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Communication by helix

Understanding the complex web of intracellular signalling pathways is brought a step closer by determinations of the structure of the SH3 domain/peptide ligand interface.

THE cells that make up a multicellular organism are able to sense and respond to a vast number of signals in their environment. These signals may direct them to proliferate, alter their architecture or their metabolism, differentiate or even die. This barrage of information must be integrated and acted upon so that the cell, and as a consequence the whole organism, can continue functioning normally. How is this maze of biochemical pathways coordinated? The answer, at least for a small part of the network, seems to reside in two small independently folded protein modules, the Src-homology domains SH2 and SH3. These motifs — found in a wide range of proteins involved in signalling and, for SH3, in the cytoskeleton — are suggested to act as molecular adaptors linking and regulating the location and/or activity of molecules such as tyrosine kinases and small GTP-binding proteins. The nature of the interaction between either the SH2 and/or SH3 modules and their protein ligands — which may themselves contain SH2 and SH3 domains — is likely to be critical in determining the fate of the signal and the subsequent action taken by the cell. The first glimpse of an SH3/peptide ligand interface is presented in this month's *Nature Structural Biology*[1] and in a recent paper published in *Cell*[2].

A number of protein ligands that interact with various SH3 domains have been characterized and the binding site in each case has been localized to a sequence rich in proline residues. Similar results have been obtained from screens of combinatorial peptide libraries. At the same time several structures of SH3 domains in the absence of ligand have been determined[3], and these reveal that the core of the domain is formed by two three-stranded β-sheets which are perpendicular to one another. The most highly conserved residues form a hydrophobic platform nestling between two loops of variable length that connect strands in the sheets. The conservation of the hydrophobic residues in the platform together with NMR experiments have suggested that this pocket is the ligand binding site, a suggestion that is now confirmed[1,2].

Musacchio and colleagues have determined the X-ray crystal structures of the SH3 domains of the tyrosine kinases Abl and Fyn, either alone or complexed with proline-rich peptide from the protein ligands 3BP-1 and 3BP-2, respectively. The two different bound peptides adopt very similar conformations, the majority of the residues adopting a left-handed polyproline II helix (PPII) conformation, as is seen in the NMR structure of the phosphatidylinositol-3-OH kinase SH3 domain bound to peptide selected from a combinatorial library[2]. PPII helices are ideally suited as a target for SH3 domains. Because of their lack of internal hydrogen bonding and dependence on backbone solvation for stability, they are, for the most part, found on the surfaces of proteins making them effective substrates for protein–protein-mediated interactions.

No dramatic conformational changes are seen in the structures of any of the SH3 domains on binding ligand, suggesting that shape complementarity is important for the interaction between the protein motifs and the proline-rich peptides. The PPII helix, almost twice as long as an α-helix with the same number of residues, fits comfortably into the long and shallow groove of the SH3 binding pocket. The PPII helix has three residues per turn; every fourth residue (that is, residues *i* and *i*+3) sticks out from the peptide in roughly the same direction to form three groups of residues with similarly orientated side chains. Two of these groups of residues, all prolines in the case of 3BP-1 (*i* and *i*+1; *i*+3 and *i*+4), and equivalent residues in the NMR structure, interdigitate with two of the conserved aromatic residues in the ligand-binding site[4]. The prolines *i*, *i*+3 are known from mutagenesis studies to be particularly important for binding. Indeed comparison of the sequences of known SH3 binding motifs reveals that the PXXP (*i*, *i*+3) motif is found in all cases.

To avoid hopelessly tangling up the signal transduction network in the cell, it is important that signalling molecules and ancillary factors are recruited to the right proteins on the pathway. The van der Waals interactions involving the conserved proline residues are seen in all the complexes, and although these interactions are likely to provide most of the binding energy for complex formation, they provide little specificity of binding. Musacchio *et al.* note that the equivalent hydrogen bonds in the Abl and Fyn SH3 complexes are directed to the main-chain atoms of the peptides and are therefore not specific for the peptide sequence. However, these hydrogen bonds may be important in selecting the orientation of binding of the ligand.

The two studies come to somewhat different conclusions as to what is likely to dictate the specificity and therefore the effector function of the many SH3 domains in signalling and cytoskeletal proteins. Yu *et al.* suggest that non-proline residues on the two faces of the PPII helix that can interact with the SH3 domain may be able to make complementary interactions with the more variable residues in the vicinity of the ligand-binding site, and particularly with residues in the two variable loops that border the hydrophobic platform. For example, they observe two salt bridges between the first and sixth residues of the ligand and these loops. Mutation of the residues in SH3 and disruption of the salt bridge result in a loss of binding affinity, by fiftyfold in one case[2]. On the other hand, Musacchio *et al.* detect no non-proline interactions in the Abl or Fyn SH3 complexes. They point out that the SH3 domain may not be a functionally independent unit but may need to interact with SH2 or other SH3 domains to be fully functional. Perhaps there are still other factors to be taken into account in the analysis of these adaptor proteins: what, for example, is the role of another domain, the plekstrin-homology domain[5], which also occurs in the same families of proteins, and how does this motif influence intracellular signalling?

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