A Deubiquitinating Enzyme UBPY Interacts with the Src Homology 3 Domain of Hrs-binding Protein via a Novel Binding Motif PX(V/I)(D/N)RXXKP*

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Hrs-binding protein (Hbp) is a Src homology 3 (SH3) domain-containing protein that tightly associates with Hrs. Hbp together with Hrs is thought to play a regulatory role in endocytic trafficking of growth factor-receptor complexes through early endosomes. Association of Hbp with a binding partner(s) via the SH3 domain seems to be essential for Hbp to exert its function. In this study, we searched for Hbp-binding proteins by a far Western screening and isolated a mouse cDNA clone encoding a deubiquitinating enzyme mUBPY as an Hbp SH3-binding protein. mUBPY has two Hbp-SH3 domain binding sites. Mutagenic analysis identified a consensus sequence PX(V/I)(D/N)RXXXP as the Hbp-SH3 domain binding motif. It is a novel SH3-binding motif and does not contain the canonical proline-rich consensus binding motif, PXXP. Ubiquitination of growth factor receptors is thought to regulate their intracellular degradation. Thus, UBPY may play a regulatory role in the degradation by interaction with the SH3 domain of Hbp via the novel SH3-binding motif.

The binding of growth factors to their receptors on the cell surface causes the initiation of signal transduction cascades, which eventually result in biological effects on target cells. After growth factor binding, growth factor-receptor complexes are internalized and delivered to early endosomes. Thereafter, they escape recycling to the cell surface and are sorted to the degradation pathway, which leads to the down-regulation of growth factor receptors (1). Little is known about the mechanism by which growth factor-receptor complexes are sorted for lysosomal degradation. Some of the early endosomal proteins may play a regulatory role in this sorting.

Hrs (hepatocyte growth factor-regulated tyrosine kinase substrate) is an early endosomal protein that is tyrosine-phosphorylated in cells stimulated with growth factors (2, 3). Since Hrs is likely to be a mammalian homologue of the yeast Vps27p, which is essential for vacuolar and endocytic trafficking through a prevacuolar compartment (4), Hrs may be a regulator of endocytic trafficking through early endosomes in mammalian cells. Hrs tightly associates with Hrs-binding protein (Hbp)† (5). Hbp has an N-terminal VHS domain, a Src homology 3 (SH3) domain, a coiled-coil motif, and a ITAM motif and associates with Hrs through the coiled-coil motif. Overexpression of deletion mutants of Hbp lacking either the SH3 domain or the coiled-coil motif inhibits the intracellular degradation of a growth factor and its receptor (5). Thus, Hbp together with Hrs appears to play a regulatory role in the intracellular degradation of growth factor-receptor complexes through early endosomes, and the association of Hbp with a binding partner(s) via the SH3 domain may be essential for Hbp to exert its function. Hrs also associates with STAM (6), which is homologous to Hbp and has the same domain structures as Hbp (7). An SH3 domain deletion mutant of STAM shows dominant negative effects on DNA synthesis stimulated by interleukin-2 and granulocyte-macrophage colony-stimulating factor (8), suggesting that the SH3 domain of STAM is essential for its function. AMSH has been identified as a binding partner of the SH3 domain of STAM and has been shown to be involved in signaling for DNA synthesis stimulated by interleukin-2 and granulocyte-macrophage colony-stimulating factor (9).

SH3 domains are present in many proteins involved in tyrosine kinase signaling and the organization of cytoskeletal matrices and mediate protein-protein interactions that regulate signal transduction (10, 11). SH3 domains recognize proline-rich peptides containing the core PXXP. Structural and mutagenic analysis of peptide-SH3 complexes revealed that proline-rich peptides associated with SH3 domains adopt a polyproline type II helix conformation with 3 residues per turn (12). Recently, it has been shown that peptide sequences other than proline-rich peptides are involved in binding to SH3 domains. The SH3 domain of p53BP2 interacts with a peptide sequence in p53 that is not proline-rich, and the interaction is essential for p53 to associate with p53BP2 (13). Moreover, a PXXDY consensus sequence is indispensable for binding to the SH3 domain of Eps8, and the domain does not bind to canonical PXXP-containing peptides (14). Thus, interaction of SH3 domains with peptide sequences without the PXXP core plays a crucial role in the functional association of some SH3 domain-containing proteins with their binding partners.

Since the SH3 domain of Hbp seems to be essential for the degradation of growth factor-receptor complexes, identification

* This work was supported in part by research grants from the Ministry of Education, Science, Sports and Culture of Japan. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1744 solely to indicate this fact.

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBankTM / EBI Data Bank with accession number(s) AB045709.

The abbreviations used are: Hbp, Hrs-binding protein; SH3, Src homology 3; SBB, SH3 domain binding motif; RACE, rapid amplification of cDNA ends; mUBPY, mouse UBPY; GST, glutathione S-transferase; PCR, polymerase chain reaction; HA, hemagglutinin; UBP, ubiquitin-specific protease; GAB, Grb2-associated binder; BLNK, B cell linker protein.
of its binding partners is necessary to elucidate how Hbp is involved in the degradation pathway. Therefore, we searched for Hbp-binding proteins by a far Western screening of a mouse cDNA library and isolated a cDNA clone encoding mouse UBPY (mUBPY) as an Hbp3-binding protein. We found by mutational analysis that a novel peptide sequence PXV/IV/D/NRRXXKP is essential for the association of mUBPY with the SH3 domain of Hbp, and PXXP-containing sequences are not involved in this association. Furthermore, we demonstrate that this novel SH3-binding sequence mediates other protein-protein interactions.

**EXPERIMENTAL PROCEDURES**

Cloning of a mUBPY cDNA—A ZAP mouse liver cDNA library was screened by a far Western method with the \(^{32}P\)-labeled Hbp as a probe. To prepare probe, mouse Hbp cDNA was inserted into the NcoI site of the pGEX-2TK vector (Amersham Pharmacia Biotech). The GST fusion protein was expressed in *Escherichia coli* and was bound to glutathione-Sepharose 4B beads (Amersham Pharmacia Biotech). The fusion protein bound to the beads was incubated with human thrombin (Sigma) at room temperature for 2 h. After incubation, the Hbp protein cleaved from the GST tag was incubated with protein kinase (Sigma), 10 mM MgCl\(_2\), and 0.4 mCi/ml \(^{32}\)P-ATP (\(3000 \text{ Ci/mmol}\); Amersham Pharmacia Biotech) for 30 min at 30 °C. Free ATP was removed by a gel filtration on a Sephadex G-50 column, and the fraction containing the Hbp protein was used as a probe. Nitrocellulose membranes pretreated with 10 mM isopropyl-\(\beta\)-thiogalactoside were overlaid onto phase plaques and incubated at 37 °C for 4 h. After being treated with blocking buffer (20 mM Tris-HCl, pH 7.4, 150 mM NaCl, 0.05% skim milk, and 0.05% Tween 20) at room temperature for 2 h, the membranes were incubated with the probe (5 \(\times\) 10\(^{-6}\) cpm/ml) in blocking buffer for 12 h at 4 °C. After a wash in TBS buffer (20 mM Tris-HCl, pH 7.5, 0.15 M NaCl) containing 0.05% Tween 20, bound proteins were detected by autoradiography.

The missing 5'-portion of the cDNA was obtained by 5'-RACE-PCR using Cap Site cDNA\(^{TM}\) Mouse Testis (Nippon Gene) as a template. The fusion protein bound to the beads was incubated with human thrombin (Sigma) at room temperature for 4 h. After incubation, the Hbp protein cleaved from the GST tag was incubated with protein kinase (Sigma), 10 mM MgCl\(_2\), and 0.4 mCi/ml \(\gamma\)-\(^{32}\)P-ATP (3000 Ci/mmol; Amersham Pharmacia Biotech) for 30 min at 30 °C. Free ATP was removed by a gel filtration on a Sephadex G-50 column, and the fraction containing the Hbp protein was used as a probe. Nitrocellulose membranes pretreated with 10 mM isopropyl-\(\beta\)-thiogalactoside were overlaid onto phase plaques and incubated at 37 °C for 4 h. After being treated with blocking buffer (20 mM Tris-HCl, pH 7.4, 150 mM NaCl, 0.05% skim milk, and 0.05% Tween 20) at room temperature for 2 h, the membranes were incubated with the probe (5 \(\times\) 10\(^{-6}\) cpm/ml) in blocking buffer for 12 h at 4 °C. After a wash in TBS buffer (20 mM Tris-HCl, pH 7.5, 0.15 M NaCl) containing 0.05% Tween 20, bound proteins were detected by autoradiography.

**RESULTS**

Identification of Mouse UBPy as an Hbp-interacting Molecule—To isolate cDNA clones encoding Hbp-interacting molecules, we screened a mouse liver cDNA library by a far Western method with the \(^{32}P\)-labeled full-length Hbp as a probe. We isolated several cDNA clones and identified them by computer-assisted sequence homology search. One of the cDNA clones encoded a protein that shared 80.6% amino acid sequence identity with human UBPy (15). Thus, the protein is a mouse counterpart of UBPy (mUBPy). UBPy is a member of the ubiquitin-specific protease (UBP) family. UBP family members contain two conserved motifs, a Cys box and a His box, in the catalytic domains (16, 17). These motifs are located in the C-terminal region of UBPy (Fig. 1). In addition, domain or motif prediction analysis of UBPy using the SMART program (18) predicted a rhodanese homology domain (RD) (19) and two coiled-coil motifs (Fig. 1).

Interaction of Hbp with mUBPy via the SH3 Domain—Hbp contains protein-interacting domains such as the SH3 domain and coiled-coil motif (5). Because the coiled-coil motif is involved in the interaction of Hbp with Hrs (5), the interaction of Hbp with UBPy may be mediated via the SH3 domain. To examine this possibility, UBPy in lysates of NIH3T3 cells was assayed for in vitro binding to the immobilized GST-fused SH3 domain of Hbp, mUBPy bound to the GST-fused SH3 domain of Hbp (GST-Hbp-SH3) as well as the GST-fused full-length Hbp (GST-Hbp), whereas it did not bind to the GST-fused full-length Hbp lacking the SH3 domain (GST-Hbp\(\Delta\)SH3) (Fig. 2). These results indicate that Hbp interacts with mUBPy via the SH3 domain.

Mapping of the SH3-binding Region in mUBPy—mUBPy contains six proline-rich sequences that match the minimal SH3-binding motif consensus PXXP (Fig. 1A). Among them, the \(^{649}\)PSSAPSPPP motif sequence matches the consensus SH3-binding site of the dynamin family, PXXPXXPXXP (20). To determine which proline-rich sequence in mUBPy was involved in binding to the SH3 domain of Hbp, FLAG-tagged deletion mutants lacking each PXXP sequence in mUBPy were expressed in COS-7 cells and assayed for in vitro binding to the GST-fused SH3 domain of Hbp. Unexpectedly, all mutants bound to the
SH3 domain of Hbp (data not shown), suggesting that mUBPY interacts with the SH3 domain of Hbp through a sequence other than the PXXP motif. Thus, to identify the sequence, a series of FLAG-tagged deletion mutants of mUBPY were expressed in COS-7 cells and assayed for in vitro binding to the GST-fused SH3 domain of Hbp. The deletion mutant lacking 646 C-terminal amino acid residues (1–434) bound to the SH3 domain, whereas the mutant with a further 57-amino acid deletion (residues 1–377) did not (Fig. 3). These results indicate that another SH3-binding sequence is located within amino acids 378–434. Surprisingly, the deletion mutant lacking 506 N-terminal amino acid residues (507–1080) still bound to the SH3 domain, suggesting the presence of another SH3-binding sequence in the C-terminal region of mUBPY. The deletion mutant lacking 665 N-terminal amino acid residues (666–1080) bound to the SH3 domain, whereas the mutant with a further 66-amino acid deletion (732–1080) did not (Fig. 3). These results indicate that another SH3-binding sequence is located within amino acids 666–731.

Identification of the Sequence Critical for Binding of mUBPY to the SH3 Domain of Hbp—Because the SH3-binding sequences are located within amino acids 378–434 and 666–731, the two sequences were compared with search for a consensus sequence. In addition, the sequences of the corresponding regions in human UBPY were aligned with the mouse sequences. We found that all of these sequences contained 9-amino acid sequences fitting PXXXRXXKP (Fig. 4A). Moreover, the sequence was located in the region of AMSH that mediates association of the protein with the SH3 domain of STAM (9). To investigate whether the sequences mediated the binding of UBPY to the SH3 domain of Hbp, FLAG-tagged deletion mutants were expressed in COS-7 cells and assayed for in vitro binding to the GST-fused SH3 domain of Hbp. The N-terminal half (residues 1–506) and C-terminal half (residues 507–1080) of mUBPY lacking the 9 amino acid residues (ND (405–413) and CD (700–708), respectively) did not bind to the SH3 domain of Hbp (Fig. 4B). These results suggest that PXXXRXXKP is a core sequence of the SH3-binding motif (SBM) of mUBPY. To examine whether the sequences mediate the association of mUBPY with Hbp in vivo, FLAG-tagged full-length mUBPY or its deletion mutants were cotransfected with HA-tagged Hbp into COS-7 cells. Lysates of the transfected cells were immunoprecipitated with an anti-FLAG antibody, and the immunoprecipitates were immunoblotted with an anti-HA antibody. The deletion mutants lacking each SBM of mUBPY were coprecipitated with Hbp, whereas the mutant lacking both SBMs was not (Fig. 4C). These results suggest that PXXXRXXKP is a core sequence of the SH3-binding motif (SBM) of mUBPY. To examine whether the sequences mediate the association of mUBPY with Hbp in vivo, FLAG-tagged full-length mUBPY or its deletion mutants were cotransfected with HA-tagged Hbp into COS-7 cells. Lysates of the transfected cells were immunoprecipitated with an anti-FLAG antibody, and the immunoprecipitates were immunoblotted with an anti-HA antibody. The deletion mutants lacking each SBM of mUBPY were coprecipitated with Hbp, whereas the mutant lacking both SBMs was not (Fig. 4C). These results suggest that mUBPY associates with Hbp in vivo through either of the two SBMs in mUBPY.
and assayed for in vitro binding to the GST-fused SH3 domain of Hbp. Mutagenesis of the proline at position 405 or 413, the arginine at position 409, or the lysine at position 412 completely abolished binding. Mutagenesis of the valine at position 407 or the asparaginic acid at position 408 markedly reduced binding (Fig. 5). These results indicate that these amino acid residues are critical for the binding of mUBPY to the SH3 domain of Hbp.

There are a number of proteins that interact with SH3 domains of partner proteins. The novel SBM may mediate some of these interactions. Thus, we searched a data base for proteins containing the novel SBM by PatternSearch. The consensus sequence of the novel SBM is most likely PXX(V/I)(D/N)RP. Other Proteins Containing the Novel SBM—AMSH was reported to bind to the SH3 domain of STAM via the PXX motif (P227PAKP231) as a major binding site (9). In that report, the motif was identified from the result that the deletion mutant of AMSH lacking the sequence of P227PAKP231 did not associate with the SH3 domain of STAM. The proline residue at position 231 corresponds to the first amino acid of the consensus sequence of the novel SBM, PXX(V/I)(D/N)RP (Fig. 6A) and is critical for the consensus sequence to mediate binding to the SH3 domain of Hbp. Thus, it is possible that AMSH associates with STAM via the novel SBM but not the PXXP motif. To examine this possibility, FLAG-tagged deletion mutants of AMSH were expressed in COS-7 cells and assayed for in vitro binding to the GST-fused SH3 domain of STAM. The deletion mutant lacking 227PAKP230 bound to the SH3 domain. However, the deletion mutants lacking the whole SBM or the C-terminal 5 residues failed to bind to the SH3 domain of STAM (Fig. 6B). These results suggest that a major site of AMSH for binding to the SH3 domain of STAM is the SBM but not the PXXP motif.

Because the SH3 domain of Hbp is highly homologous to that of STAM (5), it is expected to bind to AMSH via the novel SBM. Thus, FLAG-tagged deletion mutants of AMSH expressed in COS-7 cells were assayed for in vitro binding to the GST-fused SH3 domain of Hbp. The deletion mutants lacking the whole SBM or the C-terminal 5 residues did not bind to the SH3 domain of Hbp, whereas the deletion mutant lacking 227PAKP230 did (Fig. 6B). These results suggest that AMSH interacts with the SH3 domain of Hbp via the SBM.
sequence of the SBM, PX(V/I)(D/N)RXXKP, was found in members of the family of Grb2-associated binder (GAB) and the family of B cell linker protein (BLNK) (Fig. 7). It is possible that the sequence mediates the association of these proteins with their binding partners.

**Interaction of mUBPY with the Other SH3 Domains**—SH3 domain-containing proteins bind to their binding partners through specific binding motifs. To determine the specificity of mUBPY for binding to SH3 domains, FLAG-tagged mUBPY expressed in COS-7 cells was transfected with expression vectors encoding the FLAG-tagged mutants of the N-terminal region (UBPYN, amino acid residues 1–506) of mUBPY in which each residue in the Hbp-SH3 domain binding motif was replaced with an alanine. The cell lysates were assayed for in vitro binding to the SH3 domain of Hbp as described in the legends to Figs. 2 and 3 (top panel). Expression of the proteins was verified by immunoblotting (bottom panel). B, binding efficiencies of UBPN mutants were quantitated by BioMax analysis and are expressed as the percentage of that of wild type UBPN. C, the consensus sequence of the novel SH3 domain binding motif.

![Fig. 5. Identification of amino acid residues critical for binding of mUBPY to the SH3 domain of Hbp. A, COS-7 cells were transfected with expression vectors encoding the FLAG-tagged mutants of the N-terminal region (UBPYN, amino acid residues 1–506) of mUBPY, in which each residue in the Hbp-SH3 domain binding motif was replaced with an alanine. The cell lysates were assayed for in vitro binding to the SH3 domain of Hbp as described in the legends to Figs. 2 and 3 (top panel). Expression of the proteins was verified by immunoblotting (bottom panel). B, binding efficiencies of UBPN mutants were quantitated by BioMax analysis and are expressed as the percentage of that of wild type UBPN. C, the consensus sequence of the novel SH3 domain binding motif.](image)

![Fig. 6. In vitro binding of AMSH to the SH3 domains of STAM and Hbp via the novel SH3 binding motif. A, amino acid sequence around the SH3 binding motif of AMSH. B, COS-7 cells were transfected with expression vectors encoding the FLAG-tagged wild type (W) or deletion mutants (Δ227–230, ΔSBM, Δ235–239) of AMSH. At 48 h after transfection, the cell lysates were assayed for in vitro binding to the SH3 domains of STAM and Hbp as described in the legends to Figs. 2 and 3 (top panel). Expression of the proteins was verified by immunoblotting (bottom panel).](image)

**FIG. 7.** Proteins containing the consensus sequence(s) of the novel SH3 domain binding motif.

| Protein    | Amino Acids | Sequence   |
|------------|-------------|------------|
| GAB family |             |            |
| human      | 517–525     | PPVDRLMKP  |
| human      | 473–481     | PPVWRLKP   |
| Drosophila | 640–648     | PSVDRKLKP  |
| SLP-76     | 692–700     | PVYDKEKLP  |
| BLNK family|             |            |
| human      | 204–212     | PMVIGSTKPP |
| human      | 233–241     | PS1DRTKPP  |
| chicken    | 219–227     | PPDVRKTP   |
| Other proteins |         |            |
| mouse      | 405–413     | PQVRSTKPP  |
| human      | 699–708     | PMVIGSTKPP |
| human      | 405–413     | PQVRSTKPP  |
| human      | 736–746     | PTVHRENKP  |
| human      | 231–239     | PVDRESLKP  |
| yeast      | 322–330     | PQVLDSRTPK |

Hbp aligned with that of the C-terminal region of Grb2 without any gaps. In addition, there are some amino acid residues that are common in the SH3 domains of Hbp and STAM and the
C-terminal SH3 domain of Grb2 but are distinct from those in other SH3 domains.

**DISCUSSION**

In this study, we have identified mUBPY as a Hbp-SH3 domain-binding protein. UBPY is a deubiquitinating enzyme (15). Deubiquitinating enzymes are subdivided into two groups: ubiquitin C-terminal hydrolases and ubiquitin-specific processing proteases (UBPs) (21). UBPY is a member of the UBP group. UBPs are able to cleave both linear and isopeptide-linked ubiquitin chains. Some UBPs negatively regulate protein degradation by removing ubiquitin from substrates before they are destroyed by proteasomes (22), while other UBPs seem to positively regulate proteolysis.

Yeast Ubp14 and its mammalian homologue isopeptidase T stimulate substrate degradation by the 26 S proteasome both in vitro (23) and in vivo (24). Yeast Doa4 is required for recycling ubiquitin from both proteasome-bound ubiquitinated intermediates and membrane proteins destined for destruction in the vacuole (25). Several growth factor receptors such as platelet-derived growth factor receptor (PDGFR), epidermal growth factor receptor, and hematocyte growth factor receptor are ubiquitinated upon ligand stimulation (26–28). It has been shown that proteasome inhibitors suppress degradation of PDGFR and hematocyte growth factor receptor (28, 29), suggesting that in addition to the lysosomal pathway, the ubiquitin-proteasome pathway is involved in proteolytic degradation of growth factor receptors. Because the deletion mutant of Hbp lacking the SH3 domain shows dominant negative effects on degradation of PDGFR (5), it is unknown whether the motif alone is sufficient or another sequence is required for the association.

Similar sequences to UBPY SBMs are also found in AMSh and members of the BLNK family and the GAB family. AMSh binds to the SH3 domain of STAM. Although a PXXP motif in AMSh was suggested to mediate the interaction of AMSh with the SH3 domain of STAM (9), our in vitro binding assay showed that the consensus sequence of SBM in AMSh contributes to the interaction. By a similar analysis, we showed that AMSh binds to the SH3 domain of Hbp via the SBM. Thus, the novel SBM is essential for mUBPY to associate with Hbp. It remains unknown whether the motif alone is sufficient or another sequence is required for the association.

UBPY has two Hbp-SH3 domain binding sites. Mutagenic analysis identified a consensus sequence, PXV(D/N)RXXXP, as the Hbp-SH3 domain binding motif. It does not contain the proline-rich consensus binding motif, PXXP (30). It is also distinct from the SH3-binding motif, PXXPY, which is found in Eps8-interacting proteins (14). There are six proline-rich sequences in mUBPY fitting the consensus sequence, PXV(D/N)RXXXP, but they do not contribute to the interaction of mUBPY with the SH3 domain of Hbp, because the deletion mutant lacking the Hbp-SH3 domain binding motifs failed to bind. Thus, the novel SBM is essential for mUBPY to associate with Hbp. It remains unknown whether the motif alone is sufficient or another sequence is required for the association.

![Image](https://example.com/image.png)
Grb2-binding sites in Gab1 were identified. The first site matches the proline-rich consensus binding motif PXXP, while the second binding site constitutes the consensus sequence of SBM (35). Thus, the SBM in Gab1 may play an important role in Gab1-Grb2 interaction required for growth factor signaling. The novel SBM appears to be a crucial sequence to mediate protein-protein interactions through SH3 domains in some intracellular signaling pathways.

We found by in vitro binding assay that the SBM of UBPy binds to the SH3 domains of Hbp and STAM and also to the C-terminal SH3 domain of Grb2 but not to other SH3 domains examined. We also found that the SBM of AMSH binds to the SH3 domains of Hbp and STAM. These findings suggest that these SH3 domains specifically recognize the novel SBM. Thus, the SBMs of SLP-76 and Gab1 probably mediate association of these proteins with Grb2 through the C-terminal, but not N-terminal, SH3 domain. Amino acid sequence comparison shows that the SH3 domains of Hbp and STAM and the C-terminal SH3 domain of Grb2 constitute three-dimensional structures similar to other SH3 domains, suggesting that the proline-rich consensus sequence PXXP is also recognized by these SH3 domains. In fact, the proline-rich consensus sequences in some proteins bind to the C-terminal SH3 domain of Grb2 (36–38), although such sequences recognized by the SH3 domains of Hbp and STAM have not been identified yet. Although similar three-dimensional structures are suggested, some amino acid residues are characteristic to the SH3 domains of Hbp and STAM and to the C-terminal SH3 domain of Grb2 and are distinct from those in other SH3 domains. The SH3 domain of p53BP2, although it is able to recognize the proline-rich consensus sequences (39), interacts with p53 via a distinct peptide sequence (13). Crystal structural analysis revealed that 3 amino acid residues in the RT loop of the SH3 domain of p53BP2 are critical to recognize the binding site of p53. Tyr<sup>469</sup> makes hydrogen bonds to Asn<sup>247</sup> of p53, and Asp<sup>475</sup> and Glu<sup>476</sup> make charge-stabilized hydrogen bonds to Arg<sup>248</sup> of p53 (13). Similarly, amino acid residues characteristic in the SH3 domains of Hbp and STAM and the C-terminal SH3 domain of Grb2 may contribute to interaction of the SH3 domains with the SBM. Crystal structural analysis will identify critical amino acid residues in the SH3 domains that interact with individual amino acids in the SBMs.

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J. Biol. Chem. 2000, 275:37481-37487.
doi: 10.1074/jbc.M007251200 originally published online September 11, 2000

Access the most updated version of this article at doi: 10.1074/jbc.M007251200

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