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Critical Review

PTB or Not to Be: Promiscuous, Tolerant and Bizarro Domains Come of Age

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Summary

PTB domains are protein modules that usually interact with the cytoplasmic tail of a wide variety of growth factor receptors. In so doing, they mediate the transduction of extracellular information to specific downstream targets within the cell that ultimately determine the fate of a number of important biological processes such as cell growth and differentiation, cell cycle regulation and apoptosis. Recent structural and functional studies of PTB domains from a variety of cellular proteins have begun to shed light on the molecular mechanisms of action of these important protein modules. In the present review, we provide an account of such studies and suggest that PTB domains can be subdivided into three distinct categories on the basis of their topological differences. We also discuss the various mechanisms employed by the PTB domains in recognition of a diverse set of ligands without a consensus sequence. Finally, we discuss the role of molecular plasticity as a possible determinant of functional versatility of PTB domains.

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INTRODUCTION

Growth factor receptors (GFRs) utilize a number of mechanisms in the transmission of information from the outside to the inside of the cell (*1, 2*). Upon the binding of extracellular stimuli such as hormones, growth factors, cytokines and antigens, the activated GFRs can either directly recruit and activate effector enzymes such as phospholipase Cγ and phosphatidylinositol-3′-kinase, or alternatively, they may activate downstream effector enzymes through the recruitment of a plethora of adaptor proteins and protein modules (*3*). One such module is the PTB domain, present in a wide variety of cellular proteins, that plays a critical role in the transduction of exracellular information from the activated growth factor receptors to downstream targets (Fig. 1). For example, the activated epidermal growth factor (EGF) receptor recruits the adaptor protein Shc through specific interaction with its PTB domain (*4–7*). This enables Shc to bind to the SH2 domain of Grb2, which in turn interacts with the guanine nucleotide exchange factor SOS (*8–10*). The activated SOS causes GDP-GTP exchange within the Ras, allowing it in turn to activate the three-tier mitogen-activated protein (MAP) kinase module Raf→MEK→MAPK (*11, 12*). Upon activation, MAP Kinases such as ERK mediate key cellular events in the cytoplasm including phosphorylation of membrane-associated and cytoplasmic proteins such as kinases, cytoskeletal elements, phospholipase A_2 and stathmin (*13*). MAPKs may also translocate to the nucleus to phosphorylate specific transcription factors such as c-Jun, c-Fos, Elk-1 and c-Myc (*14–18*).

PTB domain was initially identified in the adaptor protein Shc in the early 1990s as an alternative protein module for recognizing substrates containing phosphotyrosine (pY) within the consensus motif −NPXpY- (*4, 5, 19, 20*). 'Alternative' in that prior to its discovery, the SH2 domains had already been identified and confirmed as protein modules specifically designed to interact with a multitude of protein targets containing the motif −NPXpY- (*21, 22*). However, a number of major differences distinguish these two ubiquitous protein modules. These include:

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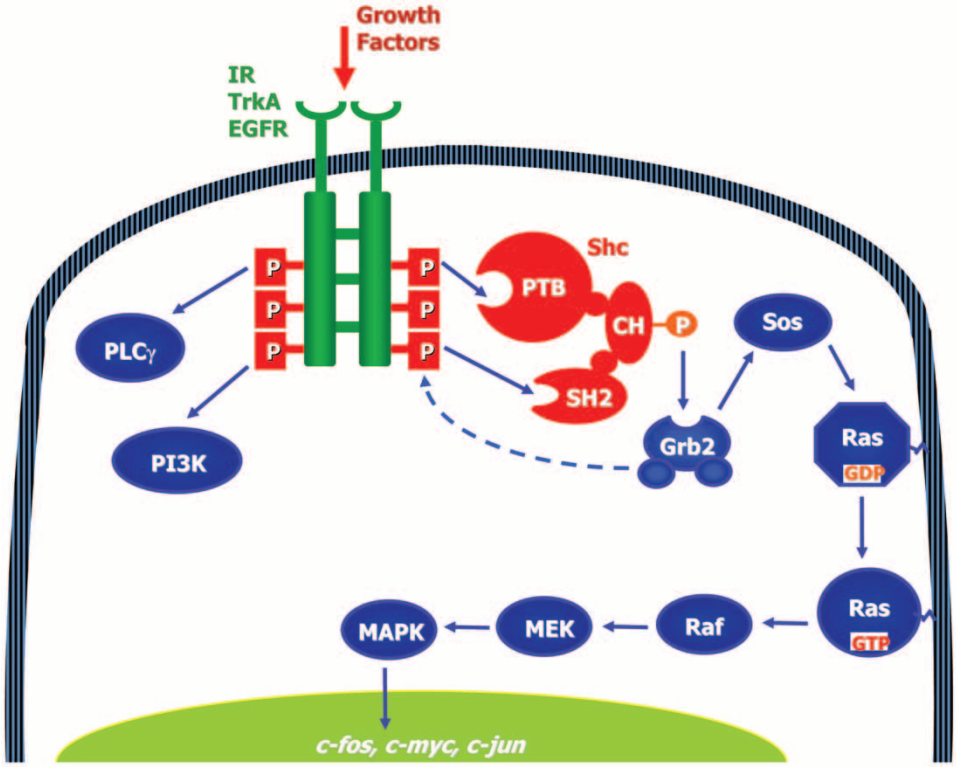

Figure 1. Transmission of information from the activated growth factor receptors (GFRs) to downstream targets by the Shc PTB domain. Upon receptor stimulation, Shc is recruited to the membrane surface via direct interaction between its PTB domain and the tyrosine-phosphorylated tail of GFRs and becomes phosphorylated at specific tyrosine residues. This enables Shc to bind to the SH2 domain of Grb2, which in turn interacts with the guanine nucleotide exchange factor SOS. The activated SOS causes GDP-GTP exchange within the Ras allowing it in turn to activate the three-tier mitogen-activated protein (MAP) kinase module Raf→MEK→ERK.

(i) Specificity. In the case of the interactands containing the −NPXpY motif, the specificity of PTB domain interaction is determined by residues located either N-terminal or C-terminal to the pY, while only residues C-terminal to the pY determine the specificity of the SH2 domains.

(ii) Topology. The PTB and SH2 domains are structurally distinct. The PTB domain is constructed on a core β-sandwich comprised of two roughly orthogonal anti-parallel β-sheets and a C-terminal α-helix, while the SH2 domains are constructed on a central anti-parallel β-sheet sandwiched between helices.

(iii) Binding. Although pY is not essential in the recognition of ligands by either the PTB or the SH2 domains, the mechanism by which they interact with their ligands is distinct. In SH2 domains, the ligand binds in an extended conformation perpendicular to the central β-sheet. In contrast, the ligand binds parallel to the second β-sheet of the β-sandwich and employs a variety of mechanisms from extended to helical conformations in binding to the PTB domain.

The above differences clearly suggest that the SH2 and PTB domains are evolutionarily distinct protein modules and that the latter have simply acquired the ability to bind substrates containing pY. In the current review, we provide an account of the recent studies that are beginning to shed further light towards understanding the PTB domain function at structural level. We suggest that PTB domains can be subdivided into three distinct categories on the basis of their topological differences. We also discuss the various mechanisms employed by the PTB domains in recognition of a diverse set of ligands without a consensus sequence. Finally, we discuss the role of molecular plasticity as a possible determinant of functional versatility of PTB domains.

STRUCTURAL AND FUNCTIONAL DIVERSITY OF PTB DOMAINS

Although PTB domains may be alternative to SH2 domains for recognizing proteins containing the motif −NPXpY-, it is now well established that neither the pY nor the motif −NPXpY- are de rigueur for the PTB domain function (*23*).

The demonstration that the PTB domain of the oncogenic protein Shc is capable of interacting with the non-phosphotyrosine based sequence -NPLH- located within the regulatory domain of the PTP-PEST phosphatase provided the first clue to the promiscuous nature of PTB domains (*24–26*). Further studies revealed that the PTB domain of the X11 protein interacts with a 14-mer peptide, derived from X11 binding site within the β-amyloid precursor protein (βAPP), containing the sequence −NPTY-, underlying the dispensability of pY (*27, 28*). More recently, the PTB domain of the cell fate determinant Numb has been shown to bind the peptides, derived from Numb interactands LNX and NAK, containing the sequences, -GPpY-, -NPAY- and −NMSF- with high affinity (*29–31*). Taken together, these observations suggest that the recognition of non-phosphotyrosine ligands is a general feature of PTB domain function rather than an isolated case.

Despite low sequence homology, PTB domains share a remarkably similar structural architecture based upon a β-sandwich capped with one or more helices (*23*). This topology was first identified in the PH domain – a specialized protein module for recognizing a variety of different phospholipids with varying specificity (*32–34*). To date, high resolution structures of PTB domains from seven critically vital cellular proteins have been solved. These include the PTB domains of Shc (*6, 35*), IRS1 (*36, 37*), X11 (*38*), Numb (*39, 40*), SNT (*41*), Dab1 (*42*) and Dok1 (*43*). Although the minimal PTB domain fold is based on a β-sandwich comprised of two roughly orthogonal anti-parallel β-sheets and a C-terminal α-helix, insertion of additional helices and strands within this minimal β-sandwich not only imparts structural variation upon PTB domain but also provides a recipe for their subgrouping into three distinct types. These are discussed as follows:

(i) Type I. In this category, exemplified by the PTB domains of IRS1 (*36, 37*), SNT (*41*) and Dok1 (*43*), the two β-sheets of the 7-stranded β-sandwich are constructed in a chronological order with the strand arrangement of 1234/567 – the first β-sheet is assembled from the first four β-strands (β1–β4) and the second β-sheet from the last three β-strands (β5–β7). The ligand binds as an additional β-strand and forms an anti-parallel β-sheet with the strand β5 and this interaction is further stabilized by the C-terminal helix α2 (Fig. 2a and Table 1). This core topology of Type I PTB domains may be further decorated with the insertion of additional helices and strands. For example, the PTB domains of IRS1 and Dok1 contain an additional one turn helix α1 between strands β1 and β2, while the PTB domain of SNT contains an additional strand β8 that lies C-terminal to helix α2 and forms anti-parallel β-sheet with the ligand in addition to the strand β5 – the ligand is flanked between strands β5 and β8.

(ii) Type II. In this category, examples of which include the PTB domains of X11 (*38*), Numb (*39, 40*) and Dab1 (*42*), the two β-sheets of the 8-stranded β-sandwich are constructed in a non-chronological order with the strand arrangement of 1345/2876 – the first β-sheet is assembled from strands β1 and β3–β5, while strand β2 forms a part of the second β-sheet which also contains strands β6–β8. The ligand binds as an additional β-strand and forms an anti-parallel β-sheet with the strand β6 and this interaction is further stabilized by the C-terminal helix α3 (Fig. 2b and Table 1). The strand β6 and helix α3 are respectively analogous to strand β5 and helix α2 in Type I PTB domains discussed above. An additional helix α2, located between strands β2 and β3, also packs against the back of the second β-sheet, possibly providing a supporting role for the PTB domain architecture. This core topology of Type II PTB domains may be further customized with the insertion of additional helices and strands. For example, the PTB domain of Numb contains an additional N-terminal helix α1, while the PTB domain of Dab1 additionally contains both the helix α1, and helix α4 that lies C-terminal to helix α3.

(iii) Type III. In this category, the sole example of which is the PTB domain of Shc (*6, 35*), the two β-sheets of the 9-stranded β-sandwich are constructed in a non-chronological order with the strand arrangement of 1456/23987 – the first β-sheet is assembled from strands β1 and β4–β6, while strands β2 and β3 form a part of the second β-sheet which also contains strands β7–β9. The ligand binds as an additional β-strand and forms an anti-parallel β-sheet with the strand β7 and this interaction is further stabilized by the C-terminal helix α3 (Fig. 2c and Table 1). The strand β7 and helix α3 are respectively analogous to strand β5 and helix α2 in Type I PTB domains, and strand β6 and helix α3 in Type II PTB domains discussed above. Furthermore, the N-terminal helix α1 and a second helix α2, located between strands β3 and β4, are analogous to helices α1 and α2 in Type II PTB domains. Availability of structures of further PTB domains will clearly reveal which elements of the Type III PTB domains constitute the core domain.

For the sake of simplicity, the binding of ligand is only discussed as formation of an additional anti-parallel β-strand, although it must be noted that the ligand binding can employ other mechanisms as discussed below.

MECHANISMS OF LIGAND BINDING TO PTB DOMAINS

Given their topological and functional diversity, the PTB domains employ a number of distinct mechanisms for binding to their partners depending upon the nature of the ligand (Table 1). These can be divided into four major categories on

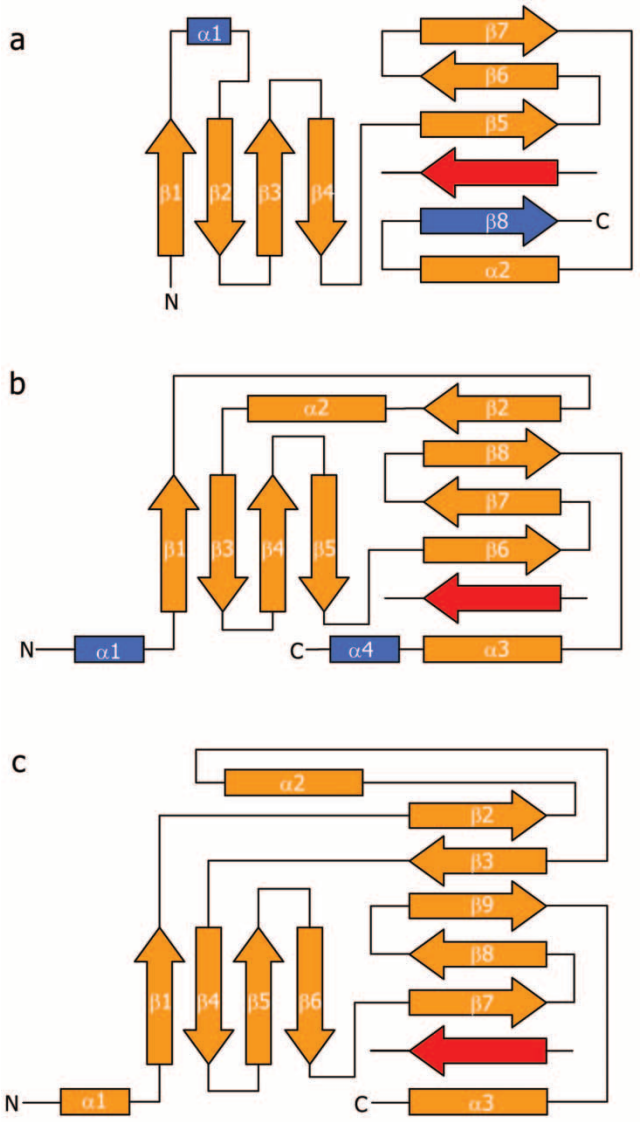

Figure 2. Topologies of PTB domain. PTB domains can be divided into three distinct categories on the basis of their topological differences observed in the β-sandwich. In Type I PTB domains (a), the first β-sheet of the 7-stranded β-sandwich is comprised of the first four β-strands ($\beta1-\beta4$), while the second β-sheet is comprised of the last three β-strands ($\beta5-\beta7$) giving the fold an overall topology of 1234/567. In Type II PTB domains (b), the first β-sheet of the 8-stranded β-sandwich is comprised of the strands $\beta1$ and $\beta3-\beta5$, while the second β-sheet is comprised of the strands $\beta2$ and $\beta6-\beta8$ giving the fold an overall topology of 1345/2678. In Type III PTB domains (c), the first β-sheet of the 9-stranded β-sandwich is comprised of the strands $\beta1$ and $\beta4-\beta6$, while the second β-sheet is comprised of the strands $\beta2$, $\beta3$ and $\beta7-\beta9$ giving the fold an overall topology of 1456/23789. The ligand is shown in red as a β-strand though it may adopt one of the four conformations discussed in the article.

the basis of the conformation that the peptide ligand adopts in complex with the β-sandwich of the PTB domain:

Extended Conformation

In this mechanism, the peptide ligand inserts largely in an extended conformation as an additional β-strand against the strand $\beta5$, $\beta6$ or $\beta7$, depending upon the type of PTB domain, in an antiparallel fashion and contacts helix $\alpha2$ or $\alpha3$ that runs along it, leading to formation of an hydrophobic peptide binding groove (Fig. 3a). This mode of binding appears to be largely favored by ligands containing the -NPXpY- or -NPXY- motifs. These include peptides from the receptors TrkA, IL4, RET, βAPP and ApoER2, which respectively bind to the PTB domains of Shc (*6*), IRS1 (*36, 37*), Dok1 (*43*), X11 (*38*) and Dab1 (*42*) (Table 1). Note that the proline in the -NPXpY- motif is substituted by a glutamate in the RET sequence. Intramolecular hydrogen bonding between the sidechain oxygen of the asparagine at the -3 position and the backbone amide of the residue at the -1 position relative to pY or Y imparts a β-turn conformation upon the -NPXpY- and $-$NPXY- motifs at the C-terminus of the peptide ligands. Hydrogen bonding between the backbone carbonyls and amides of the peptide and the strand $\beta5$, $\beta6$ or $\beta7$ also accounts for a major stabilizing force for the ligand within the β-sandwich. In addition, peptides derived from TrkA, IL4 and RET, that contain the -NPXpY- motif, are further stabilized by the coordination of their phosphotyrosine by two highly conserved arginine residues in the PTB domain. These include R112 and R227 for IRS1, R207 and R222 for Dok1, and R67 and R175 for Shc – the PTB domains that preferentially bind to tyrosine-phosphorylated ligands. Mutation of these arginines either diminishes or completely abrogates peptide binding to these PTB domains as does the dephosphorylation of pY (*6, 36, 43*). For βAPP and ApoER2, that contain the motif $-$NPXY- and respectively bind X11 and Dab1 PTB domains, phosphorylation of tyrosine is dispensable (*38, 42*). To compensate for the loss of specific interactions between phosphotyrosine in the peptide and arginine residues in the PTB domain, the peptides derived from βAPP and ApoER2 appear to employ a novel mechanism whereby residues C-terminal to Y are required to engage in specific intermolecular hydrophobic contacts.

In addition to the stabilization of the peptide ligand through intermolecular hydrogen bonding, coordination of phosphotyrosine to arginines and engagement of residues C-terminal to Y in intermolecular hydrophobic contacts, further stabilization of the peptide is achieved through proline and asparagine residues located at positions -2 and -3 relative to Y or pY, which make strong hydrophobic contacts with specific hydrophobic residues within the PTB domain, particularly those from helices $\alpha2$ or $\alpha3$. Peptide binding specificity is achieved through residues located both in the N- and C-terminal to Y or pY. For example, the IRS1 PTB favors a small hydrophobic residue such as alanine at the -1

Table 1.
Subgrouping of PTB domains from various cellular proteins

Subgroup	Substrate	Peptide	Kd/μM	PDB
Type I				
IRS1	IL4R	LVIAGNPApYRS	2.0	1IRS
	IR	LYASSNPEpYLS	90	
SNT	FGFR	HSQMAVHKLAKSIPLRRQVTVS	10	1XRO
	TrkA	HIIENPQpYFSDA	5.0	
	TrkB	PVIENPQpYFGIT	5.0	
Dok1	RET	STWIENKLpYGMSDGGK	3.0	1P5T
Type II				
X11	βAPP	QNGEYNPTYKFFEQ	0.5	1AQC, 1X11
Numb	NAK	GFSNMSFEDFP	2.0	2NMB, 1DDM
	LNX	LDNPAY	2.0	
	Mdm2	YIGQYI	—	
Dab1	ApoER2	TKSMNFDNPVYRKT	2.0	1NTV, 1NU2
Type III				
Shc	TrkA	HIIENPQpYFSDA	0.1	1SHC, 1OY2
	ErbB3	SAFDNPDpYWHSRLF	0.3	
	EGFR	SLDNPDpYQQDFF	2.0	
	IR	LYASSNPEpYLS	4.0	
	PTP-PEST	LLKAPLSFTNPLHSDDWHSDG	—	

position (*7, 36*), the Dok1 PTB domain prefers a large hydrophobic residue such as leucine at the -1 position (*43*) and the Shc PTB domain requires a large hydrophobic residue such as isoleucine at the -5 position (*6, 7*). Residues at positions -4, -5, -7, -8, $+2$ and $+3$ relative to Y all appear to be critical for high affinity binding of the X11 PTB domain to the βAPP peptide (*38*). While tyrosine in the $-$NPXY- motif does not appear to be critical for the recognition of the βAPP peptide by the X11 PTB domain, as its substitution to alanine results in no significant loss of binding affinity (*27*), the Dab1 PTB domain appears to have strong preference for this tyrosine (*44*).

Sandwiched Conformation

This mechanism is elegantly demonstrated by the binding of the FGFR peptide to the SNT PTB domain (*41*). Briefly the FGFR peptide, that is completely devoid of the $-$NPXY- motif (Table 1), wraps around the β-sandwich in a snake-like manner with the five C-terminal residues -QVTVS- of the peptide forming an additional β-strand sandwiched between the strands β5 and β8 in an antiparallel fashion (Fig. 3b). The peptide is largely stabilized through intermolecular hydrogen bonding between backbone carbonyls and amides on both sides of the segment -QVTV-S and strands β5 and β8 of the PTB domain. In addition, extensive hydrophobic contacts between the peptide segments -MAVH- and -QVTVS- (see Table 1) and the PTB domain tether the peptide tightly to the protein. Electrostatic interactions between basic residues in the peptide segments and specific acidic residues in the PTB domain further contribute to the free energy of binding. At the N-terminus of the peptide lysines in the segment $-$KLAK- form salt bridges with D68, E114 and E119, while at the C-terminus of the peptide, arginines within the segment $-$LRRQ- interact closely with a contiguous patch of triple aspartates -DDD- corresponding to residues 27–29 in the PTB domain. Substitution to alanines of valines within the segments -MAVH- and -QVTVS-, leucines within the segments $-$KLAK- and $-$LRRQ-, and the second arginine in the segment $-$LRRQ- either reduces or completely abrogates binding of the FGFR peptide to the SNT PTB domain (*41*).

β-Turn Conformation

This mechanism is observed in the case of the binding of the NAK peptide, containing the sequence –NMSF- that appears to be analogous to the $-$NPXY- motif, to the PTB domain of the cell fate determinant Numb (*39*). The peptide binds in the groove formed by the strand β6 and helix α3, the same groove that is used by all PTB domains for binding to their cognate ligands, but largely in a non-extended conformation (Fig. 3c). The peptide adopts two consecutive β-turns generated by the C-terminal -NMSF- and the -EDFP- segments. The N-

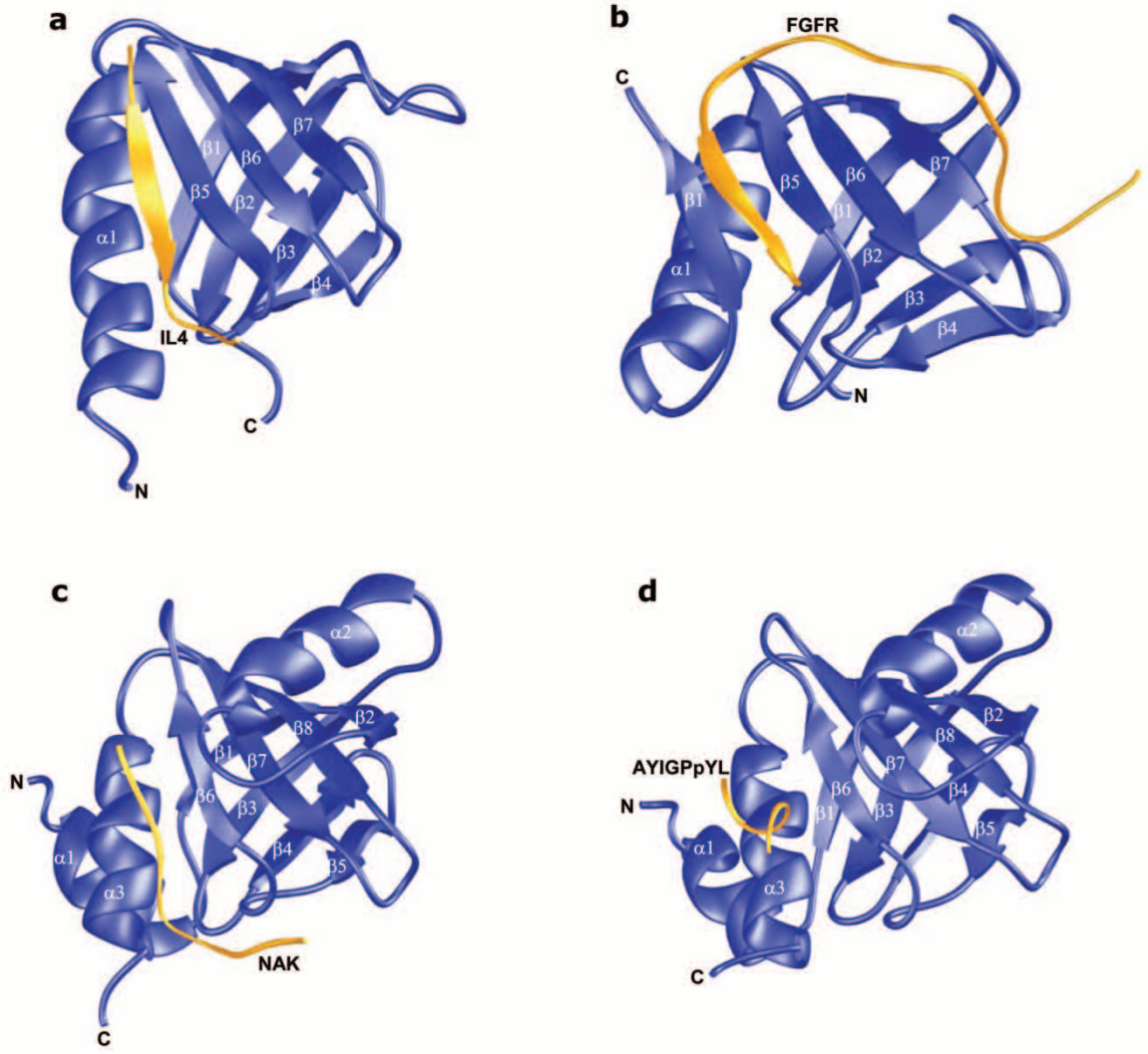

Figure 3. Mechanisms of ligand binding to PTB domain. The ligand may insert in an extended conformation as an additional β-strand with one of the strands of the second β-sheet of the β-sandwich as demonstrated by the binding of the IL4 peptide to the IRS1 PTB domain (a), it may form a sandwich by inserting between two strands of the second β-sheet of the β-sandwich as demonstrated by the binding of the FGFR peptide to the SNT PTB domain (b), it may adopt one or more β-turns in contact with the binding groove as demonstrated by the binding of the NAK peptide to the Numb PTB domain (c), or alternatively, it may adopt an helical conformation in contact with the binding groove as demonstrated by the binding of the artificial peptide AYIGPpYL to the Numb PTB domain (d). The PTB domains are shown in blue and the peptides in yellow.

terminal segment -GFS- of the peptide however assumes an extended conformation. Unlike the two mechanisms discussed above, hydrogen bonding between the backbone carbonyls and amides of the peptide and the strand β6 does not appear to play any significant role in the stabilization of the peptide with the β-sandwich. The major driving force for the stabilization of the peptide is however derived from a combination of intermolecular hydrophobic contacts and electrostatic interactions with specific residues in the strand β6 and helix α3 within the PTB domain. The sequence -NMSF- and the flanking residues on either side appear to be critical for high affinity binding of the peptide to the PTB domain. Thus, for example, substitution to alanine of phenylalanine in the segment -GFS-, asparagine and phenylalanine residues in the segment -NMSF-, and aspartate in the segment -EDFP- either diminishes or completely abolishes peptide binding (*39*). Interestingly, substitution to alanine of serine in the –GFS- segment, and methionine and serine in the -NMSF- segment augments peptide binding affinity by as much as an order of magnitude (*39*) – implying that the NAK protein is not evolutionarily optimized for binding to its partner Numb.

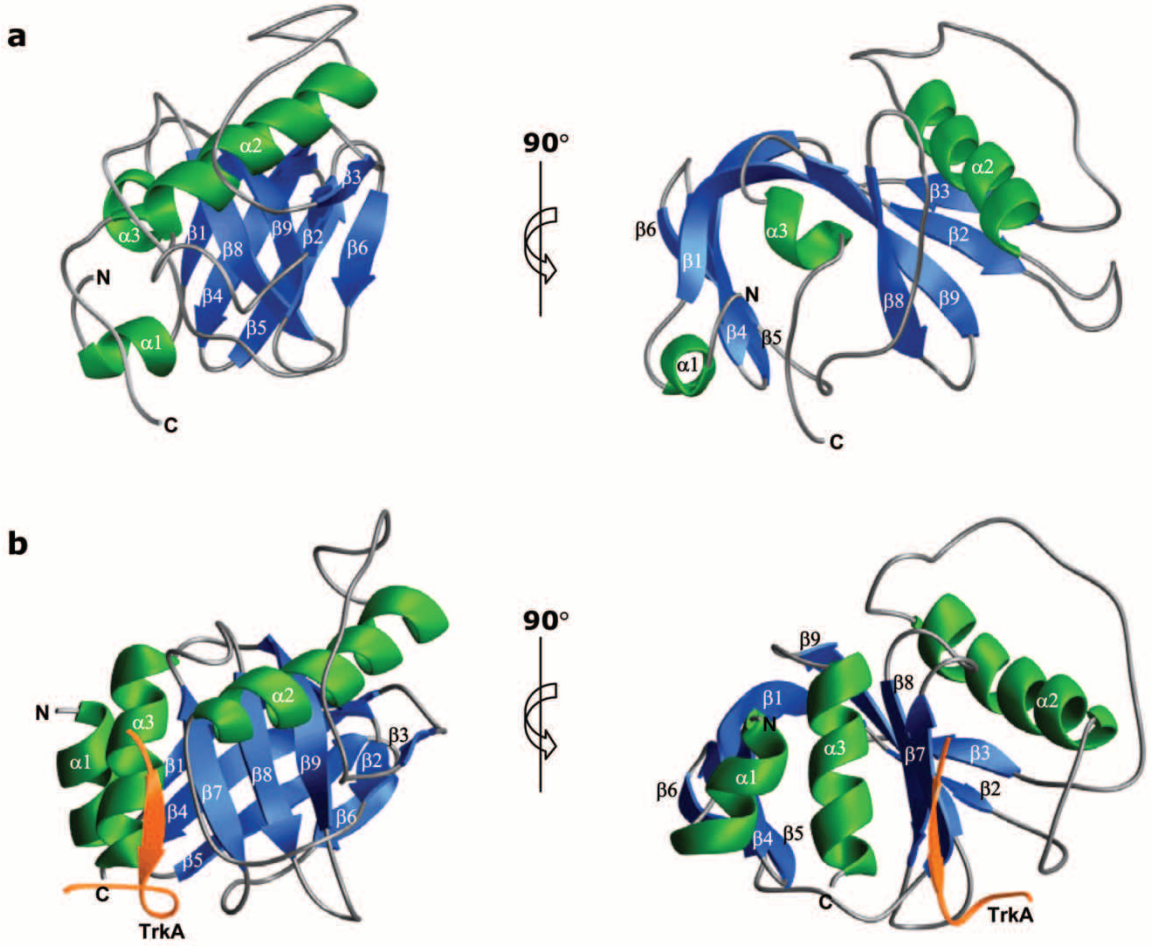

Figure 4. Coupling of folding and binding in the Shc PTB domain. Comparison of structures of the PTB domain in the unliganded form (a) and liganded to TrkA peptide (b). Alternative orientations, related by a 90° clockwise rotation about the vertical axis, of both structures are shown on the right. Helices are depicted in green, sheets in blue, loops in gray and the TrkA peptide is shown in yellow.

Helical Conformation

In this final category, the peptide binds to the PTB domain in the same groove used by all other PTB domains for interacting with their cognate ligands but in a helical conformation (Fig. 3d). This is exquisitely demonstrated by the binding of the peptide -AYIGPpYL- to the Numb PTB domain (*29, 40*). Although artificial, this peptide is very similar to the sequence -YIGQYI- present in the N-terminal region of the oncoprotein Mdm2 – the binding of which to Numb may employ a similar mechanism (*45*). Like the β-turn mechanism above, the binding of the peptide -AYIGPpYL-, in which the five central residues assume a single helical turn, to the Numb PTB domain does not involve hydrogen bonding. Instead, a tight network of hydrophobic contacts and electrostatic interactions between the residues in the peptide and specific residues in the strand β6 and helices α2 and α3 ensures high affinity binding. In addition, the phosphotyrosine is coordinated by R97 in the PTB domain.

MOLECULAR PLASTICITY UNDERLIES FUNCTIONAL VERSATILITY OF PTB DOMAINS

Our continuing efforts to understand the molecular basis of PTB domain function recently led to a surprising discovery of the coupling of folding of the PTB domain of the adaptor protein Shc with the binding of the peptide ligand (*35*). The structure of the Shc PTB domain in the unliganded form revealed that the domain is structurally disordered (Fig. 4). Interestingly, the disordered regions are largely localized to the peptide binding groove involving the strand β7. In addition, helices α1 and α3 are unwound between one to two turns each. Comparison of the structure of the unliganded form with liganded form, obtained in complex with TrkA peptide (6, 35),

reveals that the peptide binding groove is largely constructed upon peptide binding.

What is the biological significance of this salient observation? Proteins are not static structures but rather represent an ensemble of conformations that are in equilibrium exchange with each other and such a dynamic system underlies the structural and functional versatility of these molecules. This view is indeed corroborated by several studies in which protein domains have been shown to be wholly or partly unstructured in solution and become structured only upon interaction with their target molecules (*46–50*). Disordered regions in particular are very common among proteins encoded by the genomes of higher eukaryotes. Structure prediction studies indicate that as many as one in three eukaryotic proteins may be at least partially disordered in the absence of their binding partners (*51*). Thus disorder in proteins must be inherently accompanied by an intrinsic functional advantage. One argument that has been put forward is that the conformational heterogeneity resulting from the lack of intrinsic globular structure can in principle account for the binding of the same protein domain to various different target molecules (*52*). In other words, conformational heterogeneity breeds functional versatility in proteins. This scenario could not be more applicable to the Shc PTB domain for it is not only involved in binding a diverse set of growth factor receptors including TrkA, ErbB2, ErbB3, EGFR and insulin receptor but also interacts with the PTP-PEST protein tyrosine phosphatase that ultimately leads to down-regulation of Shc (*9, 24, 53, 54*). Thus the structurally disordered state of the Shc PTB domain in the unliganded form would increase its capture radius, enabling it to be easily modified accordingly in response to different molecular targets.

Given the structural similarity between various functionally different PTB domains, it is conceivable that other PTB domains may also be structurally disordered in their unliganded forms. To date, structures of only two other PTB domains in their apo forms have been solved. These include the Type I PTB domains of IRS 1 (*37*) and Dok1 (*43*). The unliganded structures of both of these domains are virtually superimposable upon their respective liganded forms – implying that little or no observable structural changes are induced upon ligand binding to these PTB domains. Whether the conformational switch observed in the Shc PTB domain upon ligand binding is unique to Type III PTB domains remains to be seen. Further structural studies of PTB domains are clearly warranted.

FUTURE PERSPECTIVES

PTB domains, present in a wide variety of cellular proteins, play a critical role in relaying the signal from the activated growth factor receptors to downstream targets such as MAP kinases (*23, 55*). Although not discussed above, it is worth pointing out that in addition to binding their protein targets, the PTB domains of Shc (*56*), Numb (*57*) and Dab1 (*42, 44*) have also evolved to interact with phosphoinositols – the membrane lipids which play an important role in the recruitment of cellular proteins to membrane surface and thereby increasing their efficiency for interacting with membrane bound proteins and receptors. Unraveling further the molecular basis of PTB domain interactions with their partners is thus an important step in not only understanding how protein modules work at molecular level, but will also pave the way for understanding the basic principles of the transmission of extracellular information to the inside of the cell and how defects in such a transmission lead to disease including cancer. Although the last decade has witnessed the emergence of structures of over half a dozen PTB domains complexed to their ligands, little is known about the dynamics of this process. Structure *per se* provides a useful starting point for understanding the biological function of a system but it is by no means the end of the story. It is the changes in the structure, or rather the dynamics of the process, in response to specific biological demands that in essence hold the clue to understanding biology. Towards this goal, the ligand-induced structural changes observed within the PTB domain of the Shc PTB domain set a new precedence. Structures of PTB domains from other cellular proteins, alone and in complex with their partners, will clearly add to our understanding of the PTB domain function at molecular level. There also remains a vacuum to be filled in understanding the kinetic mechanism of ligand binding to PTB domains. To date, virtually no data are available on this subject. Because of their ability to interact with a diverse multitude of substrates, their ability to tolerate the requirement of the motif −NPXpY-, and their ability to bind ligands in the same binding groove with strikingly different conformations, the acronym PTB perhaps best reflects Promiscuous, Tolerant and Bizarre nature rather than Phospho-Tyrosine Binding characteristic of PTB domains.

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