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PDZ domains: evolving classification

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First published online 11 January 2002

PDZ domains play a crucial role in protein targeting and assembly of macromolecular signaling complexes in cells. The PDZ domains have been identified in a large variety of proteins form bacteria to humans. The PDZ domains share a common fold and the preference for carboxy-terminal ligands. Since PDZ domains were discovered, the S/T-X-Φ carboxy-terminal motif (Φ stands for a hydrophobic residue) has been considered as 'canonical' PDZ domain binding motif. However, more recent findings show that some PDZ domains bind to a 'non-canonical' target sequence. How can we identify a ligand for a given PDZ domain and determine its binding specificity? At present, two general strategies are taken by many laboratories to search for the PDZ domain ligands. The first approach utilizes unbiased screen of potential ligand libraries by yeast two-hybrid, phage display or similar methods. For example, these are the methods used to determine CASK [1] and INDADL [2] PDZ domains ligand specificity. The second approach starts from 'educated guesses' based on information about localization, expression pattern or biological function of a PDZ domain containing protein. For example, we used a similar approach to identify the Mint1-1 PDZ domain ligand [3].

The unbiased screen is labor intensive and it is not feasible to perform a comprehensive screen for each of several hundreds of known PDZ domains. In addition, even an unbiased screen has its limitations – for example Vaccaro et al. identified only EΦΨV consensus for the hINADL-5 PDZ domain in the phage display screen [2] (hINADL-4 PDZ domain in their nomenclature, see below), but using yeast two-hybrid and GST pull-down assays in our study we show that the exact same domain has dual ligand specificity and binds both EYYV and DHWC motifs [4]. The limitations of the 'educated guess' approach are even more obvious, as only a small number of potential ligands are tested and many physiologically relevant interactions can be missed. For example, in the previous study using the yeast two-hybrid method we show that Mint1-1 PDZ domain is specific for the E/D-X-W-C/S motif [3]. In the present study, using GST pull-down and surface plasmon resonance assay we show that the same domain in fact has dual specificity and binds both EYYV and DHWC motifs [4].

Facing the fast growing amount of often conflicting experimental data regarding specificity and ligands of PDZ domains, in our paper [4] we attempted to formulate a simple rule that allows to classify PDZ domains in several groups based on their primary sequence. We reasoned that specificity of any PDZ domain may potentially be predicted by a nature of amino acids in βB5 and αB1 positions in the PDZ domain fold. The importance of these two positions in PDZ domain ligand recognition was first suggested from the crystal structure of the CASK PDZ domain complexed with the ligand [5]. Using this idea, we classified 249 PDZ domains in the SMART database [6] into groups with identical or similar amino acids in βB5 and αB1 positions, which in our paper we referred to as 'Pos1' and 'Pos2' [4].

The application of the theoretical approach to complex biological system provides a general framework for interpretation of the data and allows one to make experimentally testable predictions. However, the theoretical approach also has its limitations and almost certainly some exceptions to the theoretical rule can be found. In their correspondence Paola Vaccaro and Luciana Dente make a point that interactions between PDZ domains and their carboxy-terminal ligands involve more than just βB5 and αB1 amino acids and therefore our classification is not sufficient to describe the complexity of the PDZ domain family and to predict their ligand specificity. As an alternative, Paola Vaccaro and Luciana Dente suggest to stay at the level of empirical characterization of PDZ domain ligand specificity and to classify PDZ domains according to the classes of their ligands. It is definitely a safe approach, but unfortunately it does not have any predictive power. If this approach is taken, the specificity of every PDZ domain must be identified experimentally, presumably by an unbiased screen against a library of potential ligands. As discussed above, it is not feasible to perform unbiased screen for each of many known PDZ domains. Also, even an unbiased screen has limitations and not all potential ligands will always be detected. It is also difficult to keep track of often conflicting empirical information without some sort of organizing principle. For example the E/D-X-W-C/S PDZ domain binding motif identified in our previous study [3] is not represented among classes I–IV of PDZ domain ligands proposed by Paola Vaccaro and Luciana Dente in their correspondence.

To support their critic of our classification, Paola Vaccaro and Luciana Dente present two counter-examples. In one example, the MUPP1-1 PDZ domain ((G,H) group) was shown to bind to the carboxy-tail of proteoglycan NG2 that ends with QYWV consensus [7]. In another example the CIPP1-3 PDZ domain ((G,p) group) binds to Kir4.2 and NR2 carboxy-termini, that end with the 'canonical' S/T-X-Φ motif [8]. The example of the CIPP1-3 PDZ domain is quite interesting, as it is only PDZ domain in CIPP1 that displays dual binding specificity and also binds to neurexin carboxy-tail which ends with an EYYV sequence (table 3 in [8]). In contrast, the CIPP1-1,2,4 PDZ domains bind only to canonical S/T-X-Φ ligands (table 3 in [8]). Thus, our classification correctly predicted the specificity of CIPP1-1,2,4 PDZ domains (all from the (G,H) group) and also correctly predicted that CIPP1-3 PDZ domain will have a unique specificity for the hydrophobic ligands [4]. What we failed to predict is that the CIPP-3 PDZ domain has dual specificity and that it will also bind S/T-X-Φ ligands. The specificity of MUPP1-1 PDZ domain for the 'canonical' S/T-X-Φ motif was not tested [7] and it is not clear if this domain is an exception from the (Pos1, Pos2) rule or it also displays a dual ligand specificity similar to the CIPP1-3 PDZ domain.

PII: S0014-5793(02)02221-4

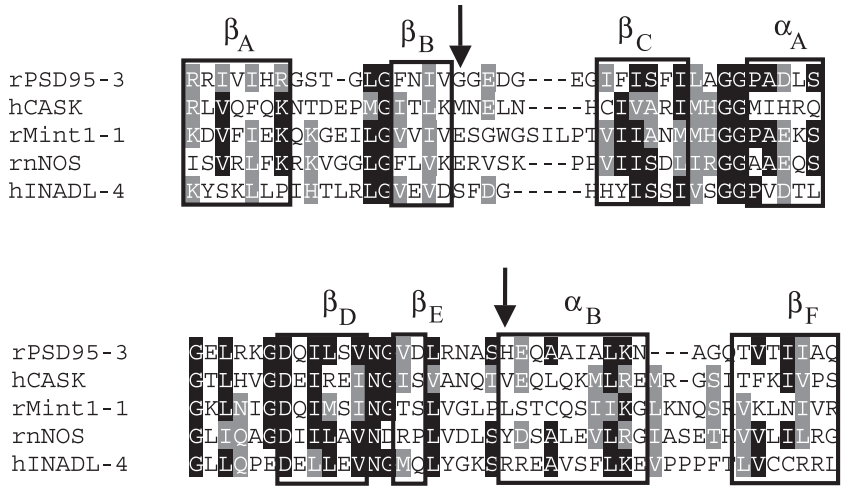

Fig. 1. Alignment of amino acids 558–639 of the human INADL protein (accession number AJ224747) with rat PSD-3, human CASK, rat Mint1-1, and rat nNOS PDZ domains. The elements of the PDZ domain fold secondary structure and (Pos1, Pos2) amino acids are indicated as in [4].

Another point raised by Paola Vaccaro and Luciana Dente concerns a number of PDZ domains in the hINADL protein. A domain structure of multi-PDZ domain protein is not a matter of 'different PDZ domain lists available on the Web', but a matter of quantitative analysis. Vaccaro et al. [2] used Pfam search that identified seven PDZ domains in hINADL protein. Using the SMART tool [6] we identified eight PDZ domains in the same protein [4]. The focus of SMART is to search for evolutionarily conserved protein domains and it provides a sensitive method of domain detection. Pfam is a more general tool based on multiple alignments of protein domains or conserved protein regions. The SMART tool scores the region of interest in the hINADL (amino acids 568–639) as PDZ domain with the *E* value of 1.72e−13. Pfam scores the corresponding region of hINADL (amino acids 555–638) as PDZ domain with the *E* value of 1.80e−02. Thus, both methods identify hINADL-4 PDZ domain, although the SMART tool does it with much higher confidence. Here we present an alignment of the corresponding region of the hINADL protein (amino acids 558–639) with the PSD95-3, CASK, Mint1-1 and nNOS PDZ domains (Fig. 1). We let a reader of this correspondence to decide from the alignment if the hINADL-4 PDZ domain really exists or not.

Because of the differences in numbering of hINADL PDZ domains between our paper [4] and the paper of Vaccaro et al. [2], the paragraph related to discussion of their results with hINADL PDZ domains 4–7 requires a revision. In fact, Vaccaro et al. analyzed hINADL PDZ domains 1–3 and 5–8 in their paper. For the hINADL domains 6, 7 and 8 (5, 6 and 7 in their nomenclature) the binding consensus fits with the 'canonical' ligand S/T-X-Φ [2], in agreement with their membership in the (G,H) group [4]. There is an unfortunate mistake on figure 7 in our paper [4] that labels hINADL-6 PDZ domain as a member of the (Sp,p) group. The entry in table 1 is correct – the hINADL-6 PDZ domain belongs to the (G,H) group. Discussion of the results of Vaccaro et al. for the hINADL-1–3 PDZ domains is not affected by skipping the hINADL-4 domain. The ligand specificity of the hINADL-4 PDZ domain ((Sp,p) group) was not determined in their paper and the contradiction with the PTPN13-3 domain data does not exist. In table 1 in our paper [4] the entry line for the ligand specificity of the (Sp,p) group must be changed to E/D-WC, a ligand of the PTPN13-3 domain [9]. For the hINADL-5 domain (hINADL-4 domain in their nomenclature) Vaccaro et al. identified EΦΨV binding consensus, which matches with our results with neurexin carboxy-tail that ends with EYYV sequence [4]. Thus, correction for the differences in hINADL PDZ domain numbering significantly improves the agreement between Vaccaro et al. their experimental results [2] and our predictions [4].

In conclusion, we would like to emphasize that the proposed classification is not meant to be a final breakdown of PDZ domains into different classes. It is rather a first attempt to organize PDZ domains by a unifying principle and to predict their ligands specificity. We hope that addition of new experimental information about PDZ domain ligands and better understanding of modes of interactions between PDZ domains and their targets will lead to further improvement in our classification. Paola Vaccaro and Luciana Dente suggest to combine (p,H), (Sp,p), (Lh,h) and (Lh,a) groups based on the common preference for the Φ/Ψ-D-Φ ligands. In our paper we separated PDZ domains into classes based exclusively on their primary sequence, but as a next step addition of information about ligand specificity will definitely help in improving the classification. Before making significant changes in the classification, we will probably need to wait for more experimental data, especially for PDZ domains outside of the (G,H) group.

References

[1] Songyang, Z. et al. (1997) Science 275, 73–77.
[2] Vaccaro, P., Brannetti, B., Montecchi Palazzi, L., Philipp, S., Helmer Citterich, M., Cesareni, G. and Dente, L. (2001) J. Biol. Chem. 276, 42122–42130.
[3] Maximov, A., Sudhof, T.C. and Bezprozvanny, I. (1999) J. Biol. Chem. 274, 24453–24456.
[4] Bezprozvanny, I. and Maximov, A. (2001) FEBS Lett. 509, 457–462.
[5] Daniels, D.L., Cohen, A.R., Anderson, J.M. and Brunger, A.T. (1998) Nat. Struct. Biol. 5, 317–325.
[6] Schultz, J., Copley, R.R., Doerks, T., Ponting, C.P. and Bork, P. (2000) Nucleic Acids Res. 28, 231–234.

[7] Barritt, D.S., Pearn, M.T., Zisch, A.H., Lee, S.S., Javier, R.T., Pasquale, E.B. and Stallcup, W.B. (2000) J. Cell Biochem. 79, 213–224.
[8] Kurschner, C., Mermelstein, P.G., Holden, W.T. and Surmeier, D.J. (1998) Mol. Cell Neurosci. 11, 161–172.
[9] Gross, C., Heumann, R. and Erdmann, K.S. (2001) FEBS Lett. 496, 101–104.

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PII: S0014-5793(02)02221-4