Structure, Vol. 10, 3–9, January, 2002, ©2002 Elsevier Science Ltd. All rights reserved. PII S0969-2126(01)00705-5

Previews

MAGUK SH3 Domains—Swapped and Stranded by Their Kinases?

Excitement in protein science comes from unexpected structural findings that shed new light on functional mechanisms. Just such a series of insights is now beginning to emerge from two recently published structures of the scaffolding protein PSD-95.

Historically, progress in protein "domainology" has occurred when a novel function is assigned to an old domain, or when a new modular signaling domain is identified. For proteins composed of multiple domains, one hopes for a molecular understanding on a deeper level, both structurally and philosophically. Exactly how do domains cooperate with their neighbors to accomplish functions exceeding those conferred by the linear sum of their parts? In this regard, several remarkable structures have greatly increased our understanding.

The structure of the Src tyrosine kinase family members reveals the cooperative nature of Src homology 2 (SH2), 3 (SH3), and kinase domain interactions [1, 2], in which independently folded domains interact through previously recognized ligand binding sites to stabilize an inactive kinase and simultaneously provide a phosphotyrosine-mediated "hair trigger" for kinase activation.

The tyrosine phosphatase SHP-2 structure illustrates how plasticity in domain folding can provide a ligand-dependent conformational switch [3]. By using one of its surfaces to nestle against the phosphatase domain, the N-terminal SH2 domain of SHP-2 blocks the substrate binding cleft, and in the process, allosterically disrupts its own phosphotyrosine binding pocket. Following recruitment to substrates via a C-terminal SH2 domain, engagement of the phosphotyrosine binding surface on the N-terminal SH2 domain simultaneously disrupts the phosphatase binding surface to relieve the inhibition.

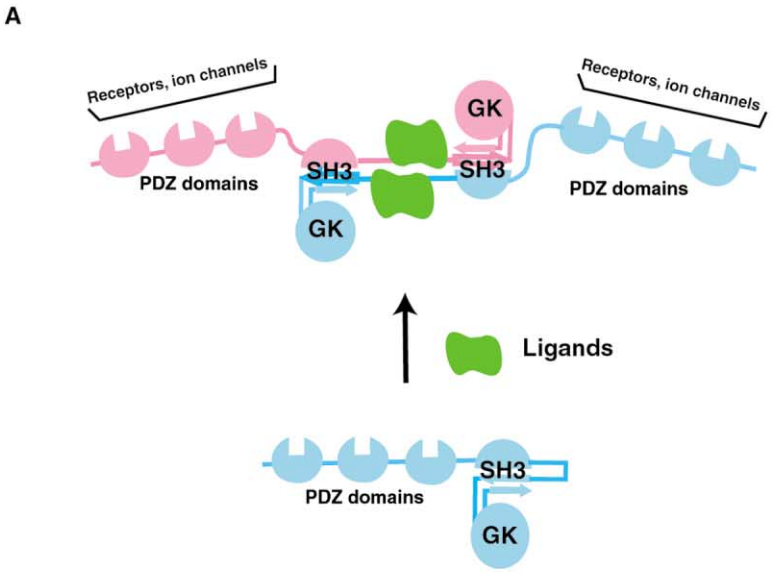
Now with the recently published studies of the SH3 domain and kinase-like domain segments of the membrane-associated guanylate kinase (MAGUK) family member PSD-95 in December's issue of *Molecular Cell* by Tavares et al. and McGee et al. [4, 5], these insights about domain-domain interactions extend into a realm where the boundaries between individual domains become blurred.

PSD-95 is a member of the MAGUK superfamily that clusters ion channels on postsynaptic membranes at excitatory synapses, and also interacts with signaling molecules and the cytoskeleton. In nonneuronal cells, MAGUK proteins such as SAP97, ZO-1, and CASK function as molecular scaffolds to assemble signaling elements at cell-cell junctions. All MAGUK proteins consist of one to three PDZ domains, an SH3 domain, a linker region, and a guanylate kinase-like domain (GK domain) that lacks catalytic activity (Figure, panel A). Several cytoplasmic proteins are known to interact with the GK domain or with the SH3-GK linker region, but the function of the SH3 domain is less clear. A variety of biochemical and yeast two-hybrid experiments, however, have identified intra- and intermolecular interactions between the SH3 and GK domains, despite the fact that the GK domain lacks the canonical PXXP motifs recognized by SH3 domains. The new X-ray structures reveal the structural basis underlying this SH3-GK interaction, and suggest a novel mechanism for PSD-95-mediated oligomerization and receptor or ion channel clustering (Figure, panel A).

The SH3 domain of PSD-95 has an unusually extended fold composed of six β strands rather than five. The first four strands that form the SH3 core, β1–β4, follow sequentially, but the fifth and sixth strands are discontinuous in sequence. The fifth strand is actually contributed from the SH3-GK linker region, and is separated from the β1–β4 core by a long α helix and a flexible loop, while the sixth β strand emerges after the C terminus of the GK domain. This arrangement, which knits the two domains together through the β5/β6 interface (Figure, panel B), essentially tethers the GK domain to the SH3 domain by flanking it with β strand "pincers." Intriguingly, both β5 and β6 make independent contributions to the stability of the SH3 domain fold in guanidine denaturation experiments, yet interaction between the isolated GK and SH3 domains in yeast two-hybrid experiments shows that the β5/β6 strands must be contained on the GK domain fragment [5]. Thus, we have a case where the structure of an isolated domain cannot be disentangled from its domain-domain interactions.

One critical element of this unusual domain-domain interaction seems to be the flexibility of the helix-loop-strand linker (a HOOK domain, in the parlance of Tavares et al.) that allows β5 to fold back against the β1–β4 core. McGee et al. show that artificially stiffening this linker prevents intramolecular SH3-GK interactions, and instead leads to interactions between the SH3 domain in one molecule with the GK domains in another through β strand swapping (Figure, panel A). These authors propose that ligand binding to the linker region in vivo may accomplish the same thing, providing an elegant mechanism for dimerizing PSD-95 molecules at the plasma membrane that would explain its function in clustering receptors and ion channels. This arrangement puts a new twist on oligomerization while maintaining the basic structural principles involved.

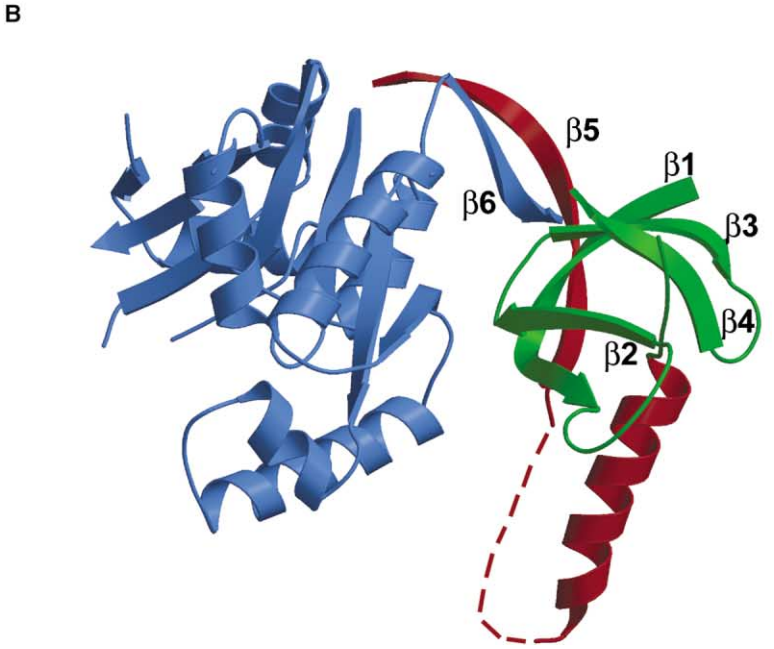
What about the functions normally attributed to SH3 and kinase domains? The peptide binding surface of the PSD-95 SH3 domain lacks a critically conserved tyrosine residue, and the site is sterically occluded by the linker, suggesting that, at least in PSD-95 monomers, binding to poly-Pro ligands may not be an important function. The GK domain lacks several critical residues involved in ATP binding and catalysis, and no kinase function has been demonstrated. But the kinase-like domain may still bind GMP. Although no binding was



Structural Models for PSD-95 Function

(A) Model for PSD-95 dimerization and receptor clustering. Ligand binding to the SH3-GK linker causes swapping of the β5 and β6 strands from an intramolecular GK-SH3 domain complex (bottom) with those from a second PSD-95 molecule (modified from McGee et al.).

(B) Structure of the PDS-95 SH3 linker-GK domain module. The core SH3 fold (green) encompassing β strands 1–4 is completed by two additional β strands coming from the linker domain (red) and the GK domain (blue), respectively.



observed in solution studies by McGee et al., Tavares and colleagues were able to obtain an X-ray structure of a GMP-bound form by judicious use of particular reagents in the crystallization solution. However, major contributions to GMP binding appeared to be contributed by those additives, including a well-ordered MPD (2-methyl-2,4-pentanediol) molecule and two guanidine molecules, one of which hydrogen bonds to the O3 atom of the GMP phosphate. Perhaps these interactions mimic interactions between the GK domain and an as yet unidentified protein that facilitates nucleotide binding. Interaction between the phosphates of bound nucleotides in small G proteins with guanidino groups from Arg "fingers" in GAP molecules, for example, is a well-established part of the catalytic mechanism [6, 7]. The fact that no significant conformational change was observed upon GMP binding, in contrast to what is observed with the catalytically active yeast guanylate kinase, means that additional experiments will be required to determine whether the observed GMP binding is physiologically relevant. Indeed, it is quite conceivable that both the SH3 and GK domains are "dead" in the classic ligand binding sense, and function instead as structural scaffolds upon which protein oligomers are assembled.

What questions do these new structures raise and

what take home messages do these new structures leave us with? In addition to the obvious questions about regulation and ligand binding to the SH3, linker, and GK domains that need to be clarified, it will be exciting to learn whether the PDZ domains also play a role in controlling PSD-95 oligomerization. Do the PDZ domains behave like balls on a string, or are they also structurally tethered to the SH3-GK domains? Does ligand engagement by the PDZ domains facilitate binding of other ligands to the linker to enhance dimerization? Do PSD-95 complexes extend beyond dimers to form higher order oligomers? How are these PSD-95 complexes disassembled during synaptic remodeling?

From a global perspective, the first thing these structures show us is that modular signaling domains contain important alternative binding surfaces that differ from the canonical ligand binding sites. This suggests that other modular domains could also use alternative surfaces for intra- or even intermolecular interactions. Second, they reinforce that spatially compact domains can form from sequentially disparate elements in multidomain proteins. Linking domain structure to domain-domain interactions allows novel mechanisms for protein function to emerge, including the type of regulated assembly of multiprotein complexes postulated for PSD-95. In addition, these observations raise provocative questions about cooperative folding transitions in multidomain proteins. Rather than observe a folding landscape populated by individual domain-folding events, the PSD-95 structure suggests that one should see a single major global folding (or unfolding) transition. This may explain how mutations within a single isolated domain of a multidomain polypeptide can result in global protein instability and human disease.

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Selected Reading

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Structure, Vol. 10, January, 2002, PII S0969-2126(01)00702-X

Phosphoserine-Dependent Regulation of Protein-Protein Interactions in the Smad Pathway

Transforming growth factor β (TGF-β) is a secreted growth factor that regulates the transcriptional program of cells via heteromeric complexes of transmembrane type II and type I Ser/Thr kinase receptors and the Smad intracellular signal transduction pathway [1]. The activity of this pathway is tightly controlled by serine phosphorylation, which plays a key role in regulating protein-protein interactions that are critical in the elaboration of transcriptional responses.

At the receptor, signaling is initiated by the phosphorylation of the Gly-Ser (GS) region of the type I receptor. This phosphorylation leads to docking of a special class of Smads called receptor-regulated Smads (R-Smads) [2]. Regulation of R-Smads by type I receptors is quite specific. R-Smad2 and R-Smad3 are phosphorylated by activin and TGF-β receptors, whereas R-Smad1, R-Smad5, and R-Smad8 are regulated by BMP receptors. In the case of TGF-β and activin pathways, activation of Smad2 and Smad3 is facilitated by the membrane-anchoring protein SARA, which preferentially binds unphosphorylated Smad2 and Smad3. Once recruited to the receptor, R-Smads are phosphorylated by the type I receptor kinase on the last two serines of a carboxy-terminal SSXS motif that is found in all R-Smads. This signals a dramatic shift in R-Smad protein partnering such that phosphorylated R-Smads dissociate from the receptor and SARA and assemble into either homomeric complexes or heteromeric complexes with the common Smad, Smad4 [3]. The Smad complex then translocates to the nucleus, where it can regulate transcription and protein stability. Thus, serine phosphorylation in this pathway functions not only to induce protein-protein interactions, but also causes their dissociation.

So how does serine phosphorylation positively and negatively regulate such diverse protein-protein interactions? Two papers appearing in the December issue of *Molecular Cell*, by Qin et al. and Wu et al., have begun to address this issue by showing that the Smad MH2 domain contains a phosphoserine binding region [4, 5]. Further, they show that this region plays a role in stabilizing the assembly of Smad homotrimers by interacting with the phosphorylated tail region of an adjacent Smad MH2 domain.

The phosphoserine binding region of the Smad MH2 domain is composed of a loop (loop 3) which emerges from a region that is enriched in basic residues, the so-called basic patch (Figure). As might be expected, the basic patch forms an extensive network of hydrogen bonds with the two phosphate groups that are present on the phosphorylated tail of the adjacent Smad MH2