Characterization of the WW Domain of Human Yes-associated Protein and Its Polyproline-containing Ligands*

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We had previously identified the WW domain as a novel globular domain that is composed of 38–40 semi-conserved amino acids and is involved in mediating protein-protein interaction. The WW domain is shared by proteins of diverse functions including structural, regulatory, and signaling proteins in yeast, nematode, and mammals. Functionally it is similar to the Src homology 3 domain in that it binds polyproline ligands. By screening a 16-day mouse embryo expression library, we identified two putative ligands of the WW domain of Yes kinase-associated protein which we named WW domain-binding proteins 1 and 2. These proteins interacted with the WW domain via a short proline-rich motif with the consensus sequence of four consecutive prolines followed by a tyrosine. Herein, we report the cDNA cloning and characterization of the human orthologs of WW domain-binding proteins 1 and 2. The products encoded by these cDNA clones represent novel proteins with no known function. Furthermore, these proteins show no homology to each other except for a proline-rich motif. By fluorescence in situ hybridization on human metaphase chromosomes, we mapped the human genes for WW domain-binding proteins 1 and 2 to chromosomes 2p12 and 17q25, respectively. In addition, using site-directed mutagenesis, we determined which residues in the WW domain of Yes kinase-associated protein are critical for binding. Finally, by synthesizing peptides in which the various positions of the four consecutive proline-tyrosine motif and the five surrounding residues were replaced by all possible amino acid residues, we further elucidated the binding requirements of this motif.

The Src homology (SH) 2 and SH3 domains have assumed essential roles in furthering the understanding of how an exo-

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s) U40825 (mouse WBP-1), U40826 (mouse WBP-2), U79457 (human WBP-1), and U79458 (human WBP-2).

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‡ The abbreviations used are: SH, Src homology; YAP, Yes kinase-associated protein; WBP, WW domain-binding protein; PY, motif containing PPPFY sequence; FISH, fluorescence in situ hybridization; DAPI, 4,6-diamidino-2-phenylindole; GST, glutathione S-transferase.
ing region of WBP-1 to a proline-rich motif with the sequence Pro-Pro-Pro-Pro-Tyr (PPPPY), which we chose to call the PY motif, interestingly, the only region of perfect homology between WBP-1 and WBP-2. Binding assays using mutant forms of the PY motif established the preliminary minimal binding consensus as XPPXY (where X signifies any amino acid) required for interaction with the WW domain of YAP. Based on these results and the observation that the PY motif failed to bind arbitrarily to selected SH3 domains, we proposed that the PY motif differed from the binding consensus determined for SH3 ligands.

Although the WW and SH3 domains are functionally similar, their three-dimensional structures are different (for review, see Ref. 11). The WW domain has its NH$_2$ and COOH termini in close juxtaposition, opposite from the ligand binding surface, allowing the modular unit to exist close to the protein surface exposed to the solvent (20). The NMR solution structure solved for the WW domain of YAP consists of a bent three-stranded anti-parallel $\beta$ sheet and a hydrophobic ligand binding pocket composed of leucine 190, tyrosine 188, and tryptophan 199 (the second conserved tryptophan). The two central proline residues of the consensus ligand (XPPXY) contact tryptophan 199 through van der Waals interactions, whereas the tyrosine residue of the PY ligand fits into a hydrophobic pocket of the WW domain formed by leucine 190 and histidine 192.

In this report, we describe the cloning and characterization of the complete cDNA clones for the murine and human orthologs of WBP-1 and WBP-2. Furthermore, the human genes encoding these two proteins were localized to chromosomes 2p12 and 17q25, respectively. To elucidate further the binding properties of the WW domain, we showed that the conservative substitution of the residues tyrosine 188, tryptophan 199, histidine 192, and proline 202 with related amino acids abolished binding, suggesting that these residues are important for binding.

**EXPERIMENTAL PROCEDURES**

cDNA Cloning and Sequencing—Mouse WBP-1 and WBP-2 partial cDNAs (17) were used as probes to screen a $\lambda$ pCEV9 cDNA library derived from M426 human lung fibroblast cells (21) (a gift from Dr. Stuart Aaronson). The low stringency conditions of hybridization were derived from M426 human lung fibroblast cells (21) (a gift from Dr. Frank and co-workers (28, 29). The lymphocytes cultures were treated with bromodeoxyuridine (0.18 mg/ml, Sigma) to synchronize the cell population. The synchronized cells were washed three times with serum-free medium to release the block and cultured at 37 °C for 6 h in minimal essential medium with thymidine (2.5 $\mu$g/ml, Sigma). Cells were harvested, and slides were made using standard procedures including hypotonic treatment, fixation, and air drying.

Chromosomal Localization by Fluorescence in Situ Hybridization (FISH)—The cDNA inserts for WBP-1 and WBP-2 were separately biotinylated with the Life Technologies, Inc. (Gaithersburg, MD) nick labeling kit at 15 °C for 1 h as described elsewhere (24). The procedure for FISH detection was performed as described previously (24, 25). Slides were baked at 55 °C for 1 h. After RNase treatment, the slides were denatured in 70% formamide in 2 × SSC for 2 min at 70 °C followed by dehydration with ethanol. The biotinylated probes were denatured at 75 °C for 5 min in a hybridization solution consisting of 50% formamide and 10% dextran sulfate. The probes were loaded on the denatured chromosomal slides. After an overnight hybridization, the slides were washed and detected as well as amplified. FISH signals and the DAPI banding pattern were recorded separately by photography, and assignment of the FISH mapping data with chromosomal bands was accomplished by superimposing FISH signals with DAPI-banded chromosomes (26).

In Vitro Site-directed Mutagenesis—The cDNA encoding the WW domain of human YAP was subcloned previously in-frame into the vector pGEX-2TK, allowing it to be expressed as a fusion protein with glutathione S-transferase (GST) (Pharmacia Biotech Inc.) (17). This vector in addition allowed the purified fusion protein to be directly labeled with $^{35}$P, at a protein kinase A phosphorylation site in the region between the GST and WW portions, as described elsewhere (17, 18, 27).

Substitution of selected amino acids in the WW domain was achieved using a double-stranded site-directed mutagenesis kit (Pharmacia). The selection primer changed the unique Scal site in the vector to a MluI site, the introduced mutation in boldface (5' -CTTGGACTGTT-GACGCGTCAACCAAGTCT-3'). The target primer was used simultaneously to effect the desired substitutions within the WW domain, the introduced mutation in boldface: (5' -CTTGFACCCGCTTGGTGCTTGCGTTTCCAATCCT-3')

The mAbs were generated against the WW domain of YAP using the “SPOTS” technique of multiple peptide synthesis on derivatized solid supports (20). The mAbs were isolated previously according to the manufacturer’s instructions. Plasmid DNA isolated from individual colonies was purified and sequenced by the Sanger method to confirm each mutation (22).

Coprecipitation and Binding Assays—HeLa cells were grown to confluence in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum in 10-cm plates and lysed in RIPA buffer (10 mM Tris-HCl, pH 7.4, 5 mM EDTA, 150 mM NaCl, 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS, 1% Trasylol, 1 $\mu$mol leupeptin, 1 $\mu$mol antipain, 1 $\mu$mol sodium vanadate). GST or GST-WW-YAP (100 $\mu$g) bound to glutathione-agarose was incubated with 200 mg of the cell lysate which was diluted 10-fold in Tris/Tween buffer (50 mM Tris-HCl, pH 7.5, 100 mM NaCl, 1 mM EDTA, 0.1% Tween 20, 1% ovalbumin, 1 mM dithiothreitol) at 4 °C overnight. The complexes were then analyzed by Western ligand blotting using $^{35}$P-GST-WW-YAP (100 $\mu$g) as a probe as described elsewhere (17). Binding assays with the bacterially expressed WW-YAP protein was performed by western ligand blotting using $^{35}$P-GST-GTP-PPPYYTVG protein as a probe (17).

**SPOTs Method of Peptide Synthesis on Derivatized Cellulose**—The peptide synthesis was accomplished following the method described by Frank and co-workers (28, 29). Fmoc (N-(9-fluorenylethoxycarbonyl) tert-amino acids, derivatized cellulose membranes, instruction manual
and computer software SPOTs, Release 1.0, for generating various schedules of amino acid applications were all purchased from Genosys Biotechnologies, Inc. Phosphorylated serine, threonine, and tyrosine were obtained from Chem Impex International. The membranes were incubated with the 32P-labeled GST-WW domain protein of human YAP under the conditions described previously (17), except the 5% milk in the blocking solution was replaced with 1/3 blocking buffer supplied (as 103 solution) by the manufacturer. After probing the membranes with 32P-labeled WW proteins, the membranes were regenerated with urea, mercaptoethanol, and acetic acid-containing buffers (as described by the manufacturer), and stained with Coomassie Blue for 10 min and destained using standard protocol for visualizing proteins on SDS-polyacrylamide gels. Uniform light blue staining was observed for the peptides with the exception of those containing Lys, Arg, or His, which were always stained more intensely with Coomassie Blue.

RESULTS

Cloning of WBP-1 and WBP-2—We described previously the isolation of partial clones for murine WBP-1 and WBP-2 which were each missing part of the 5'-coding regions (17). In this paper, the remainder of the murine cDNA encoding these proteins was determined. Additionally, the human orthologs of WBP-1 and WBP-2 were isolated by screening a human cDNA library (M426 lung fibroblasts) under low stringency conditions with the corresponding murine cDNA probes. Human and murine WBP-1 share an 84% sequence identity (Fig. 1A), whereas human and murine WBP-2 are 94% identical (Fig. 1B), not considering those residues that are biochemically similar. The WBP-1 and WBP-2 sequences are dissimilar except for the conserved PY motif. Data-base searches using the FASTA program again did not reveal any known proteins with significant homology to WBP-1. However, human WBP-2 shared 26 and 29% homology with two proteins in Caenorhabditis elegans (unnamed, with GenBank accession numbers U40413 and Z47808) respectively, as predicted from the open reading frames. These two C. elegans proteins have yet to be characterized. Interestingly, the most extensive region of homology between WBP-2 and the C. elegans proteins contains the PY motif (marked by an asterisk). The PY motif in the C. elegans proteins lacks a proline in the first position but still conforms to the XPPXY consensus that has been established as the minimal sequence that can bind to the WW domain of YAP. Additionally, another region (marked by two asterisks) contains a sequence that has the XPPXY consensus. The above sequence comparison implies that these regions have been relatively well conserved through evolution because of their functional significance, presumably in mediating protein-protein interactions.

Chromosomal Localization of WBP-1 and WBP-2—Under the conditions described, the hybridization efficiency for the WBP-1 cDNA probe was approximately 81% (among 100 mitotic figures examined, 81 showed signals on one pair of chromosomes). Similarly, the efficiency for the WBP-2 cDNA probe was approximately 76%. The detailed positions of the loci were determined further based on the summary from 10 FISH signal photographs/probe; representative hybridization pictures are shown in Fig. 2, A and D. By superimposing the signals obtained from FISH onto a representation of DAPI-banded chromosomes, we localized the WBP-1 locus to chromosome 2p12 and the WBP-2 locus to chromosome 17q25 (Fig. 2, C and F).
Some hybridization of the WBP-1 cDNA occurred at chromosome 2p12 as well, but this signal was considerably weaker compared with hybridization at region p12.

Filter Binding Assays with Mutant WW Domains—To determine the residues of the WW domain which are important for binding, we performed in vitro site-directed mutagenesis of selected residues in the WW domain fused to GST (Fig. 3). The most conserved residues in the domain were chosen, including Trp^{177}, Tyr^{188}, Phe^{199}, His^{202}, and Pro^{202}. Since we were interested in elucidating the residues directly involved in forming the binding surface and contacting the ligand, we replaced the above residues with amino acids similar in structure and size. Replacing with amino acids too dissimilar in size would have risked disturbing the natural tertiary structure of the WW fold. We conducted filter binding assays in which these GST-WW mutant proteins were transferred to a nitrocellulose membrane. The blots were then probed with ^32P-labeled GST-WW (wild type) protein. 

Binding of the WW Mutants in Solution—To determine the residues of the WW domain that was subsequently probed with ^32P-labeled GST-PY protein. The WW mutants Y188F, H192F, P202A, and W199F failed to bind to the ligand probe (Fig. 4A). However, the constructs W177F and F189Y were still able to bind to the ligand, and the former construct may bind at a slightly higher affinity than even the wild type GST-WW protein (Fig. 4A). Thus, according to these results, the residues Tyr^{188}, His^{192}, Trp^{199}, and Pro^{202} seem to be involved in establishing the binding surface of the WW domain. Alternatively, some of these residues may be critical for maintaining the three-dimensional structure of the binding site on the nitrocellulose membrane, should a renatured form of the GST-WW protein be required to retain binding function.

Mutational Analysis of the PY Motif—Each position of the target ligand, Gly^{1}-Thr^{2}-Pro^{3}-Pro^{4}-Pro^{5}-Pro^{6}-Tyr^{7}-Thr^{8}-Val^{9}, Gly^{10}, corresponding to residues 170–179 of the mouse WBP-1 sequence, was substituted with all possible amino acids using SPOTs peptide synthesis to determine the binding requirements of the YAP WW domain ligand. Certain points can be
inferred from the results of the SPOTs analysis: The replacement of the Pro4 residue with leucine, serine, valine, or alanine resulted in weak binders to the WW domain of YAP. However, substitution with other amino acids in this position abolished binding completely, in agreement with the consensus established earlier (Fig. 5). When the Pro5 position was replaced with a tyrosine (Fig. 6, spot 20), the peptide bound with moderate affinity. All other substitutions at Pro5 were negative. Tyr7 could not be replaced with phenylalanine, tryptophan, histidine, or with any other amino acid without disrupting binding activity. Also, a target peptide containing a phospho-

FIG. 5. Mutational analysis of the Gly1-Thr2-Pro3-Pro4-Pro5-Pro6-Tyr7-Thr8-Val9-Gly10 target peptide that binds to the WW domain of human YAP. The SPOTs technique was used to generate a repertoire of 10-mer peptides (28, 29). Pro3, Pro4, and Pro6 were replaced consecutively with the remaining 19 amino acids. Peptide 1 is the parent sequence. Peptides 2–20 correspond to all of the Pro3 substitutions, and peptides 40–58 correspond to Pro1 substitutions. Tyr7 was replaced by phosphotyrosine, serine, phosphoserine, threonine, and phosphothreonine (a–f), and we also assayed these peptides with reverse sequence (g–l). For blotting 3P-labeled GST-WW domain of human YAP was used. Panel A, autoradiogram of the membrane exposed for 5 min; panel B, for 15 min; panel C, orientation of the derivatized spots on which peptides were synthesized. Panel D, individual sequences of the peptides corresponding to numbered spots. Controls indicated derivatized spots onto which amino acids were not applied. There was no binding detected when the blot was probed with 3P-labeled GST protein and exposed for 5 or 15 min.

FIG. 6. Mutational analysis of the Gly1-Thr2-Pro3-Pro4-Pro5-Pro6-Tyr7-Thr8-Val9-Gly10 target peptide that binds to the WW domain of YAP. The SPOTs technique was used to generate a repertoire of 10-mer peptides (28, 29). Pro3, Pro4, and Pro6 were replaced consecutively with the remaining 19 amino acids. Peptide 1 is the parent sequence. Peptides 2–20 correspond to all of the Pro3 substitutions, and peptides 40–58 correspond to Pro1 substitutions. Tyr7 was replaced by phosphotyrosine, serine, phosphoserine, threonine, and phosphothreonine (a–f), and we also assayed these peptides with reverse sequence (g–l). For blotting 3P-labeled GST-WW domain of human YAP was used. Panel A, autoradiogram of the membrane exposed for 5 min; panel B, for 15 min; panel C, orientation of the derivatized spots on which peptides were synthesized. Panel D, individual sequences of the peptides corresponding to numbered spots. Controls indicated derivatized spots onto which amino acids were not applied.

Although the lack of perfect normalization of the SPOTs membrane because of difficulties in precise measurements of the amount of peptide in each individual spot precludes a rigorous quantitation of the binding affinities, certain conclusions can be drawn. Based on the Coomassie Blue staining of...
We assume that there is no significant difference in the amount of peptide between different spots. From the data shown in Figs. 5–7 we can observe that the replacement of Pro3, Pro6, Val9, and Gly10 with lysine, and Thr8 with proline increase the interaction with the GST-WW-YAP domain. Cysteine at position Gly1 and a cysteine or proline at position Thr2 seems to increase the binding slightly. In contrast, an isoleucine at position Pro3 and acidic residues at position Pro6, Thr8, Val9, or Gly10 reduce the interaction with the WW domain. To prove these conclusions, however, selected peptides would have to be synthesized, and the $K_d$ value of their interaction with the WW domain, in solution and on solid supports, would have to be determined. Preliminary results obtained with an isothermal titration microcalorimeter indicate that peptide analogs containing lysine and proline substitutions at the carboxyl-terminal end of the parent peptide show at least 5-fold lower binding constants compared with the $K_d$ of the parent peptide binding to the WW domain of YAP.2

**DISCUSSION**

We have cloned the human homologs of the ligands to the WW domain of YAP, WBP-1 and WBP-2. These proteins allowed us to categorize the WW motif as a novel polyproline-binding module (17). The amino acid sequences of WBP-1 and WBP-2 have been well conserved through evolution, considering the high degree of homology between mouse and human orthologs. The degree of homology shared by the orthologs, especially in the region of the PY motif, suggests that the function of these proteins has been conserved between species. The PY motif, a region mediating protein interaction, is perfectly conserved between the mouse and human forms of WBP-1 and WBP-2 and represents the most conserved region shared between WBP-2 and two C. elegans proteins. This further supports the hypothesis that the biological function of these proteins depends on their ability to participate in protein interactions.

Using the cDNA of these clones, we localized the genes encoding human WBP-1 and WBP-2 to chromosomes 2p12 and 17q25, respectively. The chromosome region 2p12 has been shown to be potentially involved in several disorders. Chromosomal abnormalities are found in the majority of cases of non-Hodgkin’s lymphoma, a subset of which shows a translocation involving the regions 3q27 and 2p12 (30). In addition, the gene for familial juvenile nephronophthisis, a leading genetic cause of juvenile end-stage renal failure, has been narrowed to the region 2p12 based on human linkage analysis (31). However, more work must be done to determine if WBP-1 can be implicated in these human conditions.

The chromosome region 17q25 has been shown to be involved in certain forms of human carcinogenesis. Some cases of alveolar soft part sarcoma show a consistent abnormality of 17q25 (32). However, the region of 17q25, also occasionally translocated in chronic myelogenous leukemia, has not yet been associated with any known oncogenes (33). Additional genetic and biochemical studies would need to be performed to demonstrate the importance of WBP-2 as a potential oncogene in the etiology of these human cancers.

Using site-directed mutagenesis we have implicated specific residues in the WW domain of YAP which are important to maintain its ability to bind to the PY motif. Our results complement the recent data on the NMR solution structure of the WW domain of YAP in complex with the proline-rich ligand (20). The three-dimensional structure consists of a three-stranded, antiparallel $\beta$ sheet with residues Tyr188 and Trp199 (the second conserved tryptophan) on the concave side and residues Phe189 and Trp177 on the convex side. The concave aspect contains an almost flat hydrophobic binding surface.

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2 M. Lemmon and M. Sudol, unpublished data.
represented by the residues Tyr\textsuperscript{188}, Trp\textsuperscript{199}, His\textsuperscript{192}, and Leu\textsuperscript{190}. Moreover, the NH\textsubscript{2} and COOH termini of the domain appose each other on the convex side in the manner of a hydrophobic buckle which presumably helps maintain the stable folded structure of the domain. This buckle forms when Pro\textsuperscript{174} and Pro\textsuperscript{202} pack against Trp\textsuperscript{177}, the interaction of which is further stabilized by the presence of Ile\textsuperscript{167}. These results from Macias and co-workers (20) support the binding consensus of the PY ligand established earlier as XPPX (17). Moreover, the mutagenesis results presented here are for the most part in agreement with the NMR structure. Conservative substitution of the residues Tyr\textsuperscript{188}, His\textsuperscript{192}, Trp\textsuperscript{199}, and Pro\textsuperscript{202} abrogated the binding function of the WW domain in our filter binding assays, implying in light of the NMR data that the hydrophobic binding surface is disrupted by the first three mutations or that the fold of the WW domain is severely compromised by the Pro\textsuperscript{202} substitution. Nuclear Overhauser effects between His\textsuperscript{192} and ligand residues Tyr\textsuperscript{7} and Val\textsuperscript{9}, Trp\textsuperscript{199}, and Pro\textsuperscript{6}, and between Tyr\textsuperscript{188} and the carbonyl group of Pro\textsuperscript{6} provide corroborating evidence for the importance of these residues. The replacement of the histidine residue at position 192 with a phenylalanine suggests the participation of a hydrogen bond located between the imidazole nitrogen acceptor of histidine and the hydroxyl hydrogen donor of Tyr\textsuperscript{7} (Fig. 8). Elimination of this hydrogen bond likely destabilizes this structure, as does the introduction of the bulkier phenylalanine residue into such a confined space. Lack of ligand binding by the W199F and P202A mutants are not manifested through variations in charges or hydrogen bonds but likely reflect alteration in the architecture of the surface topology and changes in side chain packing. The removal of tryptophan at position 199 and replacement with a phenylalanine may eliminate enough of the surface recognition site to inhibit ligand binding. Alternatively, the newly introduced phenylalanine residue may not pack efficiently into the cavity designated by Pro\textsuperscript{4} and Pro\textsuperscript{5} (Fig. 8). Likewise, molecular van der Waals packing interactions are central to the P202A mutation. The incorporation of an alanine diminishes this tight hydrophobic packing which may ultimately destroy the molecular "fastener" thought to be responsible for maintaining the integrity of the core domain \(\beta\) sheet (Fig. 8). One should point out that the stability of the WW domain could be considered marginal (20) because the domain is only one \(\beta\) sheet layer thick and thus may not have a well defined hydrophobic core.

In contrast, mutations of Trp\textsuperscript{177} or Phe\textsuperscript{189} had no observable effect in the binding assays, suggesting that structural changes possibly imparted by these substitutions were insignificant to the overall function of the domain. The replacement of Tyr\textsuperscript{188}
with phenylalanine failed to attenuate the binding ability of the domain in solution, as illustrated by the coprecipitation assays (Fig. 4B). Based upon NMR data, the hydroxyl proton of this tyrosine residue is within hydrogen bonding distance of the carbonyl oxygen of ligand residue Pro6 (Fig. 8, A and B). The ligand binding studies presented within suggest that such an interaction is not required to manifest ligand binding in solution. Comparison of the mutant Y188F structure with that of the wild type does not reveal any significant structural distinction. We also cannot rule out the possibility that another portion of the domain may compensate for or “mask” this mutation, depending on the conformation of the domain while bound to nitrocellulose membrane compared with its state in solution.

Mutational analysis of the target peptide (GTPPPPPTVGG) for the WW domain of human YAP led us to the following conclusions. The data confirm our preliminary consensus Xaa-Pro4-Pro5-Xaa-Tyr7 generated by the “alanine scan” mutagenesis (17). In addition, these results are in agreement with the structure of the WW domain of human YAP in complex with the target peptide (20). The PY motif ligand (peptide GTPPPP-PYTVGG) fits into the binding surface in the form of a proline-rich type II helix. The Pro4 and Pro5 of the ligand are involved in the binding interface by forming van der Waals contacts with the second conserved Trp199. Moreover, the Tyr1 of the ligand peptide is accommodated by a hydrophobic surface containing conserved residues including Leu190 and His192 in the WW domain. Interestingly, mutations P5L and Y7H in the PY motif are analogous to those found in the amiloride-sensitive sodium channel β subunit of two Liddle’s syndrome patients (34, 35). The proline-rich motif of amiloride-sensitive sodium channel is a target of deletions and point mutations resulting in Liddle’s syndrome of hypertension. It is likely that the two mutations P616L and Y618H (Refs. 34 and 35), which are analogous to P5L and Y7H, result in the complete lack of binding to the WW domain of Nedd4. In addition, the fact that the WW domain does not bind to a target peptide with the reverse sequence provides suggestive evidence that the YPPPP motif in the WBP-2 protein (amino acids 179–183 of human WBP-2) may be nonfunctional in terms of binding to the WW domain of YAP.

The WW domain appears to be involved in various physiological and pathophysiological processes. The disruption of the WW-PY interaction may in fact be the molecular deficiency present in a form of inherited hypertension known as Liddle’s syndrome (36–39). Furthermore, dystrophin, a protein implicated in Duchenne’s and Becker’s muscular dystrophy, forms a stable complex via its WW domain to a proline-rich motif in β-dystroglycan in vitro (40). In addition, the Gag protein of retroviruses contains a PY motif that when mutated severely curtails the ability of the virus to bud from the host cell membrane (40–43).

Because of the relatively small size of the PY motif, low molecular weight mimotopes may be designed to fit into the binding pocket of the WW domain, to interfere with the viral life cycle, for example. Additional work on the WW domain will provide answers to fundamental biological questions and will ultimately lead to the development of therapeutic applications.

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