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COMMENTARY

Disrupting specific PDZ domain-mediated interactions for therapeutic benefit

Miles D Houslay

Neuroscience and Molecular Pharmacology, Wolfson and Davidson Buildings, Faculty of Biomedical & Life Sciences, University of Glasgow, Glasgow, UK

The past two decades have seen an immense increase in our appreciation of the vast range of signalling processes and supporting machinery that occur in cells. Pivotal to this is the notion of signal compartmentalization (compartmentation). Targeting by protein domains is critical in allowing signalling complexes to be assembled at defined intracellular locales so as to confer correct function. This issue of the *BJP* contains two intriguing articles that address functional protein–protein interactions involving PDZ domains [Post-synaptic density protein-95 (PSD95), Drosophila disc large tumour suppressor (DlgA) and Zonula occludens-1 protein (zo-1)] and their implications for signalling. One involves targeting of neuronal nitric oxide synthase to the N-methyl D-aspartic acid (NMDA) receptor via the PDZ-containing signal scaffold, PSD95. The other involves controlling multiple receptor inputs into regulation of epithelial Na^+K^+-ATPase through the PDZ-containing signal scaffold Pals-associated tight junction. Highlighted is not only the use of dominant-negative strategies to identify the importance of targeting at specific types of PDZ domains but also the exciting notion that small molecule disruptors of interaction at specific PDZ domains can be generated for potential therapeutic application.
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Abbreviations: IC87201, 2-((1H-benzo[d][1,2,3]trizol-5-ylamino)methyl)-4-6-dichlorophenol; NMDA, N-methyl D-aspartic acid; nNOS, neuronal nitric oxide synthase; PATJ protein, Pals-associated tight junction protein; PDZ domains, Post-synaptic density protein-95 (PSD95), Drosophila disc large tumour suppressor (DlgA) and Zonula occludens-1 protein (zo-1); PSD95, post-synaptic density protein-95; Tat peptide, transactivator of transcription peptide

Cells are complex three-dimensional entities that respond to a host of environmental cues. These are detected by a panoply of receptors, generating signals that are integrated in both time and space to yield an appropriate response.

The past two decades have seen an immense increase in our appreciation of the vast range of signalling processes and supporting machinery that occur in cells. However, it is only relatively recently that the implications of the spatial complexity of signalling have become apparent. The notion of signal compartmentalization (compartmentation) arose originally from studies done on the archetypal second messenger, cAMP (see Houslay *et al.*, 2007). Although regarded as heretical at the time, it is now well established that cAMP signalling is compartmentalized in cells. Genetically encoded sensors detect cAMP gradients sculpted by spatially constrained degradation achieved by phosphodiesterase isoforms sequestered to distinct signalling complexes. Such sculpted gradients are then sampled by sequestered cAMP effector systems.

The concept of compartmentalization was transformed by the generation of Ca^{2+} sensors that allowed appreciation that small molecules could form gradients in cells and provide unique functions at spatially distinct locales (Berridge, 2006). Pivotal insight, however, came from studies on tyrosyl kinases, yielding the protein domain concept shown as critical in allowing signalling complexes to be assembled at defined intracellular locales so as to confer function (Pawson and Nash, 2003). Subsequent advances in cloning, sequencing and bio-informatics led to a wealth of functional protein domains being identified, conferring interaction not only between defined sets of proteins but also with lipids, DNA, RNA and sugars. Furthermore, post-translational modification by phosphorylation, ubiquitination and SUMOylation can provide regulatory control, conferring a dynamic input into certain systems.

Correspondence: Miles D Houslay, Neuroscience and Molecular Pharmacology, Wolfson and Davidson Buildings, Faculty of Biomedical & Life Sciences, University of Glasgow, Glasgow, G12 8QQ, UK. E-mail: M.Houslay@bio.gla.ac.uk
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At one time, the concept of disrupting protein–protein interactions was considered anathema by the pharmaceutical industry. However, it is now becoming apparent that at least some of these interactions may well be 'druggable' (Dev, 2004) using so-called 'biologics' and small, that is, low MW, molecules. This is likely to be true at least for those situations where interaction is dynamic or where a small molecule or biologic may bind a nascent protein domain with such high affinity so as to prevent subsequent interaction with a partner protein. Greatly aiding in conceptualizing this has been the 'dominant-negative' concept to achieve inhibition of proteins whose functionality depends upon targeting. This is where overexpression of a protein containing a functional input binding site, but an inactivated output site, for example, a catalytically inactive enzyme, is used to disrupt a signalling system by displacing the endogenous active species and replacing it with a bound but functionally impotent one. The mere fact that a phenotype can arise from such an experiment shows that protein–protein interaction can be disrupted in cells with a consequential functional change. Indeed, mapping the interaction surfaces between partners, such as by using peptide array technology (Houslay *et al.*, 2007), can allow mutants in specific binding surfaces to be generated that disrupt targeting to defined partner proteins and, thereby, selectively ablate specific 'dominant-negative' functions.

This issue of the *BJP* contains two intriguing articles (Chen *et al.*, 2009; Florio *et al.*, 2009) that address functional protein–protein interactions involving PDZ domains. Such domains were named after Post-synaptic density protein-95 (PSD95), Drosophila disc large tumour suppressor (DlgA) and Zonula occludens-1 protein (zo-1); proteins that were first discovered to share this domain. The PDZ domain is a widespread protein module that serves multiple functions (van Ham and Hendriks, 2003; Kim and Sheng, 2004). While their primary sequence can differ markedly, their three-dimensional structure is remarkably conserved. This consists of two α-helices (α1, α2) and five to six β-strands (β1–5/6), where a peptide from the partner protein binds as an anti-parallel β-strand in a positively charged groove formed between α2 and β2. The C-terminus of the partner protein was originally thought to provide the portion interacting with the PDZ domain. However, an increasing number of interactions have now been reported with internal protein sequences. The PDZ domain groove ends with a hydrophobic cavity to which the predominantly, but by no means exclusively, hydrophobic side chain of the C-terminal end residue of the partnering protein inserts. Indeed, the nature of this pocket appears to have considerable influence on the specificity of partnerships that a particular PDZ domain can undergo. In addition to this, the conserved 'signature' of a PDZ domain is a Arg-X-X-Gly-Leu-Gly-Phe linear motif that forms a connecting loop between the first two β-strands and which is critically involved in binding the interacting peptide. There are 400+ PDZ domains encoded by the human genome, and those analysed to date show distinct specificities for the linear peptide motifs that they bind. This has been evaluated by probing both peptide and phage display libraries, and an *in vitro* directed evolution approach has been used to generate PDZ domains of heightened affinity for specific ligands (Ferrer *et al.*, 2005). Intriguingly, certain PDZ domains appear able to bind phosphatidylinositol 4,5-bisphosphate (PIP2) and cyclic peptides (van Ham and Hendriks, 2003), which gave the first indication that small molecules and biologics might be able to manipulate PDZ domain functioning.

Invariably PDZ domains are arranged in tandem or greater repeats in proteins and often occur along with other protein interaction domains, such as Ankyrin repeats, WW, LIM, FERM and RGS domains, so as to form scaffold proteins. The multi-valency of such scaffolds allows them to assemble multi-protein complexes for coordinating signalling events in spatially distinct locales and offering potential for cooperative interactions between PDZ repeats and regulation by post-translational modification.

The study by Florio *et al.* (2009) relates to the archetypal PDZ-containing protein, PSD95, in the context of using a novel small molecule, IC87201 [2-((1H-benzo[d][1,2,3]trizol-5-ylamino)methyl)-4-6-dichlorophenol], and also a cell permeable fusion protein formed from a portion of nNOS (residues 1–299), targeted to disrupt specifically the binding of neuronal nitric oxide synthase (nNOS) to PSD95. They show that such disruption results in the inhibition of the N-methyl D-aspartic acid (NMDA)-nNOS signalling pathway and suggest that such targeted disruption may provide a novel approach to obtain selective anti-hyperalgesics, while mitigating side effects observed using NMDA receptor antagonists that ablate channel function. In this regard PSD95 employs its multiple PDZ domains to recruit nNOS to the NMDA receptor, forming a signalling module that spatially localizes Ca^{2+}/calmodulin-activated nNOS adjacent to the NMDA receptor Ca^{2+} entry system. The importance of PSD95 to this system is evident from studies of its knockdown, which confer decreased NMDA-induced NO production and NMDA-mediated excitoxicity in neurons, without affecting NMDA receptor channel properties. IC87201 showed dose-dependent inhibition of both nNOS–PSD95 interaction and NO signalling by the NMDA receptor, as well as potential efficacy in a model of neuropathic pain. Such inhibitory effects were recapitulated by a dominant-negative approach using an N-terminal fragment of nNOS that contained the PDZ binding region but lacked the catalytic site. This was fused to an 11-mer HIV-1 Tat (transactivator of transcription) peptide, which conferred cell entry allowing the chimeric fusion protein to displace endogenous active nNOS from the PSD95/NMDA receptor complex, thereby uncoupling the NMDA receptor from nNOS activation. As PSD95 binds to many other proteins in addition to nNOS (Kim and Sheng, 2004), disrupting the PSD95/nNOS interface is likely to provide less potential for side effects than targeting the PSD95/NMDA receptor interface.

The Pals-associated tight junction (PATJ) scaffold protein contains 10 PDZ domains plus a single N-terminal MRE domain and in epithelial cells plays a pivotal role in tight junction formation, cell polarity and migration (Shin *et al.*, 2005). Chen *et al.* (2009) uncover a novel role for PATJ in transporting epithelia, namely in mediating regulation of the basolateral Na^+K^+-ATPase with three distinct signalling inputs, involving the receptors for dopamine, insulin and angiotensin-II. In this they used a deletion/dominant-negative strategy to suggest a functional role for specific PATJ PDZ domains. Their studies indicate that PATJ, through

certain of its PDZ domains, may serve to organize components of the dopamine, angiotensin-II and insulin signalling pathways in order to control cross-talk between these various stimulatory and inhibitory inputs and to confer precise spatial and temporal control.

These reports add to the growing body of evidence indicating that specific PDZ domains have particular functional roles. Importantly they also add validation to the concept that disruption of protein targeting is a practical and novel approach to manipulate more precisely specific cellular functions while avoiding adverse effects associated with the wider panoplies of responses that may emanate from drugs aimed at the binding/active site targeted receptor and enzymes. Indeed, along with the studies on IC87201 (Florio *et al.*, 2009), another small molecule disruptor of PDZ domain interaction has recently been reported (Grandy *et al.*, 2009). This describes a molecule suggested to be of potential therapeutic use in prostate cancer that acts by disrupting PDZ functioning in Dishevelled, an essential protein in the Wnt signalling pathway. Biologics also offer potential in disrupting PDZ domain interactions, with a small cyclic peptide being designed to allow disruption of the PSD95 interaction with GluK2 kainate receptors that may provide a route for novel therapeutic agents for addressing drug addiction and epilepsy (Piserchio *et al.*, 2004). These various studies all add strong support to the notion that interactions involving PDZ domains are potentially druggable and may provide for a new generation of novel medicines targeted at important disease states.

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