Cell PRESS
Structure
Previews

Fibronectin Structure: A New Piece of the Puzzle Emerges

Richard J. Bingham[1] and Jennifer R. Potts[2,*]
[1]Department of Chemical and Biological Sciences, University of Huddersfield, Queensgate, Huddersfield, HD1 3DH, UK
[2]Departments of Biology and Chemistry, University of York, York, YO10 5YW, UK
*Correspondence: jp516@york.ac.uk
DOI 10.1016/j.str.2010.05.003

In the structure of the gelatin binding domain (GBD) of fibronectin reported by Graille et al. (2010), both the zinc-mediated dimerization and the rearrangement of 8F_I from the canonical F_I fold are unexpected, suggesting interesting new directions for researchers in the field.

Fibronectin is an ~220 kDa (monomer molecular weight) protein that is found in a dimeric soluble form in body fluids, including plasma, and in an insoluble multimeric form as part of many extracellular matrices. It contains binding sites for a wide range of molecules including cell surface receptors such as integrins. It is thought to play an important role in processes that involve cell migration (such as wound healing and development), and emphasizing its importance in embryogenesis, fibronectin null mutations in mice are embryonic lethal (George et al., 1993). In addition to its physiological roles, a number of pathogenic bacteria appear to use fibronectin as a bridge to the host cell surface to adhere to and to invade human cells (Schwarz-Linek et al., 2004). Fibronectin is a modular protein and contains three types of modules that were first identified in fibronectin (fibronectin type I, II, and III modules) and have since been found in other proteins. Traditionally, due to proteolytic stability, fibronectin has been described as containing an N-terminal domain (NTD) (a string of five type I modules; F_I) followed by a gelatin binding domain (GBD) (containing type I and II modules, I-II-II-I-I-I) at the N terminus of the molecule and then a string of type III modules making up the central region; there are three more type I modules at the C terminus (Potts and Campbell, 1994). Iain Campbell's laboratory at the University of Oxford pioneered the high-resolution structural characterization of fibronectin modules with the structure of the seventh type I module (determined using nuclear magnetic resonance [NMR] spectroscopy), published in 1990 (Baron et al., 1990). Since then, the structure, dynamics, and ligand-binding of many single modules, module pairs, or larger constructs have been published.

This dissection approach, using studies of single modules and module pairs, allowed a picture of fibronectin structure and function to be partially assembled. However, especially in the GBD, where the structure of a triple module fragment (I-II-II), published in 2001 (Pickford et al., 2001), showed for the first time a nonlinear arrangement of fibronectin domains (Figure 1A), it was clear that the structures of larger fragments would be required to take the picture nearer to completion. However, as the size of the fragments increases, the NMR structural studies become more challenging and intermodule flexibility is likely to hamper structure determination using crystallography. So the article, in this issue of *Structure*, from Marc Graille, Herman van Tilbeurgh, and coworkers (Graille et al., 2010) of the structure of the intact GBD as a zinc-mediated dimer is an important advance. The fact that key features of this structure are unanticipated is very exciting, as they will provide new directions, and perhaps some explanations, for researchers in the field.

The rearrangement of the eighth type I module in the GBD dimer is unexpected. Previously, structures had been determined of all the type I modules from the NTD and GBD as single modules or within module pairs (sometimes in complex with a peptide). Thus, the canonical type I fold of a short antiparallel double-stranded β sheet that forms a sandwich with an antiparallel triple-stranded β sheet enclosing a small hydrophobic core was well established. In the module pairs, inter-type I interfaces (even most recently in the $^{8}F1^{9}F1$ module pair (Erat et al., 2009)) involve a tandem arrangement in which the individual type I structures are maintained and a relatively small amount of surface area is buried (Figure 1B). None of these structure determinations, as far as we are aware, hinted at the ability of the first β sheet of a type I module to be completely subsumed into a larger and rearranged triple-stranded sheet, as observed in the GBD dimer. Whether this is a process determined by the presence of three tandemly arranged type I modules, interactions with the other GBD modules, zinc-binding, and/or dimerization is yet to be determined. Certainly this study demonstrates very clearly the importance of expanding the structural and functional studies of fibronectin to larger fragments.

The important role of zinc in the rearranged GBD dimer is also unexpected, although previously published data have suggested a role for cations in cryptic proteolytic activity detected in fibronectin (Houard et al., 2005; Schnepel and Tschesche, 2000). Here, the authors show that the rearrangement of 8F_I forms zinc-binding sites that are not present in the canonical F_I fold; two of these sites involve histidine residues previously implicated in proteolytic activity (Houard et al., 2005). The GBD modules, and specifically IGD sequences in 7F_I and 9F_I, are involved in the cell migration-stimulating activity of migration stimulating factor (MSF), a truncated isoform of fibronectin produced by breast cancer cells and associated fibroblasts (Millard et al., 2007; Schor et al., 2003); the potential role of zinc, and the concomitant structural changes in 7-9F_I that occur on zinc binding, on this process can now be tested. Importantly, fibronectin is a compact dimer in solution with intramonomer and/or intradimer interactions

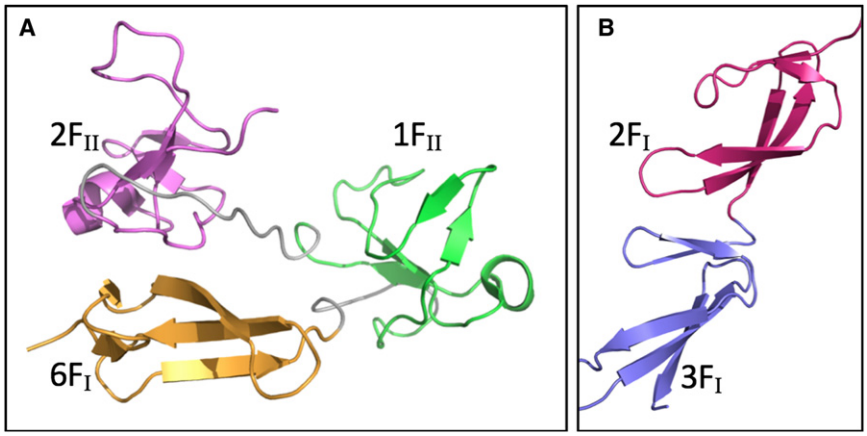

Figure 1. Intermodule Interfaces in the GBD and NTD of Fibronectin
(A) Ribbon diagram of the gelatin binding domain of fibronectin determined by NMR showing the nonlinear arrangement of 6F$_{I}$-1F$_{II}$-2F$_{II}$ domains (PDB accession code 1E8B).
(B) Ribbon diagram of the linear module pair interaction between the second and third F$_{1}$ domains (PDB accession code 2CG7).

that are likely to play an important regulatory role in fibronectin function. That is, in this compact state, many of the key binding sites appear to be somewhat cryptic and only exposed as a result of conformational changes during processes such as fibril assembly (Mao and Schwarzbauer, 2005). The demonstration that two GBD monomers can dimerize in the presence of zinc suggests an additional mechanism of regulation.

Of course, more work has yet to be done to understand the physiological relevance of the zinc-mediated dimerization, in particular to determine whether it occurs at zinc concentrations found in relevant tissues. Overall, the potential for zinc-mediated dimerization and reorganization of 8F$_{I}$ demonstrated by this exciting structure will need to be considered in future studies of fibronectin and MSF function.

REFERENCES

Baron, M., Norman, D., Willis, A., and Campbell, I.D. (1990). Nature *345*, 642–646.

Erat, M.C., Slatter, D.A., Lowe, E.D., Millard, C.J., Farndale, R.W., Campbell, I.D., and Vakonakis, I. (2009). Proc. Natl. Acad. Sci. USA *106*, 4195–4200.

George, E.L., Georges-Labouesse, E.N., Patel-King, R.S., Rayburn, H., and Hynes, R.O. (1993). Development *119*, 1079–1091.

Graille, M., Pagano, M., Rose, T., Ravaux, M.R., and van Tilbeurgh, H. (2010). Structure *18*, this issue, 710–718.

Houard, X., Germain, S., Gervais, M., Michaud, A., van den Brûle, F., Foidart, J.-M., Noël, A., Monnot, C., and Corvol, P. (2005). Int. J. Cancer *116*, 378–384.

Mao, Y., and Schwarzbauer, J.E. (2005). Matrix Biol. *24*, 389–399.

Millard, C.J., Ellis, I.R., Pickford, A.R., Schor, A.M., Schor, S.L., and Campbell, I.D. (2007). J. Biol. Chem. *282*, 35530–35535.

Pickford, A.R., Smith, S., Staunton, D., Boyd, J., and Campbell, I. (2001). EMBO J. *20*, 1519–1529.

Potts, J.R., and Campbell, I.D. (1994). Curr. Opin. Cell Biol. *6*, 648–655.

Schnepel, J., and Tschesche, H. (2000). J. Protein Chem. *19*, 685–692.

Schor, S.L., Ellis, I.R., Jones, S.J., Baillie, R., Seneviratne, K., Clausen, J., Motegi, K., Vojtesek, B., Kankova, K., Furrie, E., et al. (2003). Cancer Res. *63*, 8827–8836.

Schwarz-Linek, U., Hook, M., and Potts, J.R. (2004). Mol. Microbiol. *52*, 631–641.

Topoisomerase IB-DNA Interactions: X Marks the Spot

Lynn Zechiedrich[1] and Neil Osheroff[2,*]
[1]Department of Molecular Virology and Microbiology, Verna and Marrs McClean Department of Biochemistry and Molecular Biology, Department of Pharmacology, Baylor College of Medicine, Houston, TX 77030, USA
[2]Departments of Biochemistry and Medicine, Vanderbilt University School of Medicine, Nashville, TN 37232, USA
*Correspondence: neil.osheroff@vanderbilt.edu
DOI 10.1016/j.str.2010.05.002

The observation made twenty years ago that type IB topoisomerases bound DNA helix-helix juxtapositions was unexpected, given the controlled helical rotation mechanism of the enzyme. In this issue, Patel et al. (2010) provide an elegant structural explanation for this interaction.

Topoisomerases are essential enzymes that regulate DNA supercoiling and remove knots and tangles from the genome (Leppard and Champoux, 2005; Liu et al., 2009; Deweese and Osheroff, 2009). Type I topoisomerases act by creating transient single-stranded breaks in the double helix, followed by passage of the opposite intact strand through the break (type IA)