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PDZ Dimerization Brings Proteins Together

Kimberly Mendes[1] and Thomas Kodadek[1,*]
[1]Departments of Chemistry and Cancer Biology, The Scripps Research Institute, Scripps Florida, 130 Scripps Way, Jupiter, FL 33458, USA
*Correspondence: kodadek@scripps.edu
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The dimerization of different PDZ domains suggests that these protein modules evolved to guide the assembly of multiprotein complexes. In this issue, Chang et al. (2011) describe a massively parallel approach for identifying all PDZ dimerization events that reveals the prevalence of this protein-protein interaction.

Protein-protein interactions are instrumental in guiding the assembly of complexes that perform most of the critical molecular processes in the cell. Protein-protein interactions can either be essential for assembling catalytically active complexes from individual poypeptides that have no independent activity or drive the associations of regulatory or accessory factors with a protein displaying a particular catalytic activity. Some of the most common examples of large molecular machines built from a number of different protein and other components include the DNA replication complex, the ribosome for protein synthesis, and the proteasome for molecular degradation (Hartwell et al., 1999). Many other protein-protein interactions are dynamic and regulated by posttranslational modifications such as protein phosphorylation found in, for example, the signal transduction pathways that transmit extracellular signals to the nucleus, resulting in changes in gene expression. (Oda et al., 2005).

The sequencing of many genomes, coupled with a great deal of fundamental research, has made it clear that there exist a number of relatively small domains that specialize in mediating protein-protein interactions. One of the most common of these protein interaction modules is the PDZ domain, which is constituted of approximately 80–90 residues. PDZ domains recognize a short stretch of 5–7 amino acids usually at the carboxyl terminus of its partner protein (Songyang et al., 1997), which is most commonly a transmembrane receptor or an ion channel. Thus, the PDZ domain aids in localizing these proteins to specific subcellular regions, leading to the formation of multiprotein complexes to mediate processes such as cell signaling. In addition to this canonical binding mode, there have been a few observations of stable dimers of two different PDZ domains. One example of such an interaction is the PDZ domain of neuronal nitric oxide synthase (nNOS), which interacts specifically with the PDZ domain of α1-syntrophin (Hillier et al., 1999). The physiological relevance of this association is to localize nNOS in the dystrophin complex of muscle cells, thereby coupling NO production to muscle contraction. Hence, the production of NO increases blood flow, compensating for the exertion of muscle contraction (Hillier et al., 1999). Given this precedent, one can suspect that there are many more important PDZ-PDZ interactions in cells that have yet to be elucidated.

In the current issue of *Chemistry & Biology*, Chang et al. (2011) have completed the arduous task of surveying all of the PDZ dimerization partners across the mouse proteome. Every PDZ domain encoded in the mouse genome (157 in total) was cloned, expressed, purified, and printed onto a glass microarray. Microarray technology is ideal for large-scale screening because it enables many different samples, in this case PDZ domains, to be analyzed rapidly and simultaneously, as well as allowing for the experiment to be repeated under varying conditions. MacBeath and Schreiber (2000) have successfully developed functional protein microarrays that attach the protein of interest to surface of the slide covalently but maintain the protein's native properties, including the ability to interact specifically with other proteins. The PDZ domain microarray was incubated with each of the 157 different mouse PDZ domains and analyzed to determine whether each PDZ domain bound specifically to any of other PDZ domains within the proteome. This resulted in the identification of approximately 300 PDZ-PDZ interactions.

The positive hits from the protein microarray were validated using solution-phase fluorescence polarization. Fluorescence polarization is a versatile technique to measure equilibrium binding that is highly sensitive, reproducible, and requires modest quantities of sample, thereby providing a rapid means to characterize the binding affinities of each of the PDZ domain interactions (Heyduk et al., 1996). From the fluorescence polarization results, 15 PDZ domain interactions were identified with a K_D <5 μM. Although low micromolar affinity interactions between two purified protein domains are observed in isolation, these interactions may not be relevant in the significantly more complex cellular environment. Therefore, the PDZ domains observed to dimerize in the fluorescence polarization assay were returned to the context of their full-length proteins, and the interactions were analyzed in a cellular environment using coaffinity purification (coAP). Gratifyingly, the coAP assays scored positive for all seven novel protein-protein interactions that were tested. Furthermore, these novel interactions occurred mostly between proteins that not only exist in the same cellular component, but are also connected to each other through broader protein networks.

Overall, Chang et al. (2011) report 37 PDZ-PDZ interactions involving 46 distinct PDZ domains, suggesting that this binding mode occurs at a higher frequency than previously suspected. This noncanonical interaction may have evolved to guide the assembly of, or to strengthen, multiprotein complexes. For instance, a new PDZ-PDZ interaction between CASK and Pard3 was identified in this work. Both proteins are involved in the epithelial cell junction network and were postulated to interact only indirectly through other protein-mediated

interactions. Specifically, CASK is known to interact with ZO1, which interacts with JAM2, which interacts with Pard3, thus bringing CASK and Pard3 into proximity. However, the work presented here shows that CASK and Pard3 interact directly through dimerization of their PDZ domains. Why these two proteins interact directly requires further investigation, but now that Chang et al. (2011) have identified every PDZ-PDZ interaction from the mouse proteome, they have paved the way for establishing the biological significance of these interactions.

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Chemical Activators of ClpP: Turning Jekyll into Hyde

David A. Dougan[1,*]
[1]Department of Biochemistry, La Trobe Institute for Molecular Science, La Trobe University, Melbourne 3086, Australia
*Correspondence: d.dougan@latrobe.edu.au
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Casein lytic peptidase P (ClpP) is a serine peptidase that, when coupled to its cognate ATPase, facilitates the controlled degradation of both damaged and unwanted proteins in bacteria. In this issue of *Chemistry & Biology*, Leung et al. (2011) report a small molecule screen against ClpP, from which they identified four structurally distinct compounds that activate ClpP for unregulated proteolysis.

The treatment of bacterial infections with antimicrobial drugs was one of the most profound medical advances of the last century. The discovery of these drugs began in the 1930s and continued unabated over the next four decades. Indeed, many of the drugs we use today can be traced back to natural compounds, identified during these "golden" years of drug discovery, and their effectiveness is evidenced by our current quality of life. However, since the end of this fruitful period of drug discovery, relatively few new compounds (natural or synthetic) have been developed. Concomitantly, especially during the last decade, there has been a concerning increase in the occurrence of nosocomial infections involving drug resistant bacterial species (e.g., Methicillin-resistant *Staphylococcus aureus* [MRSA] and Vancomycin-resistant *Enterococci* [VRE]) (Levy and Marshall, 2004) that has in turn lead to the emergence of multi-drug resistant (MDR) bacteria. Hence, there is a real need for the development of new drugs, especially those that target novel mechanisms to kill bacterial cells.

In 2005, Brötz-Oesterhelt and colleagues identified a new class of natural antibiotics termed acyldepsipeptides (ADEPs) that showed remarkable promise, as they were active in the treatment of rodents infected with antibiotic resistant bacteria (Brötz-Oesterhelt et al., 2005). Surprisingly, these compounds do not kill bacteria by inhibiting an essential cellular process, but rather they target a non essential protein, the peptidase ClpP, to kill bacteria. Indeed ADEPs are proposed to kill bacteria via a unique mechanism—by triggering the widespread and unregulated degradation of nascent polypeptides and unfolded proteins (Kirstein et al., 2009). Despite their remarkable bactericidal activity, limited availability of these antibiotics has hampered progress in elucidating their mechanism of action; hence the identification of new ClpP activators of unregulated proteolysis may aid in further defining how this promising class of drug functions.

ClpP is a barrel-shaped protein composed of two heptameric rings in which the catalytic residues are sequestered inside a proteolytic chamber. In the absence of its cognate AAA+ (ATPase associated with various cellular activities) component (e.g., ClpA, ClpC or ClpX), entry into this chamber is restricted to a narrow entry portal at either end of the complex (Wang et al., 1997). In this state, although short peptides can enter the proteolytic chamber for hydrolysis, large polypeptides are generally excluded from the chamber, preventing the indiscriminate degradation of cellular proteins. Therefore, in the absence of its cognate ATPase, protein degradation by ClpP is effectively turned OFF (Figure 1). By contrast, in the presence of its cognate ATPase, ClpP-mediated protein degradation is turned ON (Figure 1). Currently, it is widely accepted that activation of ClpP results from docking of a specific loop (known as the IGF loop) on the cognate ATPase (Kim et al., 2001), which culminates in opening of the narrow entry portal located at the distal ends of the complex, supporting entry of unfolded polypeptides, into the proteolytic chamber (Burton et al., 2001).

