

Cloning, Mapping, and Characterization of the Human Sorbin and SH3 Domain Containing 1 (SORBS1) Gene: A Protein Associated with c-Abl during Insulin Signaling in the Hepatoma Cell Line Hep3B

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SH3P12/CAP/ponsin, a gene product with a sorbin homology domain and three consecutive SH3 domains in the carboxy-terminus, has been isolated from murine adipocytes and identified as an important adaptor during insulin signaling. Here we describe the cloning, mapping, and expression of the human homologue, termed SORBS1 (sorbin and SH3 domain containing 1). Multiple transcripts of this gene with different mRNA isoforms were observed among different tissues. Here we report 13 alternatively spliced exons, which were ascertained from the full-length cDNA cloned in adipose, liver, and skeletal muscle tissues. Among the major isoforms, the shortest, 2223-bp, open reading frame (ORF) encodes a protein with a predicted molecular weight of 81.5 kDa, while the longest, 3879-bp, ORF encodes a protein of about 142.2 kDa. This gene was mapped to human chromosome 10q23.3–q24.1, which is a candidate region for insulin resistance found in Pima Indians. In human hepatoma Hep3B cells, SORBS1 was partly dissociated from the insulin receptor complex and bound to c-Abl protein upon insulin stimulation. This interaction with c-Abl was through the third SH3 domain and a possible conformational change of SORBS1 induced by insulin. Our data suggest that c-Abl oncoprotein via SORBS1 might play a role in the insulin signaling pathway.

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INTRODUCTION

SH3P12 belongs to a growing family of proteins containing a sorbin homology domain and three SH3 do-

main in the C-terminal region. This protein was first cloned by screening a mouse cDNA expression library with a SH3 binding ligand (Sparks *et al.*, 1996) and subsequently isolated by a yeast two-hybrid system using c-Cbl as bait and a blot overlay method with the labeled 1-afadin (Ribon *et al.*, 1998c; Mandai *et al.*, 1999). SH3P12 was therefore denoted as CAP, a c-Cbl-associated protein, or ponsin because of its interaction with both 1-afadin and vinculin in a competitive binding manner (Kurakin *et al.*, 1998; Ribon *et al.*, 1998c; Mandai *et al.*, 1999). Moreover, SH3P12/CAP has been found to interact with insulin receptor, Sos, flotillin, and focal adhesion kinase (Ribon *et al.*, 1998a,c; Baumann *et al.*, 2000), indicating that SH3P12/CAP might be involved in the signaling pathways to the reorganization of cytoskeleton and insulin-stimulated glucose uptake processes. Interestingly, some insulin-sensitizing thiazolidinediones, which are specific ligand and activator for peroxisome proliferator-activated receptor γ , can increase the expression of SH3P12/CAP in adipose tissues (Ribon *et al.*, 1998a).

Many SH3 domain-containing proteins such as c-Crk, Nck, Abi-1, and Arg/Abl binding protein 2A (ArgBP2A) can interact with Abl and Arg via the binding of the PXXP motif and SH3 domain (Ren *et al.*, 1994; Dai and Pendergast 1995; Shi *et al.*, 1995; Wang *et al.*, 1997; Smith *et al.*, 1999). ArgBP2A proteins are expressed ubiquitously in human tissues and are extremely abundant in the heart. Interestingly, ArgBP2A and SH3P12/CAP share structural homology and each protein contains a sorbin homology domain and three adjacent SH3 domains in the C-terminal region (Wang *et al.*, 1997). c-Abl, a member of the Abelson nonreceptor protein tyrosine kinase family (Kruh *et al.*, 1990; Wang, 1993), contains three conserved PXXP motifs that can serve as binding sites for SH3 domains. c-Abl play multiple roles in cellular physiology such as in cell cycle, transcription, DNA

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damage response, apoptosis, and neuronal development (Mattioni *et al.*, 1995; Birchenall-Roberts *et al.*, 1997; Koleske *et al.*, 1998; Agami *et al.*, 1999). The function of c-Abl may involve neuron development, actin cytoskeleton structure regulation, and cell-cell signaling interactions (Lewis *et al.*, 1996; Koleske *et al.*, 1998). Whether SH3P12/CAP interacts with c-Abl oncoprotein remains to be studied.

By using mRNA differential display analysis, we had also independently found that SH3P12/CAP was up regulated during adipogenesis and its expression was further enhanced by treatment with BRL49653 in the well-differentiated 3T3-L1 adipocytes (Lin *et al.*, 1999). Based on these observations, it is therefore important to elucidate the gene structure and function of the human homologue, now termed SORBS1 (sorbin and SH3 domain containing 1) for better understanding of the mechanism of insulin signaling and pathogenesis of diabetes and obesity. With great advances in positional cloning for human diseases, it is also important to map the SORBS1 gene to its chromosomal location for advancing genetic information that might facilitate positional candidate gene studies for insulin resistance and associated disorders.

MATERIALS AND METHODS

EST database enquiry and primer design. By searching the human EST database (dbEST, NCBI) using the mouse SH3P12/CAP (U58883) cDNA sequence, four human EST sequences were found, i.e., AI452468, AA873174, U70668, and AA296029 (GenBank Accession Nos.). Ten oligonucleotide primers were synthesized for cloning of the human SORBS1 cDNA, i.e., h1f (5'-GCATG AGTTC TGAAT GTGAT GGTG-3'), m345f (5'-ATGAG TCTG AATGT GATG-3'), m1139f (5'-CTCAC TTTT GAGCG AGGCA CAG-3'), h4f (5'-GAGAT GAGAC CTGCC AGAG C-3'), h3r (5'-TTTGG CTCTG GCAGG TCTCA TCTC-3'), m2400r (5'-TCTTA TAGAT ATAAA GG-3'), h294f (5'-AACCT TCCTT CTGAC AAGTC CC-3'), Ef (5'-AGGAC AATCC TTACA CTCCC ACC-3'), h1011r (5'-TCTTT TCGTT GGTC AAGC GAAG-3'), and h2055r (5'-TCTTA TAGAT ACAA GTTT TACAT AGT-3'), as schematically shown in the Fig. 1.

Cloning and expression of SORBS1 cDNA fragments by RT-PCR. Total cellular RNA was purified from human adipose, liver, and skeletal muscle tissues using the phenol-guanidium thiocyanate extraction according to the manufacturer's protocol (TRIzol; BRL, Gaithersburg, MD). For synthesis of the first-strand cDNA, 1 μ g of total RNA was added to a buffer containing 5 mM MgCl₂, 50 mM KCl, 10 mM Tris, pH 8.3, 1 mM dNTP, 0.1 μ g 6-mer degenerate primers, 40 units RNasin, and 15 units SuperScript II reverse transcriptase in a total volume of 20 μ l. The reaction mixture was incubated at 42°C for 1 h and then inactivated by heating at 70°C for 15 min. The mixture was then incubated at 37°C for 30 min with 15 units of RNase H. The cDNA mixture was then diluted to a final volume of 60 μ l with ddH₂O. For the full-length cloning, PCR was carried out in a total volume of 50 μ l containing 2 μ l of the reverse-transcribed cDNA mixture, 3.5 mM Mg(OAc)₂, 15 mM KOAc, 40 mM Tricine-KOH, pH 8.7, 0.2 mM dNTP, 0.2 μ M each -26f (5'-TGCAG ACGAC TTGTC CTGCC AC-3') and 2119r (5'-AAGAT CCGTT CATGA ATGAT GCTTC-3') primers, and 1 μ l of 50 \times Advantage 2 Polymerase Mix (Clontech, Palo Alto, CA). The cDNA was amplified by denaturing at 95°C for 30 s, annealed, and polymerized at 68°C for 4 min for 33 cycles. The reactions were terminated by a final extension at 68°C for 5 min and then cooled down to 4°C. The

resulting PCR fragments were then purified by agarose gel electrophoresis, subcloned into pGEMT-Easy TA vector (Promega, Madison, WI), and sequenced with ABI Prism dye terminator cycle ready reaction kit (Perkin-Elmer, Foster City, CA).

Multitissue Northern blot analysis. The 1.5-kb EcoRI fragment from human SORBS1 cDNA (clone 2, Fig. 1) was labeled using the Rediprime II kit (Amersham Pharmacia, Piscataway, NJ) and hybridized to a multiple-tissue Northern blot (Clontech), the membrane of which contains mRNAs derived from various tissues in an amount of 2 μ g each. The blot was hybridized at 65°C for 1 h in QuickHyb (Stratagene, La Jolla, CA) and washed twice with 2 \times SSC, 0.1% SDS at room temperature for 20 min, followed by a final wash with 0.2 \times SSC, 0.1% SDS at 65°C for 20 min. The blot was then exposed to a Phosphor screen overnight and analyzed with a PhosphorImager (Molecular Dynamics, Sunnyvale, CA).

BAC clone isolation and fluorescence in situ hybridization (FISH). A human BAC library (Genome Systems, St. Louis, MO) was screened according to the manufacturer's protocol by using the cDNA clone 2 (Fig. 1) as a probe. FISH experiments were performed as described previously (Pinkel *et al.*, 1988). In brief, the slides containing metaphase chromosomes were first denatured in a solution containing 70% formamide/2 \times SSC at 72°C for 3 min. The probes (from BAC clones 71P24, 144F03, and 190A22, from Genome Systems) were labeled with biotin-dUTP by nick-translation, denatured at 76°C for 7 min, and then added onto the metaphase chromosomes in 10 μ l hybridization solution containing 60% formamide/2 \times SSC/10% dextran sulfate and 1 μ l human Cot-1 DNA, at 37°C for 18 h. The resulting metaphase slides were interacted with FITC-conjugated avidin (Vector Laboratories, Burlingame, CA), counterstained with diaminido-2-phenylindole, and then observed under a fluorescence microscope (Zeiss Axioskop, Oberkochen, Germany).

Cell culture and cell extract preparation. Hep3B cells (HB-8064; ATCC) were grown in DMEM containing 10% fetal bovine serum, 2 mM glutamine, 50 U/ml penicillin, and 50 U/ml streptomycin in a humidified incubator with 5% CO₂ at 37°C. Cell monolayers were washed with cold PBS, after addition of 0.1 μ M insulin, at various time intervals as indicated. For Western blotting, the cells were lysed with a buffer containing 1% Nonidet P-40, 20 mM Tris, pH 8.0, 137 mM NaCl, 1 mM phenylmethylsulfonyl fluoride, 1 mM Na₃VO₄, 0.15 U/ml aprotinin, and 1 mg/ml leupeptin at 4°C. The cell extracts were then harvested by centrifugation at 12,000g for 10 min.

Expression of the glutathione S-transferase (GST) fusion proteins. Five GST fusion proteins were generated in this study. The tagged cDNA was inserted into the prokaryotic expression vector pGEX-2TK (Amersham Pharmacia) and introduced into *Escherichia coli* BL21 (DE3) Lys for expression. Two fusion proteins, 2TK-N (nt 1-1329) and 2TK-J (containing exon J sequence), were prepared and used as antigens to generate anti-SORBS1 antibodies in rabbits. The other three fusion proteins, 2TK-SH3A (nt 1330-1553), 2TK-SH3B (nt 1552-1731), and 2TK-SH3C (nt 1860-2055), containing different SH3 domains, were prepared for protein pull-down assay according to previous report (Chuang *et al.*, 1993).

Antibody production, immunoprecipitation, and Western blot analyses. Rabbit polyclonal antibodies against GST-SORBS1 fusion proteins (2TK-N and 2TK-J) were prepared and partially purified through GST protein-bound glutathione Sepharose 4B (Amersham Pharmacia). The anti-phosphotyrosine, c-Abl, and anti-insulin receptor β subunit antibodies were from Upstate Biotechnology (Lake Placid, NY) and Transduction Laboratories (San Diego, CA). Crude cellular extracts or immunoprecipitated proteins were resolved by SDS-polyacrylamide gel electrophoresis. After separation, the proteins were transferred to nitrocellulose filter membranes and immunoblotted with specific antibodies. The proteins of interest were detected by enhanced chemiluminescence according to the manufacturer's instructions (Amersham Pharmacia).

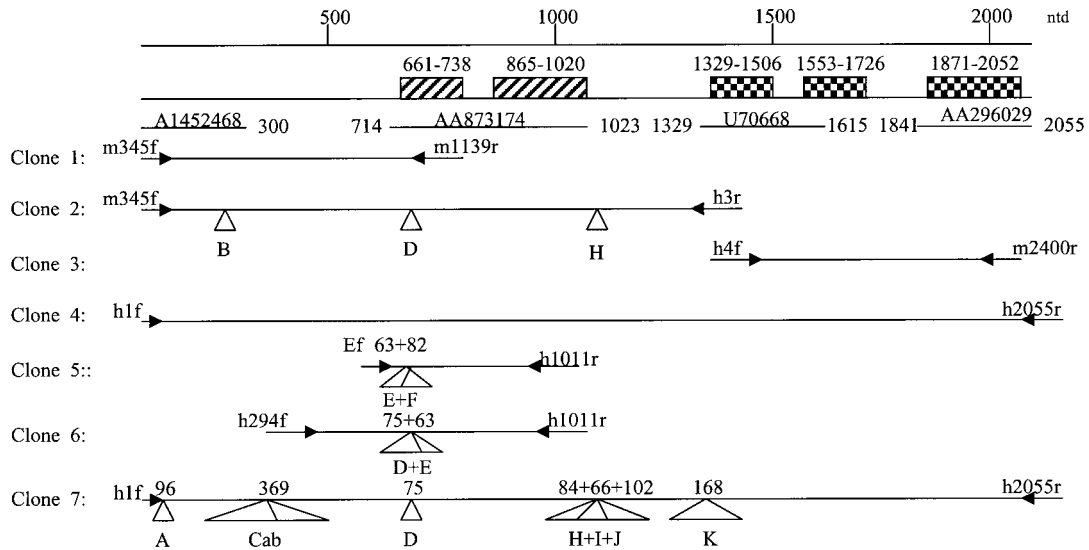


FIG. 1. Alignment of the cloned SORBS1 cDNAs. The arrowheads indicate the positions and orientations of the primers. Four homologous ESTs shown with a line and numbers on the each end indicate the positions of SORBS1 cDNA sequence (2055 bp). The striped and checkered boxes indicate the sorbin homology region and the SH3 domain, respectively. The alternative splicing exons are shown as triangles labeled from A to K with their lengths (basepairs) indicated above or under the triangles.

RESULTS

Cloning and Sequence Analysis of SORBS1 cDNA

By searching the human EST databank, four EST sequences (A1452468, AA873174, AA296029, and U70668) that were homologous to the mouse SH3P12/CAP cDNA sequence (U58883) were identified. Based on these EST sequences, 10 primers were designed to perform SORBS1 cloning from human adipose, liver, and skeletal muscle tissues. Seven cDNA clones (clones 1–7) were isolated by RT-PCR (Fig. 1). Clones 1 and 2 and clones 3 and 4 were isolated respectively from adipose and liver tissues; however, clones 5, 6, and 7 were isolated from skeletal muscle tissue. Clone 4, which contains 2055 bp had been deposited with the GenBank database under Accession No. AF136380. Compared to mouse SH3P12/CAP, there was 87.4% homology in nucleotide sequence and an 89.3% in amino acid sequence (data not shown). Detailed analyses of the domain structure revealed a sorbin homology domain (nt 661–1020) and three consecutive SH3 domains in the C-terminus, termed SH3A, SH3B, and SH3C (nt 1329–1506, 1553–11726, and 1871–2055). To elucidate further the expression of SORBS1 in different tissues, we performed RT-PCR to isolate full-length cDNA, as shown in Fig. 2B. Of the major isoforms, we had cloned and characterized 16 (Figs. 2A and 2C). Interestingly, we found another 3' donor site in exon 7 and exon 30 that resulted in exons 7b and 30b, found in M8 and M2 clones, respectively (Fig. 2D). The sequences of clones M1, M2, and M8 had been deposited with GenBank (GenBank Accession Nos. AF356525, AF356526, and AF356527). With sequence comparison by homology search, the nucleotide sequences of the

isolated exons were novel and without significant similarities to those deposited with GenBank, except that exon H was homologous to Arg/Abl binding protein 2A and exon K was homologous to Fas-ligand binding protein 2. Interestingly, exon F (82 bp) found in clone 5 (Fig. 1) was homologous to an EST sequence (BF959891) that was from a human breast tumor.

Genomic Organization of the SORBS1 Gene

To characterize the human SORBS1 genomic structure, we had used the sequences AF136380, AF356525, AF356526, AF356527, and clone 5 to search the NCBI human genomic sequence databank. Based on the search and alignment, the coding region of this gene was encoded by 34 exons (separated by 33 introns) spanning a region of more than 120 kb (Fig. 2A). As shown in Fig. 2A, intron size was derived from the human genomic sequences (NCBI). But, four intron sizes, shown as the gaps in the Fig. 2A, could not be obtained from the search.

Tissue Distribution of SORBS1 Transcripts

To determine the tissue-specific distribution, Northern blot analysis was performed on a human multiple-tissue blot. To avoid nonspecific hybridization due to the conserved SH3 domains, we use cDNA clone 2 (Fig. 1) as probe. The result showed that human SORBS1 was ubiquitously expressed in all the human tissues, including brain, heart, skeletal muscle, colon, thymus, spleen, kidney, liver, small intestine, placenta, lung, and peripheral blood leukocytes. However, SORBS1 was more abundantly expressed in the heart and skeletal muscles than in the other tissues (Fig. 3). More-

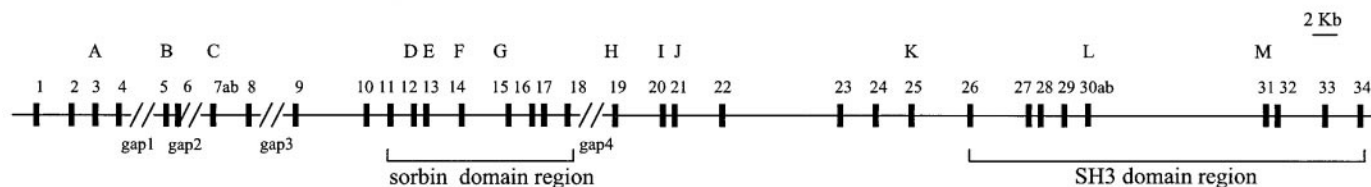
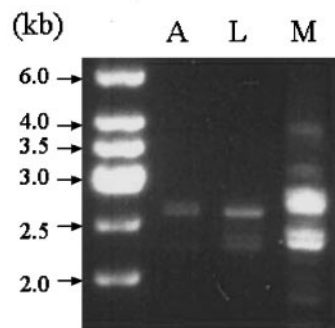
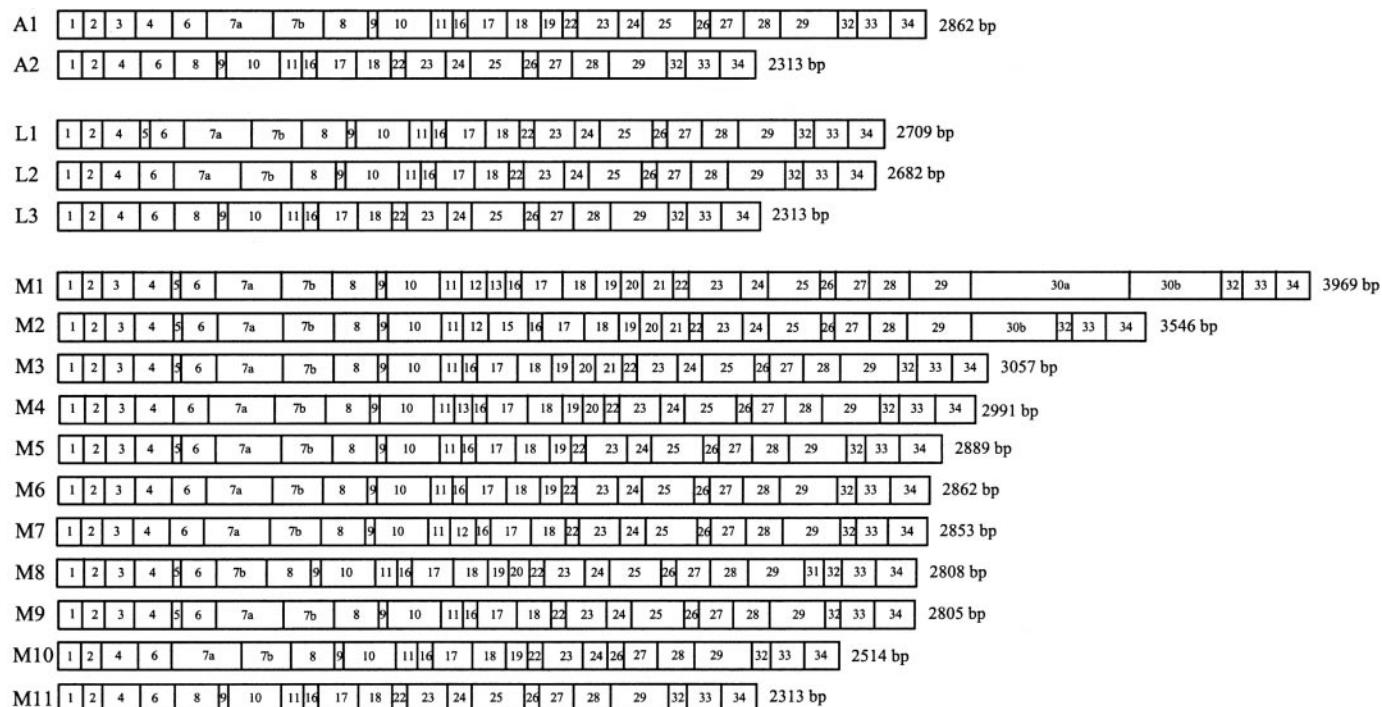
A**B****C**

FIG. 2. Genomic organization and mRNA expression of the SORBS1 gene. **(A)** Genomic structure of SORBS1. Exons are depicted as black boxes. The splicing exons named A to M are depicted over their respective boxes. The sorbin domain is encoded by exons 11, 16, 17, and 18 and the SH3 domain by exons 26, 27, 28, 29, 32, 33, and 34. The sizes of introns 4, 6, 8, and 18 are not ascertained. **(B)** The major RT-PCR fragments generated from the cDNA of human adipose (lane A), liver (lane L), and skeletal muscle (lane M) tissues. The molecular size markers are indicated on the left. **(C)** The exon organization of the 16 full-length clones isolated from adipose (A1 and A2), liver (L1–L3), and skeletal muscle (M1–M11) tissues. **(D)** The sequence of the genomic DNA fragments that contain exons 7ab and 30ab. The lowercase letters indicate intron sequence and uppercase letters denote exons 7ab and 30ab, where exons 7b and 30b are shown in boldface.

over, in heart, skeletal muscle, brain, and liver, multiple transcripts containing various alternatively spliced exons were detected, consistent with a previous report showing that SORBS1 had several splicing variants (Mandai *et al.*, 1999).

Chromosomal Localization of SORBS1

For chromosomal mapping of human SORBS1, a human BAC library was screened by using the SORBS1 cDNA. Three clones, 71P24, 144F03, and 190A22, were

D**Exon 7ab (369 bp)**

aacgaccccc ttatttccac agGATGCCAC TTCCTCCAGT GCAGCCCAGC CGGAGGTAAT AGTTGTCCCT
 CTCTACCTGG TTAATACTGA CAGAGGGCAA GAAGGCACTG CCAGACCTCC AACACCTCTG GGGCCTCTTG
 GCTGCGTCCC CACAATCCCA GCGACTGCCT CTGCCGCCTC ACCTCTGACC TTCCCGACTC TAGATGATTT
 CATTCCCCCT CATCTGCAGA GGTGGCCCCA CCACAGCCAG CCAGCCCGCG CCTCTGGCTC CTTTGCCCCC
 ATTAGCCAGA CGCCACCATC CTTCTCACCA CCACCTCCGC TGGTCCCTCC TGCCCCGGAG GACCTCCGCA
 GAGTCTCGGA GCCTGACCTC ACGGGAGCTG TTTCGAGTAC Cgtaagcttg ctggactccc ctccctcacgg

Exon 30ab (744 bp)

tcttttgttt tctctctctt tcttcttttt aagTTTTCCA GTCACAGCAA GTCATCACG CCAGCCCCCT
 CATCTCTGCC CCACTCCCGC CGAGCCCTGT CCCCCGAGAT GCACGCTGTC ACCTCTGAGT GGATCTCACT
 GACTGTGGGG GTCCCAGGCA GCGCTTCTCT GGCCCTGACC CCACCCTTGC CTCTCTGACC AGAGGCTTCT
 ATCTATAACA CTGACCACCT CGCCTTGTC CCAAGGGCCA GTCCCTCCCT GTCTCTCAGC CTCCCCATT
 TGAGTTGGTC AGATCGTCCC ACCCCACGAT CAGTAGCTTC TCCACTGGCC CTACCTTCCC CACACAAAAC
 CTACTCCCTA GCACCTACTT CCCAGGCTC CCTTCACATG AATGGAGACG GTGGTGTCCA CACGCCATCT
 TCAGGCATCC ACCAAGATAG CTTCTTGCA CTGCCGCTGG GGAGCTCTGA TAGTGTCTATC TCCCAGCTTA
 GTGATGCCTT TAGCAGCCAG AGCAAGAGGC AGCCATGGCG CGAAGAGAGT GGACAATATG AGAGGAAAGC
 AGAGAGGGGG GCAGGCGAAA GAGGCCCTGG TGGACCCAAG ATCTCTAAGA AGAGCTGCTT GAAGCCTTCA
 GACGTGGTCA GGTGCCTGAG TACTGAACAG AGACTCTCAG ATCTCAACAC CCCTGAGGAG AGCCGGCCCCG
 GCAAGCCCCT GGGTAGCGCT TTTCCAGGAA GTGAGGCTGA GCAGACAGAG CGGCATAGAG GTGGCGAGCA
 GGCGGGGAGG AAAGCTGCTC GGAGAGGTGG GAGCCAGgta ggactgcagc atgccgcgtg tgcacttggc

FIG. 2—Continued

isolated and confirmed by sequencing with h4f primer and Southern blotting (data not shown). These BAC clones were used as probes for doing the FISH experiment; 100 mitotic lymphocyte spreads all showed specific signals on one pair of chromosomes (Fig. 4). The SORBS1 gene was clearly mapped to human chromosome 10q23.3–q24.1 (Fig. 4). Recently, the mouse SH3P12/CAP (SH3d5) had been mapped to mouse chromosome 19 (35.50–37.50 cM), the syntenic region of human chromosome 10q23.1–q24.1 (Lee *et al.*, 1999).

Interactions of SORBS1 with Insulin Receptor and c-Abl

Sequence comparisons of SORBS1, especially the isoforms containing exon H, revealed a high homology with ArgBP2A, a protein that interacts with c-Abl oncoprotein (Fig. 5A). The c-Abl protein has three PXXP motifs that actually serve as an SH3 domain binding site to interact with some SH3 domain-containing protein; one of the PXXP motifs (amino acids 628–641) is a sequence that is highly conserved with the PXXP motif sequence of c-Cbl and Sos1 (Fig. 5B). It has been known that the PXXP motifs of c-Cbl and Sos1 provide the binding site for the third SH3 domain of SH3P12/CAP protein (Kurakin *et al.*, 1998). To study further the candidate protein that will interact with SORBS1 in insulin signaling, we then performed immunoprecipitation to examine the interaction between SORBS1 with c-Abl and insulin receptor in a human hepatoma cell line, Hep3B. Interestingly, SORBS1 was coprecipitated with the insulin receptor β subunit but not c-Abl in the basal state. After insulin stimulation, SORBS1 tended to dissociate from the receptor complexes (Fig. 6), as similarly reported in the 3T3-L1 adipocytes (Ribbon *et al.*, 1998c). As immunoprecipitated with anti-SORBS1 antibody after insulin stimulation, the amount of the binding of c-Abl oncoproteins to the SORBS1 proteins was remarkably increased (Fig. 7A). To map further the region responsible for the interac-

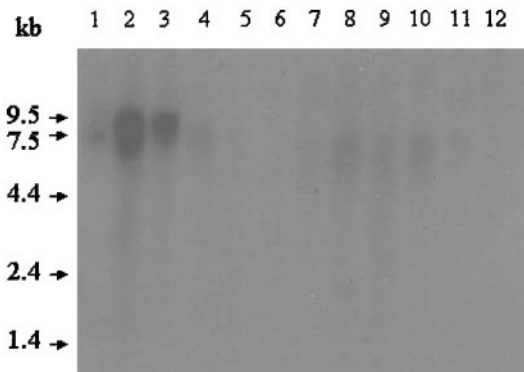


FIG. 3. Tissue distribution of the SORBS1 mRNA. The human mRNA blot was probed with radioactive SORBS1 cDNA (clone 2). Lane 1 indicates brain; 2, heart; 3, skeletal muscle; 4, colon; 5, thymus; 6, spleen; 7, kidney; 8, liver; 9, small intestine; 10, placenta; 11, lung; and 12, peripheral blood leukocytes. Size markers are indicated on the left.

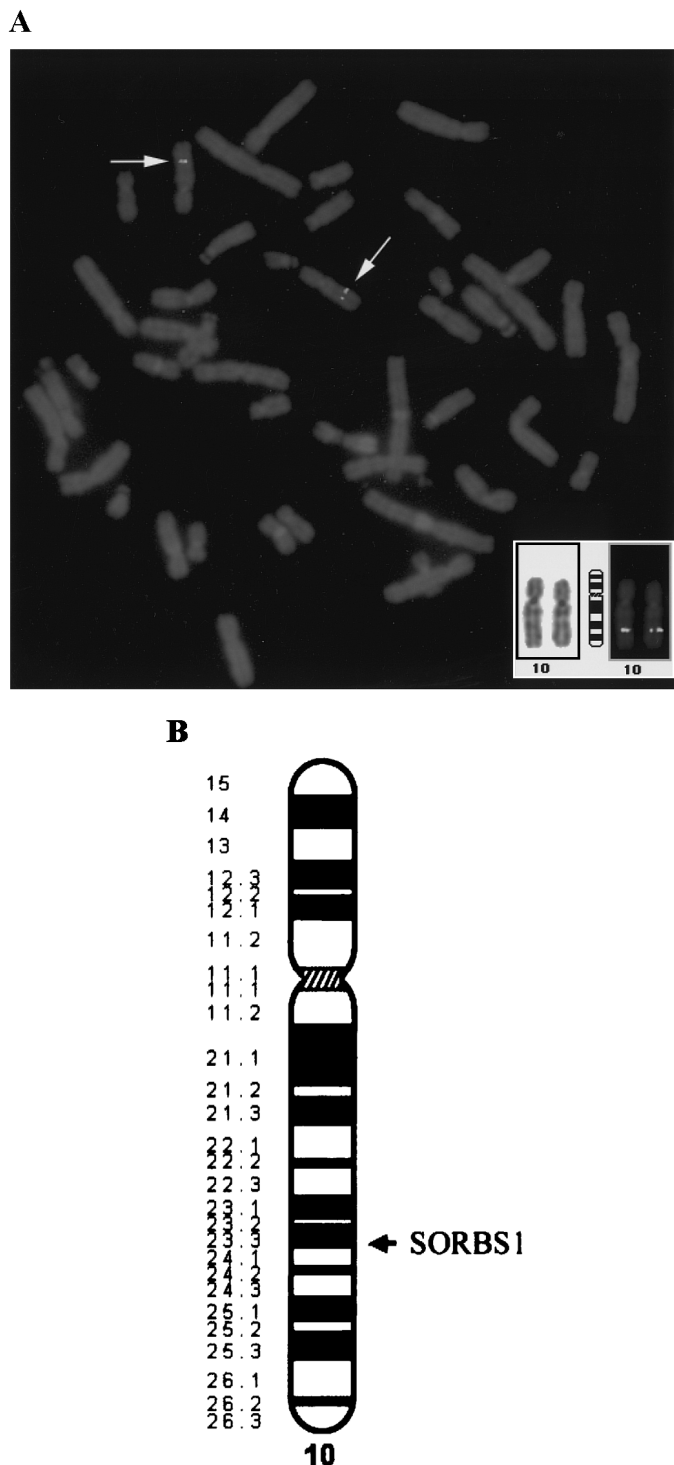


FIG. 4. Fluorescence *in situ* hybridization of SORBS1. (A) A BAC clone containing human SORBS1 gene was hybridized to normal human metaphase chromosomes as described under Materials and Methods. Arrows point to the site of hybridization of the SORBS1 probe; both sister chromatids show hybridization at that site. (B) Idiogram of human chromosome 10 shows the map of localization of the human SORBS1 gene at 10q23.3–q24.1 (arrow).

tion with c-Abl oncoprotein, we expressed the GST-fusion proteins containing the three SH3 domains of the SORBS1 gene separately. We found that the third SH3 domain, namely SH3C, was responsible for the

binding of c-Abl (Fig. 7B). It is interesting to note that the isolated GST-SH3C protein was capable of binding the c-Abl proteins present both in the control and in the insulin-stimulated cell lysates, implying that the binding between SORBS1 and c-Abl inside the intact cells requires a modification of SORBS1 protein upon insulin stimulation.

DISCUSSION

We have identified the human SORBS1 cDNA from adipose, liver, and skeletal muscle tissues by RT-PCR. SORBS1 is a member of the novel SH3P12/ArgBP2A/vinexin adaptor family. In this study, we isolated 13 alternatively spliced exons and demonstrated that the isoforms with these exons in the three insulin-sensitive tissues are different. The observation of multiple splicing isoforms of SORBS1 is similar to the discovery of more than 12 variants found in mouse tissues (Mandai *et al.*, 1999). The expression of SORBS1 is ubiquitous in all tissues, but it is preferentially expressed in the heart and skeletal muscle. The high expression of SORBS1 in heart is similar to that of ArgBP2A, an Abl/Arg tyrosine kinase binding protein (Wang *et al.*, 1997). Therefore, we speculate that SORBS1 might exert a special function related to ArgBP2A in heart and skeletal muscle tissues.

SORBS1 protein is structurally related to ArgBP2A and vinexin, a vinculin binding protein. This family of proteins can interact with vinculin, 1-afadin, and other associated proteins. The specific SORBS1 isoforms, i.e., those containing spliced exon H, have a high homology to ArgBP2A. Based on the sequence similarity between SORBS1 and ArgBP2A, and the consensus motifs (three PXXP motifs) identified in c-Abl for SH3 domain binding, we investigated the interaction between SORBS1 with insulin receptor and c-Abl in Hep3B cells. We found that SORBS1 associated with insulin receptor β subunit but not c-Abl in the unstimulated state. However, following insulin stimulation, SORBS1 was dissociated from insulin receptor while the binding to c-Abl was increased. Interestingly, the binding between SORBS1 and c-Abl was through the third SH3 domain (SH3C) in the C-terminus, as demonstrated in the GST-SH3C fusion protein pull-down assays with the cell lysates either from basal or after insulin stimulation. Taken together, our data suggest that some structural modification occurs in SORBS1 rendering it capable of binding c-Abl in intact cells when treated with insulin.

Different cellular functions might occur due to the different subcellular localizations of these interacting proteins. Previously, it is known that CAP/SH3P12 protein can associate with insulin receptor, flotillin, c-Cbl oncoprotein, Sos adaptor, and focal adhesion kinase (FAK), suggesting that SORBS1 might be involved in the insulin signaling pathway and cytoskeleton organization. It has been demonstrated that nuclear localization of c-Abl plays a role in controlling

A

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ArgBP2A    138  DRPKDWYKTMFKQIHMVHKPDDDDTDMYNTFYTYNAGLYNPPYSAQSHPAAKTQTYRPLSK
              +R KDWYKTMFKQIH +++ DDD+D+Y+ Y+++ +P S P +K++

SORBS1     225  ERSKDWYKTMFKQIHKLNLR-DDSDLYSPRYSFSEDTKSP----LSVPRSKSEM-----

              198  SHSDNSPNAFKDASSPVPPPHVPPVPPPLRPRDRSSTEKHDWDPPDRKVDTRKFRSEPRS
              S+ D      + A+ P+P                      +SS+E++DW+PPD+KVDTRK+R+EP+S

              274  SYIDGKVVVKRSATLPLPARSS-----SLKSSSERNDWEPPDKKVDTRKYRAEPKS

              258  IFEYEPGKSSILQHERPTDRINPDDIDLLENEPWYKFFSELEFGRP
              I+EY+PGKSS+L +E+ + I+P++IDL+NEPWYKFFSELEFG+P

              325  IYEQPGKSSVLTKEKMSRDISPEEIDLKNEPWYKFFSELEFGPK
  
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B

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              CONSENSUS          P×PP×R×SSL

c-Cbl (821-834 a.a.)----LPPPPPPARHSLIE--

Sos1 (1287-1300 a.a.)---IAGPPVPPRQSTSQ--

c-Abl (628-641 a.a.)----KTAPTPPKRSSFR--
  
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FIG. 5. (A) Comparison of primary protein structure of ArgBP2A with SORBS1 containing exon H sequences. The amino acid sequence of exon H is indicated in bold. (B) Alignment of the primary protein structure of c-Cbl, Sos, and c-Abl with the consensus sequence.

cell cycle, stress response, DNA repair, transcriptional activity, and apoptosis. However, the function of cytoplasmic c-Abl is far less understood. It has been shown that cytoplasmic c-Abl is associated with the actin cytoskeleton through its C-terminal actin-binding domain following growth factor and integrin signaling (Lewis *et al.*, 1996; Plattner *et al.*, 1999; Lanier and Gertler, 2000). SH3P12/CAP protein interacts with FAK through its middle SH3B domain (Ribon *et al.*,

1998a). Taken together, our data suggest a possibility that the interaction of FAK and actin is via the interaction of SORBS1 and c-Abl.

In the FISH experiments, we found that the SORBS1 gene was a single-copy gene and mapped to the human chromosome 10q23.3–q24.1 region. Recently, an interesting chromosomal region linked to insulin action was also mapped to the 10q23–q24 region in the Pima Indian population (Pratley *et al.*, 1998). However, a search for mutation of a candidate gene, the protein targeting to glycogen/PPP1R5, in this region had been pursued in vain. No contribution of this gene to insulin sensitivity was concluded (Permana *et al.*, 1999). Since SH3P12/CAP was associated with insulin receptor in several insulin-sensitive tissues (Ribon *et al.*, 1998c, and present study), and up regulated in 3T3-L1 adipocytes/adipose tissues after treatment with insulin sensitizer (Ribon *et al.*, 1998b; Lin *et al.*, 1999), collectively, these data suggest that SORBS1 represents a positional and functional candidate gene for insulin sensitivity.

In conclusion, the SORBS1 gene is expressed in all of tissues but with a tissue-specific pattern. We have cloned and identified different isoforms that were due to the alternative splicing mechanism. Interaction between SORBS1 protein with c-Abl is insulin-dependent and via its C-terminal SH3 domain, implying a role for SORBS1/c-Abl interaction in the insulin signaling pathway. SORBS1 is mapped to the chromosome 10q23.3–q24.1 region, a candidate region for insulin sensitivity found in Pima Indians. Thus, SORBS1

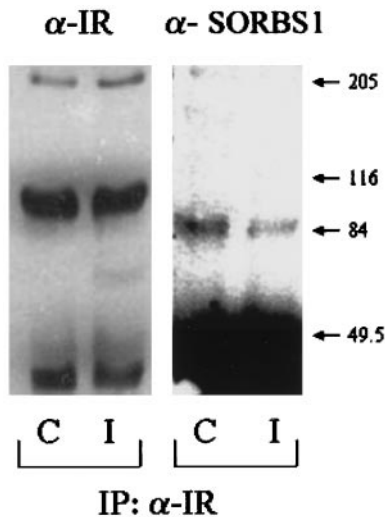


FIG. 6. Interaction of insulin receptor and SORBS1. Total cellular extracts from the Hep3B cells, control (C) and insulin stimulated for 5 min (I), were first immunoprecipitated with anti-IR antibody and then blotted with anti-IR (left) and anti-SORBS1 (right). Arrows (right) indicate the molecular weight markers in kDa.

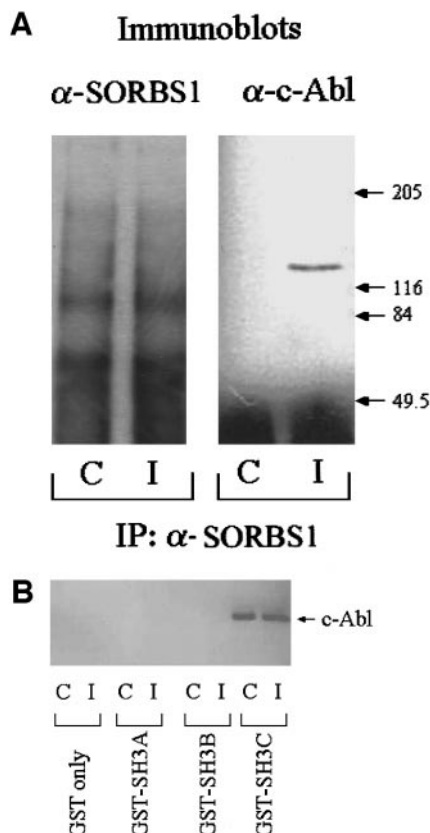


FIG. 7. Interaction of SORBS1 and c-Abl. **(A)** Immunoprecipitation. Total cellular extracts from the Hep3B cells, control (C) and insulin stimulated for 5 min (I), were immunoprecipitated with anti-SORBS1 antibody and immunoblotted with anti-SORBS1 (left) and anti-c-Abl (right). Arrows (right) indicate the molecular weight markers in kDa. **(B)** GST-fusion protein pull-down. Total cellular extracts from the Hep3B cells, control (C) and insulin stimulated for 5 min (I), were incubated with GST alone and GST fusion proteins of the three SH3 domains (SH3A, SH3B, and SH3C) of the SORBS1. The bound proteins were then transferred and blotted with anti-c-Abl antibody (indicated by arrow on the right).

serves as a positional and functional candidate for insulin sensitivity and the associated phenotype such as obesity and/or diabetes mellitus.

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