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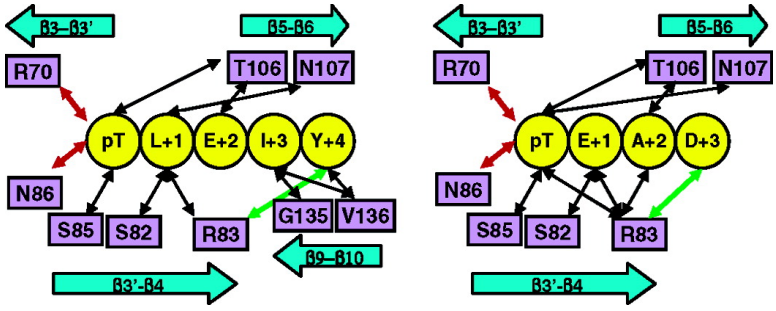

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FHA Domain−Ligand Interactions: Importance of Integrating Chemical and Biological Approaches

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The forkhead-associated (FHA) domain, the only one known to specifically recognize phosphothreonine (pT), has been subjected to intensive studies for its roles in mediating protein−phosphoprotein interactions in cellular signal transduction.[1] The FHA1 and FHA2 of *Saccharomyces cerevisiae* Rad53—a DNA damage checkpoint kinase—are among the first and most studied ones.[2−11] Two groups have independently used different sets of combinatorial phosphopeptide libraries to conclude that FHA1 and FHA2 recognize pTXXD and pTXX(I/L),[4,5,10] respectively, and have subsequently solved structures of both types of FHA−pT peptide complexes by both NMR[7,10] and X-ray.[4,12] Some of the pT−peptides used in the structures are based on the actual sequences (containing the possible sites suggested by the consensus motif) in binding partner proteins. These results led to a well recognized dogma that there are two classes of FHA domains, both recognizing pT but differing in the recognition of the pT+3 residue.[4,5,10,13] Site-specific mutagenesis has been used to engineer the pT+3 specificity successfully.[11]

However, a few recent papers have reported paradoxical exceptions to the "pT+3 rule." Rad9 T390, which was demonstrated to be the recognition site of Rad53FHA1 in vivo and in vitro, is followed by V393.[8] In another report,[14] human Ki67FHA showed no consensus in library screens and could not bind to short phosphopeptides; tight binding was shown for a 44-residue phosphopeptide fragment from its binding partner hNIFK, but this fragment has a cysteine at pT+3. Most strikingly, the recently identified checkpoint target Mdt1 was shown by in vivo experiments to bind to Rad53 via a pT(305)XXI motif, but in sharp contrast to the consensus sequence predicted from library screens, Mdt1 only binds to the FHA1 domain (which should bind Asp at pT+3) and not to the FHA2 (which should have a clear preference for Ile at pT+3).[9] This result prompted us to check binding and determine the structure of the FHA1 complex with a phosphopeptide encompassing the pT305 of Mdt1, ^{301}NDPD(pT)LEIYS^{310}, designated as pT(Mdt1) hereafter. The results are compared with the previous results of the FHA1 complex with ^{188}SLEV(pT)EADATFVQ^{200}, designated as pT(Rad9). The latter was one (with tightest binding) of the five phosphopeptides containing the five pTXXD sites of Rad9.

The ^{13}C/^{15}N-labeled FHA1 (residues 14−164) sample was purified as described.[5] The K_d value of pT(Mdt1) was determined by peptide titrations monitored by HSQC to be 15 μM, 42-fold higher than the reported value of 0.36 μM for pT(Rad9).[7] Comparing the two complexes, the cross-peaks of two highly conserved residues NH$^{\epsilon}$/R70 and NH/S85 are shifted to virtually the same magnitude and direction, and NH/N86 being absent in the free form

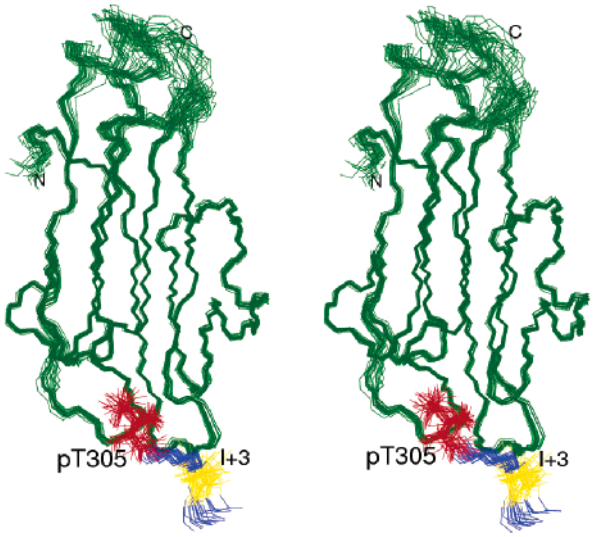

Figure 1. Stereoview of 20 overlaid structures of FHA1−pT(Mdt1). The backbone traces of residues 28−158 and pT through the +4 position are shown. The side chains for pT305 and I308 are shown in red and yellow, respectively.

shows up in both complexes, all indicating a similar binding mode for the phosphate group.[4,7] However, differences can also be discerned, most noticeably in H$^{\epsilon}$/R83 that shifts significantly smaller (~0.13 ppm) with respect to the large perturbation (~2.1 ppm) observed in FHA1-pT(Rad9), reflecting the absence and presence, respectively, of Asp at pT+3 (Supporting Information Figure 1).

The structure of FHA1−pT(Mdt1) was solved as for FHA1−pT(Rad9),[7] by performing a series of 2D and 3D NMR experiments (Supporting Information Figure 2). The ensemble of structures and the structural statistics are provided in Figure 1 and Supporting Information Table 1, respectively, and the assigned intermolecular NOEs are listed in Supporting Information Table 2. Though the pT residue binds to the same site, and the peptide also adopts an extended conformation evident from strong H$^{\alpha}$(i)−H^{N}(i+1) sequential NOE (Supporting Information Figure 3) and the trans-conformation of P303, the orientation of the pT(Mdt1) peptide is substantially different from that of the pT(Rad9) peptide (Supporting Information Figure 4). The structure of FHA1−pT(Mdt1) is the first of an FHA complex with a pT−peptide encompassing a biologically demonstrated binding site. Comparison of specific interactions in the two complexes is shown in Figure 2, and detailed analyses are provided here. First, the FHA1−pT(Mdt1) complex indeed retains the features of FHA1−pT(Rad9) in recognizing the pT moiety, which is anchored by hydrogen bond or salt bridge with R70, S85, N86, and T106, evidenced by comparable intermolecular NOEs of these residues to H$^{\gamma 2}$/pT and the aforementioned chemical

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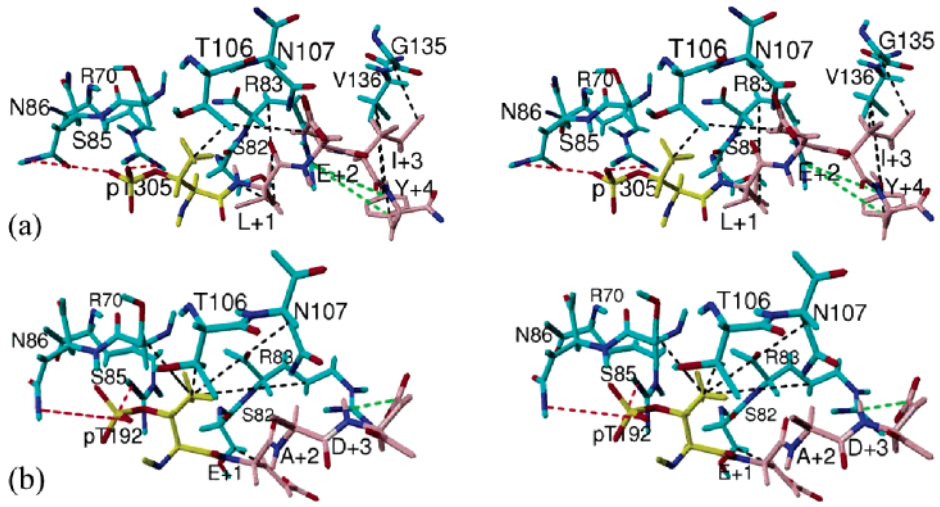

Figure 2. Stereoview of detailed binding interactions of FHA1−pT(Mdt1) (a) in comparisons with those of FHA1−pT(Rad9) (b). The ionic interactions involving phosphate group, hydrophobic interactions, and comparable electrostatic/amino−aromatic interactions involving R83 are indicated in red, black, and green, respectively.

shift perturbations. Second, while in FHA1−pT(Rad9) the Asp at pT+3 is only involved in a strong charge−charge interaction with R83 of FHA1,[4] in FHA1-pT(Mdt1), the residue Ile at (pT+3) may contribute significantly to the binding affinity by hydrophobic interactions with G135 and particularly V136 located in the β9−β10 loop. Most importantly, the aromatic ring of Y(pT+4) makes hydrophobic interaction with V136 as well as amino−aromatic interaction with R83. As a result, the residues C-terminal to pT move from the β5−β6 loop somewhat toward the β9−β10 loop. Last, while in the complexes reported previously E(pT+1) may be involved in a side chain H-bond with S82,[7] the corresponding residue L(pT+1) in pT(Mdt1) makes more hydrophobic contact with three FHA1 residues, including the R83 side chain. The side chain of E(pT+2) also makes hydrophobic interactions with $H^{\gamma 2}$/T106. All of these suggest extensive hydrophobic interactions between FHA1 and peptide residues C-terminal to pT in the FHA1−pT(Mdt1) complex. This is further highlighted by surprising observation of slowly exchanging amide protons of I(pT+3), Y(pT+4), and S-(pT+5) in 2D isotope-filtered NMR experiments conducted in D_2O (Supporting Information Figure 5). These features are different from those in the FHA1−pT(Rad9) complex, where the ionic interaction between D(pT+3) and R83 plays an important role in binding.

Peptide libraries have been widely used in the analysis of ligand binding specificity and affinity.[13] The results presented here led us to raise and discuss the following important questions: (a) How could the library screens fail to select pTXXI for FHA1? There are two possible reasons. One is that the FHA−protein interaction may involve a large number of residues, as has been shown for Ki67FHA.[14] The other is that using binding affinity for selection may not necessarily identify biologically relevant targets. Compared with hydrophobic interaction that needs more subtle fine-tuning, electrostatic interactions may be more effective in playing a major role in small interacting surfaces.[15] As mentioned above, the peptide pT(Mdt1) binds significantly less tightly, relative to pT(Rad53), to FHA1. The binding affinity could be significantly higher between native proteins; however, ionic interactions could prevail when the limited peptide library is used. (b) Does Rad53FHA1 recognize only one or both of the binding motifs represented by pT(Rad9) and pT(Mdt1) in nature? The best way to address this question is to perform more biological studies to determine whether pTXXD is a Rad53FHA binding site in vivo and to solve structures of FHA complexes with longer versions of the two phosphopeptides and others, such as one centered on pT390 of Rad9.[8] Such structural studies are challenging due to the need to obtain correctly phosphorylated protein samples (and with $^{13}C/^{15}N$ isotope labeling for NMR). (c) What values are combinatorial peptide libraries in discerning selectivity of signal transduction domains? It can be used to identify biological binding (phospho)proteins, but our results serve as a reminder that it can only provide leads; only when chemical and biological screens come together do we get the whole story.

Finally, the results from this work and a new structure of a complex of Ki67FHA[16] suggest that FHA domains are highly flexible in accommodating diverse ligands, which could explain the diverse biological functions of FHA domains.

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Supporting Information Available: Two tables and five figures. PDB accession code: 2A0T. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Li, J.; Lee, G. I.; Van Doren, S. R.; Walker, J. C. *J. Cell Sci.* **2000**, *113*, 4143−9.
(2) Sun, Z.; Hsiao, J.; Fay, D. S.; Stern, D. F. *Science* **1998**, *281*, 272−4.
(3) Liao, H.; Byeon, I. J.; Tsai, M. D. *J. Mol. Biol.* **1999**, *294*, 1041−9.
(4) Durocher, D.; Taylor, I. A.; Sarbassova, D.; Haire, L. F.; Westcott, S. L.; Jackson, S. P.; Smerdon, S. J.; Yaffe, M. B. *Mol. Cell* **2000**, *6*, 1169−82.
(5) Liao, H.; Yuan, C.; Su, M. I.; Yongkiettrakul, S.; Qin, D.; Li, H.; Byeon, I. J.; Pei, D.; Tsai, M. D. *J. Mol. Biol.* **2000**, *304*, 941−51.
(6) Wang, P.; Byeon, I. J.; Liao, H.; Beebe, K. D.; Yongkiettrakul, S.; Pei, D.; Tsai, M. D. *J. Mol. Biol.* **2000**, *302*, 927−40.
(7) Yuan, C.; Yongkiettrakul, S.; Byeon, I. J.; Zhou, S.; Tsai, M. D. *J. Mol. Biol.* **2001**, *314*, 563−75.
(8) Schwartz, M. F.; Duong, J. K.; Sun, Z.; Morrow, J. S.; Pradhan, D.; Stern, D. F. *Mol. Cell* **2002**, *9*, 1055−65.
(9) Pike, B. L.; Yongkiettrakul, S.; Tsai, M. D.; Heierhorst, J. *Mol. Cell Biol.* **2004**, *24*, 2779−88.
(10) Byeon, I. J.; Yongkiettrakul, S.; Tsai, M. D. *J. Mol. Biol.* **2001**, *314*, 577−88.
(11) Yongkiettrakul, S.; Byeon, I. J.; Tsai, M. D. *Biochemistry* **2004**, *43*, 3862−9.
(12) Li, J.; Williams, B. L.; Haire, L. F.; Goldberg, M.; Wilker, E.; Durocher, D.; Yaffe, M. B.; Jackson, S. P.; Smerdon, S. J. *Mol. Cell* **2002**, *9*, 1045−54.
(13) Yaffe, M. B.; Smerdon, S. J. *Annu. Rev. Biophys. Biomol. Struct.* **2004**, *33*, 225−44.
(14) Li, H.; Byeon, I. J.; Ju, Y.; Tsai, M. D. *J. Mol. Biol.* **2004**, *335*, 371−81.
(15) Tsai, C. J.; Maizel, J. V., Jr.; Nussinov, R. *Crit. Rev. Biochem. Mol. Biol.* **2002**, *37*, 55−69.
(16) Byeon, I.-J. L.: Li, H.; Song, H.; Gronenborn, A. M.; Tsai, M.-D. *Nat. Struct. Mol. Biol.* **2005**, in press.

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