBP antibody followed by a FITC-coupled anti-rabbit Ig and Texas Red-coupled anti-CD3; magnification 10×. Stars indicate germinal centers. A higher magnification (40×) of the area contained in the white square is displayed in the right lower corner insert. (**B**) Costaining of IBP with Pax-5. Paraffin-embedded tissue sections were stained with a rabbit polyclonal anti-IBP antibody followed by a FITC-coupled anti-rabbit Ig and Texas Red-coupled anti-Pax-5; magnification 10×. Stars indicate germinal centers. A higher magnification (40×) of the area contained in the white square is displayed in the right lower corner insert. (**C**) Costaining of IBP with anti-CD68. Paraffin-embedded tissue sections were stained with a rabbit polyclonal anti-IBP antibody followed by a FITC-coupled anti-rabbit Ig and Texas Red-coupled anti-CD68; magnification 10×. Stars indicate germinal centers. A higher magnification (40×) of the area contained in the white square is displayed in the right lower corner insert.

sections with IBP and CD68 (a macrophage marker) [28] antibodies revealed no staining for IBP in these macrophages (Figure 6C). These studies thus confirmed that IBP is strongly expressed in T lymphocytes and suggested that its expression in B cells may be confined to specific stages of B-cell differentiation.

DISCUSSION

During a search for proteins interacting with the lymphoid-restricted transcription factor IRF-4, we isolated a human cDNA encoding a novel protein, which we have named IBP (IRF-4 binding protein). A homology search analysis revealed that the 5′ portion of the human IBP cDNA was highly homologous to the Def-6 cDNA fragment that Hotfilder *et al.* [12] had isolated during a search for genes which are downregulated as a murine progenitor cell line (FDCP-Mix A4) differentiates into either myeloid or erythroid lineages. Isolation of the complete coding sequence of the murine counterpart of IBP cDNA confirmed that the 5′ portion of the murine IBP cDNA is identical to the murine Def-6 cDNA fragment. These results thus indicate that IBP is the human orthologue of Def-6. Consistent with this notion, the gene for the human IBP maps to chromosome 6p21.31 in a region syntenic to murine chromosome 17 where the Def-6 gene is located. The high degree of homology between the human and murine IBP cDNA sequences suggests that IBP is highly conserved in evolution. Indeed ESTs sharing $> 80\%$ similarity with the human IBP cDNA are present in multiple species, including Oryzias latipes and Xenopus laevis. Interestingly, however, no sequence homologous to IBP could be identified in *Drosophila melanogaster,* or *Caenorhabditis elegans* suggesting that IBP may be unique to vertebrates.

The deduced human IBP polypeptide consists of 631 amino acids and has a predicted molecular mass of ~74 kDa. Indeed *in vitro* transcription/translation analysis of the human IBP cDNA as well as Western blot analysis of both the expression plasmid-produced recombinant protein and the native proteins with anti-IBP antibodies clearly demonstrated the predicted IBP protein with an estimated molecular size of ~74 to 75 kDa. The IBP protein displays a significant similarity, in both primary sequence and overall molecular structure, to SWAP-70, which was recently described as a novel type of Rac-GEF [7]. Although the amino-terminal portions of SWAP-70 and IBP are highly homologous and are predicted to

contain an EF-hand, it is unclear at present whether this motif can impart to this region the capability to bind calcium. Both SWAP-70 and IBP proteins also contain a central PH domain and a long α-helical region at the carboxyl-terminus, which was found to contain the GEF activity. The unusual location of the DH module at the C-terminus, rather than the N-terminus, of the PH domain in both SWAP-70 and IBP molecules, coupled with their low-degree of homology with other DH domain-containing proteins support the notion that these two proteins may represent a distinctive class of GEFs. Interestingly, a search of the human genome did not reveal any additional sequences sharing extensive homology to SWAP-70 and/or IBP suggesting that SWAP-70 and IBP may be the only two members of this unique family of Rho-GEFs.

Although SWAP-70 has been revealed to contain the GEF activity, additional studies have suggested that its function may not be restricted to the activation of Rho GTPases. Indeed SWAP-70 was originally cloned as a component of a multi-protein complex believed to be involved in immunoglobulin class-switch recombination in B cells [8]. SWAP-70 was shown to contain three nuclear localization signals and to translocate to the nucleus upon B cell activation [13]. Interestingly, Vav1, another GEF for Rho GTPases known to play a critical role in the immune system, has recently been reported to translocate to the nucleus upon stimulation and to participate in the transcriptional regulation of genes by complexing with NFAT and NF-κB transcription factors [29]. Since IBP, like SWAP-70 and Vav1, contains a potential nuclear localization signal, it is likely that IBP can also translocate to the nucleus upon appropriate stimulatory conditions. It is intriguing to speculate that in the nucleus IBP can modulate gene expression by interacting with the lymphoid-restricted transcription factor IRF-4. Consistent with the yeast two-hybrid screen, preliminary results have indeed confirmed that IBP and IRF-4 can physically interact (S. Gupta, unpublished observations), and studies are now in progress to determine whether IBP can functionally modulate IRF-4 activity.

The studies investigating the expression pattern of IBP revealed that IBP is highly expressed in the immune system. Remarkably, IBP expression in lymphoid tissues is broader than that of SWAP-70 (Figure 5C). Consistent with previous reports [9], the T-cell compartment appears to preferentially express IBP and not SWAP-70. Furthermore, our immunohistochemical studies indicate that IBP is largely absent in germinal center B cells, which have previously been shown to strongly express SWAP-70. These results thus suggest that B cells at distinct stages of differentiation may exclusively express one or the other member of this unique family of GEFs.

ACKNOWLEDGMENTS

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REFERENCES

1. Symons M, Settleman J: Rho family GTPases: more than simple switches. Trends Cell Biol 10:415, 2000.
2. Ridley AJ: Rho family proteins: coordinating cell responses. Trends Cell Biol 11:471, 2001.
3. Takai Y, Sasaki T, Matozaki T: Small GTP-binding proteins. Physiol Rev 81:153, 2001.
4. Zheng Y: Dbl family guanine nucleotide exchange factors. Trends Biochem Sci 26:724, 2001.
5. Hoffman GR, Cerione RA: Signaling to the Rho GTPases: networking with the DH domain. FEBS 513:85, 2002.
6. Schmidt A, Hall A: Guanine nucleotide exchange factors for Rho GTPases: turning on the switch. Genes Dev 16:1587, 2002.
7. Shinohara M, Terada Y, Iwamatsu A, Shihora A, Mochizuki N, Higuchi M, Gotoh Y, Ihara S, Nagata S, Itoh H, Fukui Y, Jessberger R: SWAP-70 is a guanine-nucleotide-exchange factor that mediates signalling of membrane ruffling. Nature 416:759, 2002.
8. Borggrefe T, Wabl M, Akhmedov AT, Jessberger R: A B-cell-specific DNA recombination complex. J Biol Chem 273:17025, 1998.
9. Borggrefe T, Masat L, Wabl M, Riwar B, Cattoretti G, Jessberger R: Cellular, intracellular, and developmental expression patterns of murine SWAP-70. Eur J Immunol 29:1812, 1999.
10. Borggrefe T, Keshavarzi S, Gross B, Wabl M, Jessberger R: Impaired IgE response in SWAP-70-deficient mice. Eur J Immunol 31:2467, 2001.
11. Gross B, Borggrefe T, Wabl M, Sivalenka RR, Bennett M, Rossi AB, Jessberger R: SWAP-70-deficient mast cells are impaired in development and IgE-mediated degranulation. Eur J Immunol 32:1121, 2002.
12. Hotfilder M, Baxendale S, Cross MA, Sablitzky F: Def-2, -3, -6, -8, novel mouse genes differentially expressed in the hematopoietic system. Br J Haematol 106:335, 1999.
13. Masat L, Caldwell J, Armstrong R, Khoshnevisan H, Jessberger R, Herndier B, Wabl M, Ferrick D: Association of SWAP-70 with the B cell antigen receptor complex. Proc Natl Acad Sci USA 97:2180, 2000.
14. Collum RG, Brutsaert S, Lee G, Schindler C: A Stat3-interacting protein (StIP1) regulates cytokine signal transduction. Proc Natl Acad Sci USA 97:10120, 2000.
15. Frangioni JV, Neel BG: Solubilization and purification of enzymatically active glutathione S-tranferase (pGEX) fusion proteins. Analyt Biochem 210:179, 1993.

16. Gupta S, Xia D, Jiang M, Lee S, Pernis A: Signaling pathways mediated by the TNF- and cytokine-receptor families target a common cis-element of the IRF-1 promoter. J Immunol 161:5997, 1998.
17. Cattoretti G, Schiro R, Orazi A, Soligo D, Colombo MP: The bone marrow stroma in humans: anti nerve growth factor receptor antibodies selectively stain reticular cells in vivo and in vitro. Blood 81:1726, 1993.
18. Eisenbeis C, Singh H, Storb U: Pip, a novel IRF family member, is a lymphoid-specific, PU.1-dependent transcriptional activator. Genes Develop 9:1377, 1995.
19. Yamagata T, Nishida J, Tanaka T, Sakai R, Mitani K, Taniguchi T, Yazaki Y, Hirai H: A novel interferon regulatory factor family transcription factor, ICSAT/Pip/LSIRF, that negatively regulates the activity of interferon-regulated genes. Mol Cell Biol 16:1283, 1996.
20. Grossman A, Mittrucker H, Nicholl J, Suzuki A, Chung S, Antonio L, Suggs S, Sutherland G, Siderovski D, Mak T: Cloning of human lymphocyte-specific interferon regulatory factor (hLSIRF/hIRF4) and mapping of the gene to 6p23–25. Genomics 37:229, 1996.
21. Matsuyama T, Grossman A, Mittrucker H, Siderovski D, Kiefer F, Kawakami T, Richardson C, Taniguchi T, Yoshinaga S, Mak T: Molecular cloning of LSIRF, a lymphoid-specific member of the ineterferon regulatory factor family that binds the interferon-stimulated response element (ISRE). Nucleic Acids Res 23:2127, 1995.
22. Iida S, Rao P, Butler M, Corradini P, Boccadoro M, Klein B, Chaganti R, Dalla-Favera R: Deregulation of MUM1/IRF4 by chromosomal translocation in multiple myeloma. Nature Genet 17:226, 1997.
23. Ortiz MA, Light J, Maki RA, Assa-Munt N: Mutation analysis of the Pip interaction domain reveals critical residues for protein-protein interactions. Proc Natl Acad Sci USA 96:2740, 1999.
24. Kozak M: An analysis of 5′-noncoding sequences from 699 vertebrate messenger RNAs. Nucleic Acids Res 15: 8125, 1987.
25. Tripodis N, Palmer S, Phillips S, Milne S, Beck S, Ragoussis J: Construction of a high-resolution 2.5-Mb transcript map of the human 6p21.2–6p21.3 region immediately centromeric of the major histocompatibility complex. Genome Res 10:454, 2000.
26. Christophe D, Christophe-Hobertus C, Pichon B: Nuclear targeting of proteins: how many different signals? Cell Signal 12:337, 2000.
27. Nutt SL, Eberhard D, Horcher M, Rolink AG, Busslinger M: Pax5 determines the identity of B cells from the beginning to the end of B-lymphopoiesis. Int Rev Immunol 20:65, 2001.
28. Greaves DR, Gordon S: Macrophage-specific gene expression: current paradigms and future challenges. Int J Hematol 76:6, 2002.
29. Houlard M, Arudchandran R, Reggnier-Ricard F, Germani A, Gisselbrecht S, Blank U, Rivera J, Varine-Blank N: Vav1 is a component of transcriptionally active complexes. J Exp Med 195:1115, 2002.