WW domains

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WW domains are recently described protein–protein interaction modules; they bind to proline-rich sequences that usually also contain a tyrosine. These domains have been detected in several unrelated proteins, often alongside other domains. Recent studies suggest that WW domains in specific proteins may play a role in diseases such as hypertension or muscular dystrophy.

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The WW domain, also known as the WWP or Rsp5 domain [1–3], is a ~40 amino acid protein module recently identified in a variety of unrelated proteins (for example, dystrophin, Nedd4/Rsp5, YAP65) and is often found alongside other known domains (Fig. 1). It therefore joins a family of domains that includes SH2, SH3, PH/PTB/PID, C2 and other protein–protein or protein–lipid interaction modules (for a review, see [4,5]). The domain contains two highly conserved tryptophans and an invariant proline (Fig. 2), hence the name WW (WWP) domain. WW domains are often present in multiple copies in a protein (for example, in Rsp5/Nedd4 or in the mouse YAP65; Fig. 1), but sequence similarity, in some cases, is greater between WW domains of different proteins than between WW domains within the same protein [6]. This suggests a unique role for individual WW domains when present in multiple copies. Although classification of the various known WW domains is preliminary, phylogenetic distance analysis [6] and recent binding studies (see below) suggest that the Nedd4 and YAP65 WW domains belong to a different subclass from the dystrophin WW domain. Little is known about the function of WW domains, but recent evidence described below implicates this domain as a protein–protein interaction module which functions analogous to, but distinct from, SH3 domains.

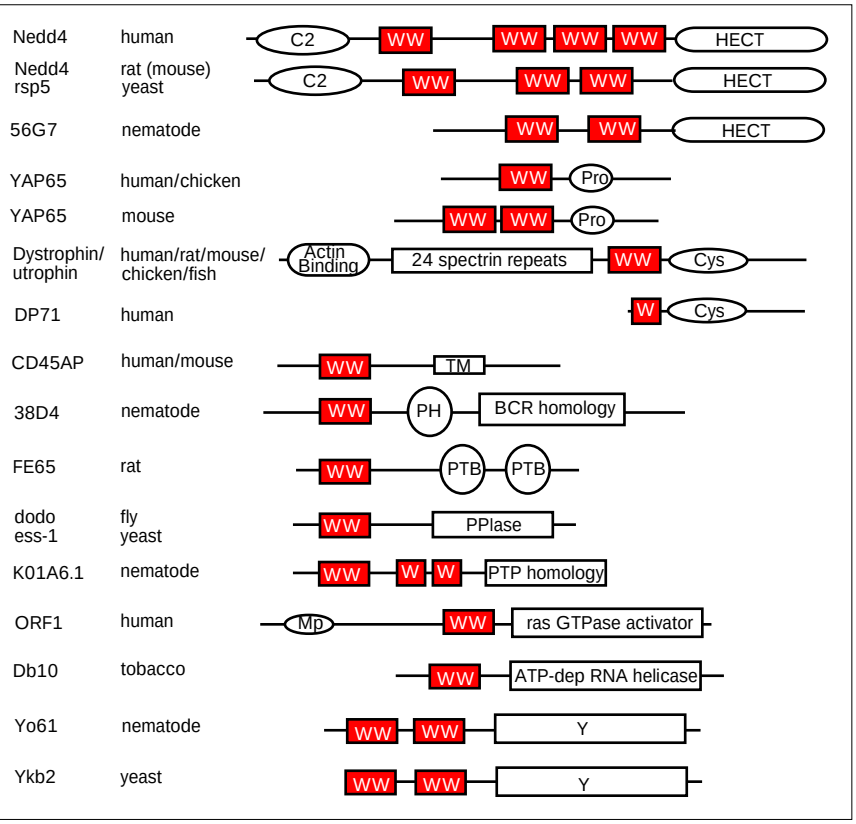
WW ligands: the PY motif

Chen and Sudol [7] have recently demonstrated *in vitro* association of the YAP65 (yes-associated protein) WW domain with short proline-rich repeats also containing a tyrosine (PPPPY) in the proteins WBP1 and WBP2. They then proposed the PY motif (xPPxY, where x is any amino acid) as a preliminary consensus for WW domain binding. Similar proline-rich PY motifs recently identified in the epithelial Na^+ channel (ENaC) [8,9] were shown to associate *in vitro* and in living cells with the WW domains of Nedd4 [8]. In the latter, each of the three subunits of the channel (α, β and γ, ENaC) contains a single PY motif located at the C terminus (PPPAY, PPPNY, PPP(R/K)Y, for α, β and γ ENaC, respectively). The biological significance of these ENaC–Nedd4 interactions is discussed below. PY motifs have been identified in many other unrelated proteins, such as viral gag proteins, interleukin receptors and several serine/threonine kinases [10], but the significance of these occurrences has still to be determined. The PY motif differs from SH3-binding (xPpxP, where p is usually also a Pro) motifs [4,11], and accordingly, preliminary *in vitro* binding studies indicate it does not bind SH3-domains [7,8]. However, exceptions may exist, as the proline-rich region in αENaC, which includes a PY motif, is able to bind both SH3 and WW domains [8,12]. Moreover, the proline-rich SH3-binding region of formin, which does not contain a PY motif, was recently demonstrated to bind both SH3 and WW domains [13]. Determination of specificity of WW binding is rudimentary. It appears, though, that unlike the WW domains of Nedd4 and YAP65, which can bind *in vitro* to the PY motifs in the sequence GTPPPxY of the Na^+ channel (β or γ ENaC) or WBP1, the dystrophin WW domain is unable to bind a similar sequence ([7]; OS and DR, unpublished data). Instead, it may bind another PY-containing peptide (see below).

Structure of WW domains

The primary structure of WW domains has not been published yet. However, preliminary NMR structure information obtained on the first WW domain of the murine YAP65 (H Oschkinat, personal communication) indicates that the domain is a compact module composed essentially of a three-stranded β sheet (β1–β3), with a turn separating each β strand, and strands β1 and β2 longer than β3. The N and C termini of the WW domain are close to each other, as is typical of a modular domain. The highly conserved residues of the domain are hydrophobic, and mostly structural. These include the first highly conserved tryptophan (located in β1), the phenylalanine/tyrosine (in β2), and the invariant proline located in the β3 loop (Fig. 2). The binding surface, composed of segments of the β1 and β3 strands, is shallow and includes the highly conserved tyrosine from β2 and the second invariant tryptophan from β3, both protruding into the binding plane. Interestingly, although the overall structure of the WW domain does not resemble that of SH3 domains, the binding surfaces of both include residues, such as tryptophan and tyrosine, ([11]; H Oschkinat, personal communication) that can form

Figure 1



Schematic representation of several proteins containing WW domains (red). A single boxed W (e.g. in DP71 and K01A6.1) represents a portion of the domain containing either the first or second conserved tryptophan. The C2 domain (a domain known to mediate Ca^{2+}-dependent association with phospholipids/membranes) is found in Nedd4/Rsp5 and also in PKC, PLA_2, PLC, rasGAP, synaptotagmin I and other proteins. The HECT domain in Nedd4/Rsp5 and 56G7 is a ubiquitin ligase (E3) enzyme also present in E6-AP, the yeast ykl162, rat p100 and UreB1. Pro represents the proline-rich (SH3 binding) region; Cys, cysteine rich region; TM, transmembrane domain of CD45 associated protein (CD45AP); PH, pleckstrin homology domain also found in dynamin, SOS, PLCγ, IRS-1, rasGAP and Btk; BCR (breakpoint cluster region) homology domain, also shared by p85 of PI-3 kinase, rhoGAP and n-chimerin; PTB/PID domains, recently suggested to be a subclass of PH domains, are also present in Shc, numb and IRS-1; PPIase, peptidylpropyl *cis-trans* isomerase, known to associate with transcription factors; PTP, homologous to protein tyrosine phosphatases. Actin binding (CH, calponin homology) domain is homologous to calponin, actinin, vav and spectrin. In human ORF1 (IQGAP1), the Mp domain is homologous to the fly muscle protein mp20, and the GTPase activator is a rasGAP domain. The Y domain, shared by Yo61 and Ykb2, has no known function. Sizes of all proteins and domains are not to scale.

hydrophobic interactions with prolines. Future peptide library screens, mutation analysis and structure determination of WW domains from unrelated proteins (e.g. dystrophin, formin-binding proteins) should help in the elucidation of the differences in binding specificity between the different WW domains.

Function of WW domains

As the WW domains were first described only recently, identification of their role in the various proteins which harbour them is in its infancy. Nevertheless, exciting clues are emerging, some related to human genetic disorders such as Liddle's syndrome or muscular dystrophy.

Liddle's syndrome is a hereditary form of systemic renal hypertension. It is characterized by increased Na^+ absorption in the distal nephron [14], which is caused by increased activity of the epithelial Na^+ channel [15]. Recent genetic linkage analyses have demonstrated that the disease is caused by effective deletion of regions within the C termini of β [16] or γ [17] ENaC, invariably causing loss of the PY motifs in these subunits. Such deletions lead to increased activity of the channel, which is probably a result of an increased number of active channels at the plasma membrane [9,15,18]. We have recently identified Nedd4 (NPC expressed developmentally downregulated) as the binding partner for ENaC. Nedd4 [19] contains a C2 domain, 3 (or 4 in the human) WW domains, and a ubiquitin-ligase HECT (homology to the E6-AP C terminus) domain (Fig. 1). ENaC–Nedd4 interaction is mediated by the WW domains of Nedd4 which bind to the PY motifs of α, β and γ ENaC [8]. Mutations within the PY motif of β ENaC have been recently identified in Liddle's patients [20,21]. These were shown to cause increased channel activity [9] and to lead to abrogation of Nedd4–WW binding [8]. As Nedd4 contains a ubiquitin-ligase domain, we speculate that this protein may be a suppressor of the epithelial Na^+ channel; in Liddle's syndrome patients, in which Nedd4-binding sites (PY motifs) in the channel subunits are lost, channel ubiquitination and degradation may be impaired, resulting in an increased number of active channels at the plasma membrane. It is interesting that a similar role, involving regulation of the number of transporters (permeases) at the plasma membrane, was recently proposed for the yeast homologue of Nedd4, Rsp5/Npi1 [22].

RSP5, an essential gene in yeast [22], was originally identified as a suppressor of mutations in the *SPT3* gene, a transcription factor interacting with the TATA-binding protein TFIID [23]; these genetic interactions are likely to be indirect as *RSP5* mutations suppress a deletion in *SPT3*. In

Figure 2

Sequence alignment of WW domains from different proteins and the suggested WW consensus sequence. Invariant residues are in bold letters. Conserved residues in the consensus sequence are in capital letters; h represents hydrophobic residues; t corresponds to polar or charged (turn-like) residues. Boxes under the consensus sequence indicate location of the β1, β2, and β3 strands of the WW domain, determined by H Oschkinat and colleagues (H Oschkinat, personal communication). Sequences shown at the bottom of the figure are of putative WW domains in which not all of the invariant amino acids are conserved, or the domain is split in two. Dots represent spaces introduced to maximize homology. Dashes (in DP71) represent an unrelated amino acid sequence. In the formin-binding proteins FBP11, 21, 23, 28 and 30, sequences surrounding the indicated amino acids [13] have not been provided.

Protein/Species	Position	Sequences of WW domains	Accession number
Nedd4/rat-1	251	PSPLPPGWEERQDVL.GRTYYVNHE........SRTTQWKRPSPEDDLT	U50842
Nedd4/rat-2	407	SSGLPPGWEEKQDDR.GRSYYVDHN........SKTTTWSKPTMQDDPR	U50842
Nedd4/rat-3	464	LGPLPPGWEERTHTD.GRVFFINHN........IKKTQWEDPRMQNVAI	U50842
Nedd4/mouse-1	39	PSPLPPGWEERQDVL.GRTYYVNHE........SRRTQWKRPSPDDDLT	P46935
Nedd4/mouse-2	195	SSGLPPGWEEKQDDR.GRSYYVDHN........SKTTTWSKPTMQDDPR	P46935
Nedd4/mouse-3	250	LGPLPPGWEERTHTD.GRVFFINHN........IKKTQWEDPRLQNVAI	P46935
Nedd4/human-1	217	PSPLPPGWEERQDIL.GRTYYVNHE........SRRTQWKRPTPQDNLT	P46934
Nedd4/human-2	374	SSGLPPGWEEKQDER.GRSYYVDHN........SRTTTWTKPTVQATVE	P46934
Nedd4/human-3	447	QGFLPKGWEVRHAPN.GRPFFIDHN........TKTTTWEDPRLKIPAH	P46934
Nedd4/human-4	499	LGPLPPGWEERTHTD.GRIFYINHN........IKRTQWEDPRLENVAI	P46934
Rsp5/yeast-1	228	YGRLPPGWERRTDNF.GRTYYVDHN........TRTTTWKRPTLDQTEA	P39940
Rsp5/yeast-2	330	LGELPSGWEQRFTPE.GRAYFVDHN........TRTTTWVDPRRQQYIR	P39940
Rsp5/yeast-3	386	LGPLPSGWEMRLTNT.ARVYFVDHN........TKTTTWDDPRLPSSLD	P39940
Yap/mouse-1	155	DVPLPAGWEMAKTSS.GQRYFLNHN........DQTTTWQDPRKAMLSQ	P46938
Yap/mouse-2	214	SGPLPDGWEQAMTQD.GEVYYINHK........NKTTSWLDPRLDPRFA	P46938
Yap/human	170	DVPLPAGWEMAKTSS.GQRYFLNHI........DQTTTWQDPRKAMLSQ	P46937
Yap/chicken	168	DVPLPPGWEMAKTPS.GQRYFLNHI........DQTTTWQDPRKAMLSQ	P46936
cl.3544mRNA/mouse	21	TVILPPGWHSYLSPQ.GRRYYVNTT........TNETTWERPSSSPGIS	U19860
F13E6.4/*C.elegans*	217	QLPMPQGWEMCYDSD.GVRYFKDHN........SKTTTWDDPRLKQQEQ	Z68105
Dodo/drosophila	4	AEQLPDGWEKRTSRSTGMSYYLNMY........TKESQWDQPTEPAKKA	U35140
Ess1/yeast	29	STGLPTPWTARYSKSKKREYFFNPE........TKHSQWEEPEGTNKDQ	P22696
Msb1/human	249	IVLPPNWKTARDPE.GKIYYYHVI........TRQTQWDPPTWESPG	?
ORF1/human	679	VGDNNSKWVKHWVKG.GYYYYHNLE........TQEGGWDEPPNFVQN	D29640
K01A6.1/*C.elegans*-1	130	EGLLPPNWETAYTEN.GDKYFIDHN........TGTTTWDDPRELPPGW	Z68750
Fe65/rat	11	DSDLPAGWMRVQDTS.GT.YYWHIP........TGTTQWEPPGRASPSQ	X60468
Ykb2/yeast-1	1	MSIWKEAKDAS.GRIYYYNTL........TKKSTWEKPKELISQE	P33203
Ykb2/yeast-2	38	LLLRENGWKAAKTAD.GKVYYYNPT........TRETSWTIPAFEKKVE	P33203
Dystrophin/human	3954	STSVQGPWERAISPN.KVPYYINHE........TQTTCWDHPKMTELYQ	P11532
DP71/human	8	-------HE........TQTTCWDHPKMTELYQ	A45255
Utrophin/human	2811	STSVQLPWQRSISHN.KVPYYINHQ........TQTTCWDHPKMTELF	P46939
C38D4.5/*C.elegans*	95	RRDLLNGWFEYETDV.GRTFFFNKE........TGKSQWIPPRFIRTPA	P46941
P9659.21/yeast	1	MRGEWQEFKTPA.GKKYYYNKN........TKQSRWEKPNLKKGSN	U40829
Yo61/*C.elegans*-1	77	SPSVESDWSVHTNEK.GTPYYHNRV........TKQTSWIKPDVLKTPL	P34600
Yo61/*C.elegans*-2	123	QPQQGQWKEFMSDD.GKPYYYNTL........TKKTQWEPPGRASPS	P34600
Yfx1/yeast	8	PPQVPSGWKAVFDDEYQTWYYVDLS........TNSSQWEPPRGTTWPR	P43582
ZK1248.15/*C.elegans*	198	TENVSPPWKAWWHTEKKRKFYTNDK........TKESLWDHPNTRKNEE	U29244
K015c11/*C.elegans*	?	QNPDDAWNEFNAPD.GRKYYFNSI........TQENTWEKPKALIDQE	D34959
56G7/*C.elegans*-1	228	QTPPESHWKTYLDAK.KRKFYVNHV........TKETRWTKPDTLNNNH	Z46793
SPAC13C5.02/*S.pombe*-1	89	RIPNNDSWVVVFTKK.NRYFFHNLK........SHESYWEPPLEISKDLK	Q09685
CD45AP/human	48	TGLALAWRR.LSRD.SGGYYHPARLGAALWGRTRRLLWASPPGRWL	A55412
CD45AP/mouse	48	TALALAWCR.LSHA.SGGYYHPARLGAALWGHTRRLLWASPAGRWL	A49957
FBP11/mouse-1	?	WTEHKSPD GRTYYYNTE........TKQSTWEKP	?
FBP11/mouse-2	?	WKEYKSDS.GKPYYYNSQ........TKESRWAKP	?
FBP21/mouse-1	?	WVEGVTAD.GHCYYYDLI........TGASQWEKP	?
FBP21/mouse-2	?	WVEGLSED.GYTYYYNTE........TGESKWEKP	?
FBP23/mouse	?	WVENKTPD.GKVYYYNAR........TRESAWTKP	?
FBP28/mouse	?	WTEYKTAD.GKTYYYNNR........TLESTWEKP	?
FBP30/mouse	?	WQEVWDEN.TGCYYYWNTQ.......TNEVTWELP	?
Consensus:		LPtGWE ttt Gt YYhNH. TtTTtW tPt t V FF D S S	
β strands:		β1 β2 β3	
Db10/Tobacco	8	PTLPKPWKGLVDGTTGFIYFWNPE........TNDTQYERPVPSSHA	D16247
56G7/*C.elegans*-2	372	TQPLPSGWE.CITMN.NRTVFLNHA........NKETSFYDPRIRRFET	Z46793
SPAC13C5.02/*S.pombe*-2	2	SQPLPPGWTEHKAPS.GIPYYWNAE........LKKSTYQRPSFIEKNH	Q09685
YJL168c/yeast	473	RVRLPPGWEIIHENGRP.LYYNAEQ........KTKLHY.PPSGSSKVF	P46995
K01A6.1/*C.elegans*-2	161	SEGLPPGWEQVDDQNYG.TFYV	Z68750
	270	NHI........NRKTQYERPYGFGGSS	Z68750

Saccharomyces cerevisiae, growth in the presence of a good nitrogen source (NH_4^+, glutamine) causes inactivation and degradation of the general amino acid permease Gap1 [24]. The degradation step is abrogated in *RSP5*/*NPI1* mutant cells [22]. Moreover, a functional *RSP5*/*NPI1* gene is also required for stress-induced degradation of the uracil permease Fur4 [22], a process which involves ubiquitination and endocytosis of the permease, with subsequent degradation in the vacuoles [25]. This provides an attractive model in which Rsp5/Npi1 may be involved in permease endocytosis, perhaps via its C2 domain, in an analogy to one of the C2 domains of synaptotagmin I previously suggested to play a role in endocytosis [26]. Subsequent ubiquitination of the internalized permease protein could be mediated by the HECT domain of Rsp5/Npi1, as speculated above for the epithelial Na^+ channel. Indeed, the Rsp5 [27] and Nedd4 HECT domains were recently shown to form a thioester bond with ubiquitin (P Howley, personal communication), implicating these proteins as functional ubiquitin ligases. However, unlike ENaC, neither Gap1 nor Fur4 contain a 'classic' PY motif [28,29], suggesting that either Rsp5/Npi1 does not interact directly with these permeases, or that direct interaction occur via regions other than the Rsp5 WW domains.

Dystrophin is a large protein absent/mutated in muscles from patients with Duchenne or Becker muscular dystrophy [30]. Its basic structure can be divided into five regions: an N-terminal actin binding domain, a spectrin-like domain, a single WW domain, a cysteine-rich domain and the C-terminal domain (Fig. 1). Dystrophin, located at the plasma membrane of skeletal muscles, forms a

complex with several extracellular, transmembrane and intracellular proteins, including a group of transmembrane glycoproteins believed to provide a link between the extracellular matrix and the actin cytoskeleton. Among these is the glycoprotein β-dystroglycan. β-dystroglycan contains several proline-rich regions in its C terminus [31], including two PY motifs (PLPPPEYP and PYRSPP-PYVPP). A recent *in vitro* study [32] has demonstrated that binding between dystrophin and β-dystroglycan is mediated by the region encompassing amino acids 3054–3271 (which includes the WW domain and the cysteine-rich domain) of dystrophin and the C-terminal final 15 amino acids of β-dystroglycan (KNMTPYRSPP-PYVPP), which include the above PY motif. These results suggest that interaction between dystrophin and β-dystroglycan is mediated at least in part by the WW domain of dystrophin and the second PY motif of β-dystroglycan. Deletions/mutations within the 3′ region of dystrophin have been associated with severe forms of muscular dystrophy, although it is not clear that any of them map to the WW domain. Nevertheless, the above binding studies [32] underscore the importance of the WW domain in maintaining an integral complex between dystrophin and its associated proteins. Interestingly, a smaller C-terminal isoform of the dystrophin protein, DP71 (apo-dystrophin), which is ubiquitously expressed unlike the muscle-specific dystrophin, lacks the first 19 amino acids of the WW domain (Figs 1,2). This causes a deletion of the first highly conserved tryptophan and the double tyrosines (β1 and β2 strands) and presumably renders the DP71 WW domain nonfunctional. We therefore anticipate reduced or impaired binding between DP71 and β-dystroglycan.

The role of WW domains in other proteins is not yet known and can only be speculated. For example, Fe65 [33], a protein homologous to retroviral integrases, is expressed in the brain and other specific regions of the nervous system, and contains a WW domain and two PTB/PID domains. Recently, its PTB/PID domains were shown to interact with the intracellular domain of the Alzheimer amyloid precursor protein (APP) by binding to its NPTY motif [34]. APP is an integral membrane protein. A small fragment of the protein (amyloid-β-protein) accumulates in the brain of Alzheimer patients forming extracellular deposits. Mutations in APP enhance the production and deposition of amyloid-β-protein. Fe65 may be an adaptor protein which connects APP via the Fe65–WW domain to an as yet unidentified protein. The role of Fe65 in the development of Alzheimer's disease remains to be demonstrated. Similar adaptor-protein-like properties have been proposed for YAP65, a yes-kinase associated phosphoprotein of no known function [35]. A proline-rich region in YAP65 associates with the SH3 domain of yes and the WW domain of YAP65 binds *in vitro* to the PY motifs in WBP1 and WBP2 [7]. It is not known in which pathway(s) YAP65 is involved, as the above interactions have not yet been demonstrated *in vivo*. Recently, a WW domain-containing protein that binds to CD45 (called CD45AP or LPAP) was isolated [36]. CD45 is a tyrosine phosphatase involved in T-cell signalling and proliferation. Although the interaction between CD45 and CD45AP/LPAP is mediated by the transmembrane domains of both proteins and not by the WW domain [37,38], it is speculated that CD45AP/LPAP may bind via its WW domain to other protein(s) important for CD45 signalling. The *Msb1* mutation (mammalian suppressor of *bck1*) contains a single WW domain and encodes a suppressor of *bck1*, a mutant of a yeast MAPKKK (MAP kinase kinase kinase). It also suppresses *mpk1* (a yeast MAP kinase mutant) [10]. As a PY motif was recently identified in MAP kinase-associated protein kinase 2 (MAPKAP2) [10], it is possible that *Msb1* may interact via its WW domain with MAPKAP2, and that this interaction may contribute to the suppressive effect of *Msb1*.

Future studies on both the structure and function of WW domains will greatly increase our understanding of the role of this important newly described protein–protein interaction domain in the function of the various unrelated proteins that harbour it.

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