Critical Review

WW or WoW: The WW Domains in a Union of Bliss

Marius Sudol[1,2], Claudia C. Recinos[3,4], Jennifer Abraczinskas[1], Jasper Humbert[1] and Amjad Farooq[3,4]
[1]*Weis Center for Research, Geisinger Clinic, Danville, Pennsylvania, USA*
[2]*Department of Medicine, Mount Sinai School of Medicine, New York, USA*
[3]*Department of Biochemistry & Molecular Biology, University of Miami Miller School of Medicine, Miami, Florida, USA*
[4]*Braman Family Breast Cancer Research Institute, Sylvester Comprehensive Cancer Center, University of Miami Miller School of Medicine, Miami, Florida, USA*

Summary

WW domains are small protein modules that recognize proline-rich peptide motifs or phosphorylated-serine/threonine proline sites in cognate proteins. Within host proteins these modules are joined to other protein domains or to a variety of catalytic domains acting together as adaptors or targeting anchors of enzymes. An important aspect of signaling by WW domains is their ability to recognize their cognate ligands in tandem. Tandem WW domains not only act in a synergistic manner but also appear to chaperone the function of each other. In this review, we focus on structure, function, and mechanism of the tandem WW domains co-operativity as well as independent actions. We emphasize here the implications of tandem arrangement and cooperative function of the domains for signaling pathways.

IUBMB *Life*, **57**: 773 – 778, 2005

INTRODUCTION

WW domains are the smallest protein modules composed of approximately 40 amino acids and fold as a stable, triple stranded beta-sheet in the absence of ligands or disulfide bridges (*1 – 3*). The name refers to two signature tryptophan (W) residues that are spaced 20 – 22 amino acids apart and are present in most of the WW domains. In some instances, however, the first or the second conserved tryptophan is

substituted by other aromatic residues (*1, 4*). WW domains recognize proline-rich peptide motifs or phosphorylated serine/threonine proline sites in the cognate proteins (*5*). Based on the ligand recognition, the WW domain family was classified into four groups. The largest group recognizes ligands with PPxY motif (where P is proline, Y is tyrosine and x is any amino acid) (*4, 6*). Proteins with WW domains are involved in a variety of cellular processes including receptor signaling, protein trafficking, RNA processing and transcription (*5*). Several WW domain-mediated complexes have been implicated in human diseases such as Liddle's syndrome of hypertension, Alzheimer's and Huntington's diseases, and cancer (*5*).

The presence of domains as tandem repeats in a wide variety of cellular proteins is an intriguing feature of several families of protein modules including the WW domain family (Fig. 1). In an attempt to understand the molecular mechanism of how tandem repeats function, structures of tandem WW domains of the yeast splicing factor Prp40 (*7*) and the suppressor of deltex Su(dx), a homolog of human Nedd-4 that encodes E3 ubiquitin ligase (*8*) have been determined to high resolution. These tandem WW domains share a number of common features but differences indicate that WW domains may have evolved unique mechanisms to operate in tandem. Thus, while the triple-stranded beta-sheets of tandem domains of both Prp40 and Su(dx) are held together by a linker, the flexibility of this region seems to hold clues to understanding how they may act in a co-operative manner. Biochemical characterization of two other proteins that contain tandem WW domains, namely YAP2 and WWOX (*9 – 11*), provided functional data on the cooperative as well as independent action of these domains within a given protein. In this article, we discuss structure, function and mechanism of tandem WW domains and how such an organization underlies their fidelity in cellular signal transduction.
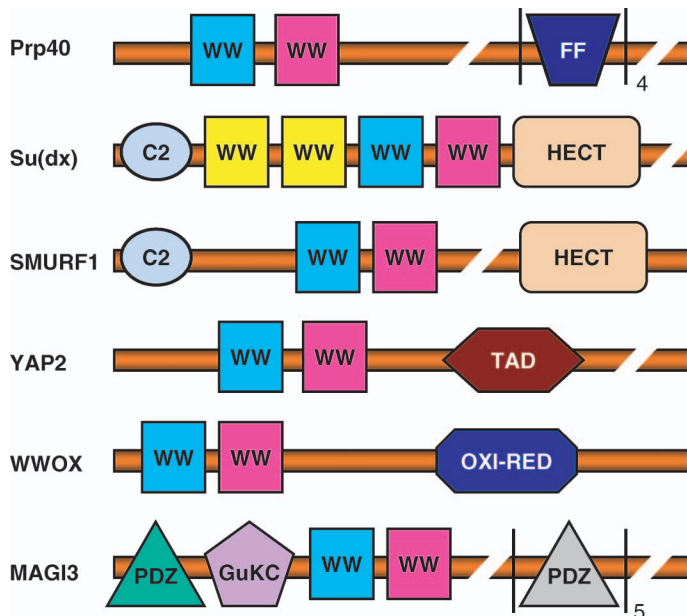
Figure 1. Schematic representation of proteins containing tandem WW domains. TAD, Transcription Activation Domain; OXI-RED, Oxidoreductase catalytic domain; GuKC, Guanylate Kinase domain; C2, calcium and lipid binding domain; and HECT, ubiquitin-ligase catalytic domain. FF and PDZ are other protein domains that frequently occur together with WW domains within the same host proteins.

LINKER REGION ORIENTS THE DOMAINS FOR OPTIMAL ACTIVITY

In Prp40, the linker region is comprised of a well-ordered helix that appears to impart strict rigidity and a fixed orientation upon its tandem domains WW1 and WW2 (7) (Fig. 2a). The two domains essentially act as a single rigid body and contain putative hydrophobic pockets on opposite faces with characteristic features for binding to proline-rich sequences. Interestingly, the sides of the two beta-stranded structures facing each other contain a highly conserved patch of hydrophobic residues such as W4, I14 and Y16 in WW1 and W45, V55 and Y57 in WW2. These residues have the potential to interact in an interdomain fashion and thus bring the two WW domains closer together in space. However, such interactions are not observed and the two domains are merely held together and maintained in a fixed orientation by the interdomain helix. Such organization of WW domains not only renders them capable of binding to distinct sites in target proteins but also fits well with the role of Prp40 in its ability to interact simultaneously and bridge precisely between target sites within the splicing machinery (7).

In contrast, the linker region between the WW3 and WW4 tandem domains of Su(dx) does not adopt helical conformation and is largely comprised of a rather flexible loop of approximately 20 residues in length (8) (Fig. 2b). The flexibility of this inter-domain loop is believed to be critical
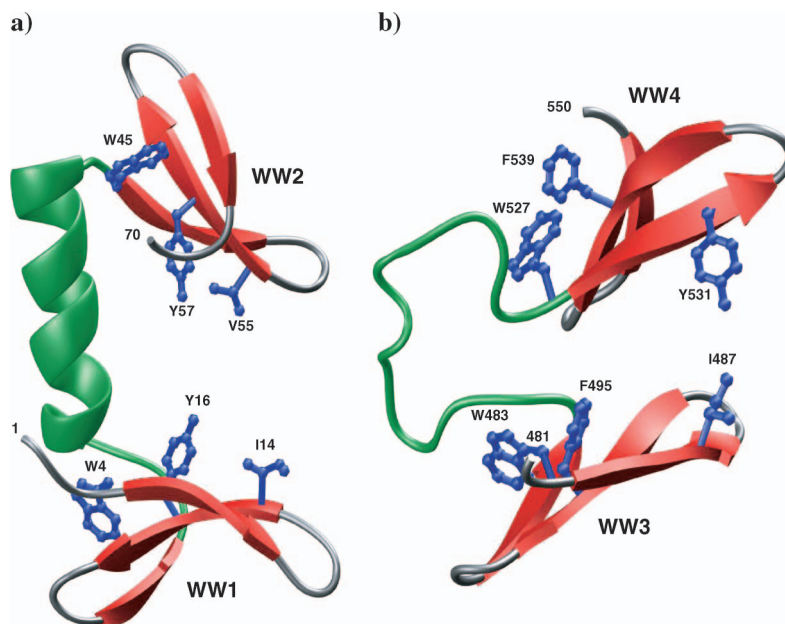
Figure 2. Solution structures of the tandem domains WW1-WW2 of the yeast splicing factor Prp40 (a) and the tandem domains WW3-WW4 of suppressor of deltex Su(dx) (b). The tandem WW domains are shown in red and the interdomain linker is in green, and sidechains of key residues constituting the hydrophobic surface on the opposite sides to the ligand binding pocket are depicted in blue. The Prp40 and Su(dx) structures displayed correspond to PDB codes 1O6W and 1TK7, respectively. Only a representative solution structure is shown.

to the function of the two WW domains in Su(dx). However, despite such flexibility of the linker loop, the two domains behave as one rigid body and their orientation relative to each other, although not as rigid as observed with the WW domains of Prp40, is also somewhat restricted. Like WW1 and WW2 in Prp40, the sides of WW3 and WW4 in Su(dx) facing each other also contain highly conserved patches of hydrophobic residues such as W483, I487 and F495 in WW3, and W527, Y531 and F539 in WW4. The orientation of the interdomain linker however prevents these from coming in close proximity to each other and engaging in interdomain interactions. Thus, like the tandem WW domains of Prp40, the tandem WW domains of Su(dx) are unable to interact with each other even though they are tethered together in a more or less fixed orientation required for their biological function.

LIGAND BINDING EMPLOYS DIFFERENT MECHANISMS

The putative hydrophobic pockets for ligand binding lie on opposite faces of the tandem domains WW1 and WW2 of Prp40 in a fixed orientation (*7*) (Fig. 3a). In WW1, the putative binding pocket is comprised of hydrophobic residues such as Y15, Y17 and W26 clustered on one face of the beta-stranded structure. In WW2, the equivalent residues are Y56, Y58 and W67. Unlike the tandem WW domains of Prp40 (*7*), the tandem WW domains of Su(dx) are able to move relative to each other and such an organization allows them to interact with their target sites independent of each other and the ligand

binding sites may not necessarily face away from each other (*8*) (Fig. 3b). In WW3, the putative binding pocket for proline-rich sequences is comprised of hydrophobic residues such as Y494, V496 and W505 clustered on one face of the triple-stranded beta-sheet structure. The equivalent residues in WW4 are F538, V540 and F549, with the latter residue mimicking the role of the second conserved tryptophan found in WW3. The fixed orientation of ligand binding pockets in Prp40 arises out of their necessity to act as a bridge between splicing factors that are held in a precise orientation and thus correlates well with the spatially-restricted target sites in the splicing machinery (*7*). In contrast, members of the Nedd4 family, that includes Su(dx), interact with a wide array of functionally diverse proteins through their WW domains (*8, 12*). Thus, the greater degree of freedom available to the WW3-WW4 tandem pair in Su(dx) renders them more adaptable to various spatial orientations imposed upon them by their substrates.

The substitution of phenylalanine for the second conserved tryptophan in Su(dx) WW4 domain does not necessarily render WW4 domain functionally distinct from classical WW domains in which both tryptophans are conserved. In support of this view is the observation that although the second tryptophan in the second WW domain of the tandem WW domains of the tight junction protein MAGI1 is replaced by a tyrosine, both WW domains interact with the proline-rich motif – PPxY- found in synaptotagmins (*13*). Unlike the tandem WW domains of Prp40, which seem to undergo little or negligible structural change upon ligand binding, the
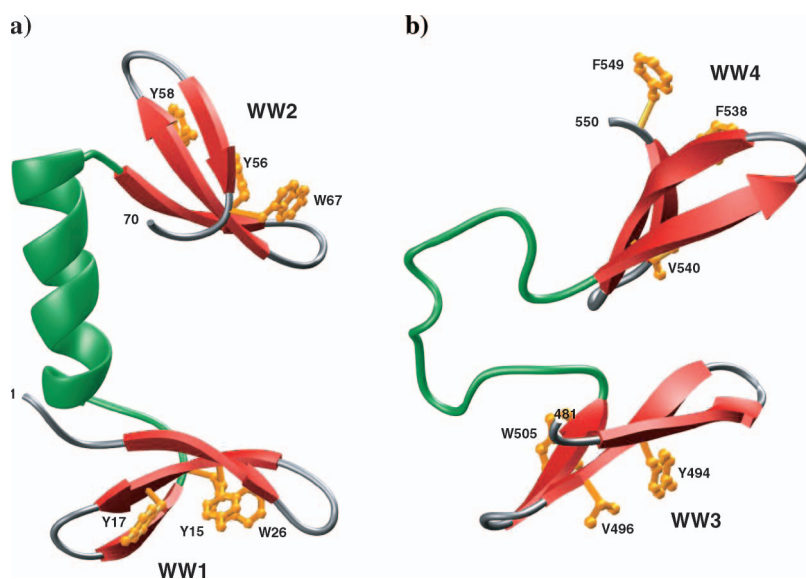
Figure 3. Ligand binding pockets within the tandem domains WW1-WW2 of the yeast splicing factor Prp40 (a) and the tandem domains WW3-WW4 of suppressor of deltex Su(dx) (b). The tandem WW domains are shown in red, the interdomain linker is in green, and the side-chains of key residues constituting the putative binding pockets for proline-rich sequences are colored yellow. The Prp40 and Su(dx) structures displayed correspond to PDB codes 1O6W and 1TK7, respectively. Only a representative solution structure is shown. The orientations of the structures are same as in Fig. 2.

tandem WW domains of Su(dx) are partially unstructured in unbound state and attain full native globular-like structure only upon binding to their cognate ligands. It is believed that the binding of ligand to WW3 not only triggers a conformational change within this domain, but also that it is transmitted to WW4 through the flexible inter-domain loop. The latter in turn may adopt a conformation optimal for ligand binding and may return the compliments by further stabilizing WW3. In this manner, ligand binding to one domain synergistically enhances the ligand binding potential of the other. It is also conceivable that ligand binding is required for stabilization of WW3 and that in the absence of ligand, interaction of WW3 with WW4 destabilizes the latter and thereby affecting its stability and structure. However, WW4 does not bind to the type I PPPY ligand recognized by WW3 and, to date, no ligands for WW4 have been identified (8). In light of this observation, we suggest that WW4 may be functionally sterile in that it is incapable of ligand binding per se but yet it is required for the ligand binding potential of WW3. In this regard, the tandem WW domains of Su(dx) may act as a single supramodule with only WW3 being capable of ligand binding while WW4 merely acts to stabilize and chaperone WW3. Such a supramodule behavior will not be unique to WW domains and it has, indeed, been previously demonstrated for a tandem pair of PDZ domains (14).

Worthy of note is also the fact that in Prp40, both WW domains are very similar to each other including the core of central aromatics. While the overall sequence identity, including the interdomain linker region, between the tandem WW domains of Prp40 and Su(dx) is 26%, the sequence identity between WW1 and WW2 tandem pair of Prp40 is 48%. The higher sequence identity between the two tandem WW domains of Prp40 further underscores the similarity in their biological function. In Su(dx), however, the WW3 and WW4 tandem domains are significantly different both at sequence level and in their binding pockets. In contrast to 48% sequence identity between the tandem WW1 and WW2 domains of Prp40, the sequence identity between the tandem WW3 and WW4 domains of Su(dx) is only 27%, implying that the WW3 and WW4 domains may act co-operatively by binding diverse sequences on a single substrate or interact with a variety of different substrates to be targeted for degradation. The latter possibility is supported by the biological role of ubiquitin ligases that are known to recognize a plethora of substrate proteins destined for proteasomal degradation.

ACTING TOGETHER OR INDEPENDENTLY

Biochemical analyses of two WW tandem-containing proteins YAP2 and WWOX that assemble on ErbB4 receptor provide suggestive evidence that tandem WW domains can act together as a true tandem pair or act independently (9, 11) (Fig. 4). The first WW domain of YAP2 and WWOX are primarily involved in recognition of PPxY motif(s) on ErbB4.

However, a sensitive functional assay of transcription has shown that at least in the case of YAP2, the presence of the intact second WW domain enhances the biological function of the YAP2-ErbB4 complex.

From the recent proteomic mapping of human WW domains we learned that the first WW domain of WWOX binds 18 human proteins whereas the second one interacts with 16 human proteins (6). Since some of the known ligands of WW domains of WWOX are common and others are unique, it is likely that many versatile proteins such as the tumor suppressing WWOX have evolved both tandem and non-tandem ways to target a large repertoire of proteins to control growth of cells in a precise way. By extrapolation, perhaps all tandem domains evolved to act in bi-modal fashion being either in 'a permanent union of bliss' by acting in unison or when required for different signaling routes 'just helping each other' a little or not at all.

FUTURE LOOKS ROSIER

The arrangement of WW domains of Prp40 (7) and Su(dx) (8) in a tandem fashion appears to present an important facet of their biology. In the case of Prp40, the tandem WW domains are held together in a fixed orientation such that their ligand binding pockets face to the outside and away from each other. Such a modular organization speaks volumes about their ability to participate and simultaneously bridge between precisely oriented splicing factors that constitute the splicing machinery (7). The need for the WW domains of Su(dx) to come together as a single functioning unit seems to be of a very different origin. These domains appear to take up their native structure only upon interaction with their cognate ligands and, as a result, they have adopted a synergistic mechanism in which ligand binding to one domain enhances the ability of the other to interact with its target sites and vice versa (8).

The occurrence of tandem WW repeats across a wide range of signaling proteins seems to be a mechanism that Nature has adopted to improve the efficiency and fidelity of cellular signal transduction. Such a mechanism is also elegantly demonstrated by the tandem bromodomains (15), the tandem SH2 domains (16, 17), the tandem PDZ domains (14, 18, 19), the BRCT tandem domains of the breast cancer associated protein BCRA1 (20), the tandem SH3 domains of NADPH oxidase (21), the tandem GAF domains of bacterial adenylyl cyclase (22), the tandem C2 domains of synaptotagmin involved in the engagement of SNAREs (23) and the tandem FF domains that occur frequently together with WW domains and are found in proteins associated with RNA splicing (24).

The extensive structural analysis of tandem domains over the last decade has begun to shed light on the molecular mechanism of their co-operative action. Yet, we have a long way to go before we can fully understand the details of such co-operation. First, the structures of tandem domains in complex with their singular or bi-dentate ligands are clearly
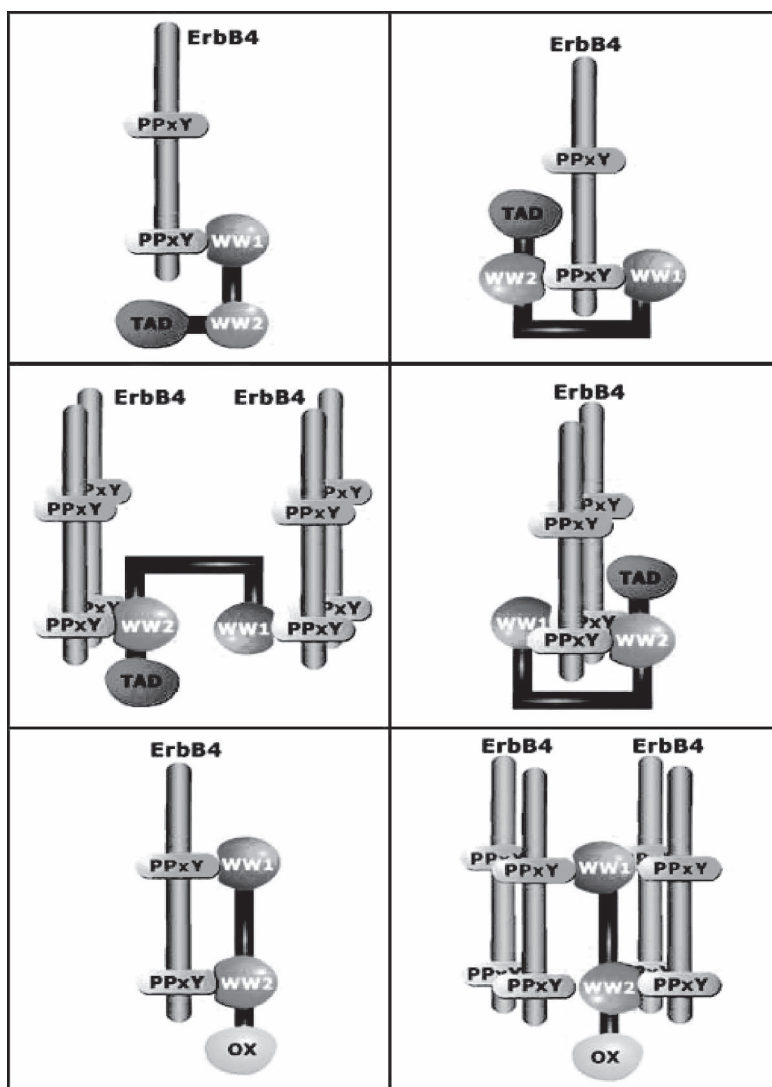
Figure 4. Schemes of interactions between the ErbB4 receptor and two of its adapters: YAP2 and WWOX (9–11). Although only the first WW domain in the tandems of YAP2 and WWOX were shown to bind directly to PPxY motifs within ErbB4 receptor, it is likely that once the complex between ErbB4 and the first WW domain of YAP2 or WWOX is formed, the second WW domain may cooperate in recognition of the same motif or could recognize other closely located PPxY cores on ErbB4. Top four panels refer to ErbB4 YAP complexes. Lower two panels refer to ErbB4-WWOX complexes. TAD, Transcription Activation Domain; OX, Oxidoreductase, catalytic domain.

warranted. Understanding the molecular basis of co-operativity observed in tandem domains in thermodynamic and kinetic terms is also an important facet of the molecular picture. Direct measurements of ligand binding to tandem versus individual domains are thus needed. The design of proteomic arrays of modular domains (WW, SH3 and PDZ domain arrays are already available from Panomics company) should contain tandems, double tandems (especially for WW, FF and PDZ) and also engineered homotypic permutations of tandem domains. The definition of tandem domains should also be

extended to consider heterotypic domains. Functional screens of arrays that contain tandems of similar but distinct domains that cooperate with each other such as WW and FF or WW and PDZ domains could be quite informative. By including in such screens permutations of tandems, completely new signaling complexes could be uncovered.

In essence, the study of tandem domains is the study of functional co-evolution of closely located domains. The analysis of naturally occurring and engineered tandem domains could provide new molecular tools for re-directing

signaling events for therapeutic purposes. A combined venture between academia and industry will undoubtedly provide further insights into how tandem domains work at the molecular level and may pave the way for the development of a new generation of drugs aimed at exploiting the unique features employed by tandem domains. The ever-increasing myriad of tandem repeats clearly poses a challenge in understanding cellular signal transduction at a new level and there is indeed a lot at stake.

ACKNOWLEDGEMENTS

REFERENCES

1. Bork, P., and Sudol, M. (1994) The WW domain: a signalling site in dystrophin? *Trends Biochem. Sci.* **19**, 531–533.

2. Chen, H. I., and Sudol, M. (1995) The WW domain of Yes-associated protein binds a proline-rich ligand that differs from the consensus established for Src homology 3-binding modules. *Proc. Natl. Acad. Sci. USA* **92**, 7819–7823.

3. Macias, M. J., Wiesner, S., and Sudol, M. (2002) WW and SH3 domains, two different scaffolds to recognize proline-rich ligands. *FEBS Lett.* **513**, 30–37.

4. Sudol, M., Chen, H., Bougeret, C., Einbond, A., and Bork, P. (1995) Characterization of a novel protein-binding module – the WW domain. *FEBS Lett.* **369**, 67–71.

5. Sudol, M. (2004). WW domain. In *Modular Protein Domains*, Cesareni, G. Gimona, M., Sudol, M., and Yaffe, M., eds. pp. 59–71, Wiley VCH, Verlag GmbH & Co., Weinheim.

6. Hu, H., Columbus, J., Zhang, Y., Wu, D., Lian, L., Yang, S., Goodwin, J., Luczak, C., Carter, M., Chen, L., James, M., Davis, R., Sudol, M., Rodwell, J., and Herrero, J. J. (2004) A map of WW domain family interactions. *Proteomics* **4**, 643–655.

7. Wiesner, S., Stier, G., Sattler, M., and Macias, M. J. (2002) Solution structure and ligand recognition of the WW domain of the yeast splicing factor Prp40. *J. Mol. Biol.* **324**, 807–822.

8. Fedoroff, O. Y., Townson, S. A., Golovanov, A. P., Baron, M., and Avis, J. M. (2004) The structure and dynamics of tandem WW domains in a negative regulator of notch signaling, suppressor of deltex. *J. Biol. Chem.* **279**, 34991–35000.

9. Komuro, A., Nagai, M., Navin, N. E., and Sudol, M. (2003) WW domain-containing protein YAP associates with ErbB-4 and acts as a co-transcriptional activator for the carboxyl-terminal fragment of ErbB-4 that translocates to the nucleus. *J. Biol. Chem.* **278**, 33334–33341.

10. Ludes-Meyers, J. H., Kil, H., Bednarek, A. K., Drake, J., Bedford, M. T., and Aldaz, C. M. (2004) WWOX binds the specific proline-rich ligand PPXY: identification of candidate interacting proteins. *Oncogene* **23**, 5049–5055.

11. Aqeilan, R. I., Donati, V., Palamarchuk, A., Trapasso, F., Kaou, M., Pekarsky, Y., Sudol, M., and Croce, C. M. (2005) WW domain-containing proteins, WWOX and YAP, compete for interaction with ErbB-4 and modulate its transcriptional function. *Cancer Res.* **65**, 6764–6772.

12. Ingham, R. J., Gish, G., and Pawson, T. (2004) The Nedd4 family of E3 ubiquitin ligases: functional diversity within a common modular architecture. *Oncogene* **23**, 1972–1984.

13. Patrie, K. M., Drescher, A. J., Welihinda, A., Mundel, P., and Margolis, B. (2002) Interaction of two actin-binding proteins, synaptopodin and alpha-actinin-4, with the tight junction protein MAGI-1. *J. Biol. Chem.* **277**, 30183–30190.

14. Feng, W., Shi, Y., Li, M., and Zhang, M. (2003) Tandem PDZ repeats in glutamate receptor-interacting proteins have a novel mode of PDZ domain-mediated target binding. *Nat. Struct. Biol.* **10**, 972–978.

15. Jacobson, R. H., Ladurner, A. G., King, D. S., and Tjian, R. (2000) Structure and function of a human TAFII250 double Bromodomain Module. *Science* **288**, 1422–1425.

16. Hatada, M. H., et al. (1995) Molecular basis for interaction of the protein tyrosine kinase ZAP70 with the T-cell receptor. *Nature* **377**, 32–38.

17. Fütterer, K., Wong, J., Grucza, R. A., Chan, A. C., and Waksman, G. (1998). Structural basis for syk tyrosine kinase ubiquity in signal transduction pathways revealed by the crystal structure of its regulatory SH2 domains bound to a dually phosphorylated ITAM peptide. *J. Mol. Biol.* **281**, 523–537.

18. Long, J.-F., Tochio, H., Wang, P., Fan, J.-S., Sala, C., Niethammer, M., Sheng, M., and Zhang, M. (2003) Supramodular structure and synergistic target binding of the N-terminal tandem PDZ domains of PSD-95. *J. Mol. Biol.* **327**, 203–214.

19. Kang, B. S., Cooper, D. R., Jelen, F., Devedjiev, Y., Derewenda, U., Dauter, Z., Otlewski, J., and Derewenda, Z. S. (2003) PDZ tandem of human syntenin: crystal structure and functional properties. *Structure* **11**, 459–468.

20. Glover, J. N., Williams, R. S., and Lee, M. S. (2004) Interactions between BRCT repeats and phosphoproteins: tangled up in two. *Trends Biochem. Sci.* **29**, 579–585.

21. Yuzawa, S., Ogura, K., Horiuchi, M., Suzuki, N. N., Fujioka, Y., Kataoka, M., Sumimoto, H., and Inagaki, F. (2004) Solution structure of the tandem Src homology 3 domains of p47phox in an autoinhibited form. *J. Biol. Chem.* **279**, 29752–29760.

22. Bruder, S., Linder, J. U., Martinez, S. E., Zheng, N., Beavo, J. A., and Schultz, J. E. (2005) The cyanobacterial tandem GAF domains from the cyaB2 adenylyl cyclase signal via both cAMP-binding sites9. *Proc. Natl. Acad. Sci. USA* **102**, 3088–3092.

23. Earles, C. A., Bai, J., Wang, P., and Chapman, E. R. (2001) The tandem C2 domains of synaptotagmin contain redundant Ca^{2+} binding sites that cooperate to engage t-SNAREs and trigger exocytosis. *J. Cell Biol.* **154**, 1117–1123.

24. Bedford, M. T., and Leder, P. (1999) The FF domain: a novel motif that often accompanies WW domains. *Trends Biochem. Sci.* **24**, 264–265.