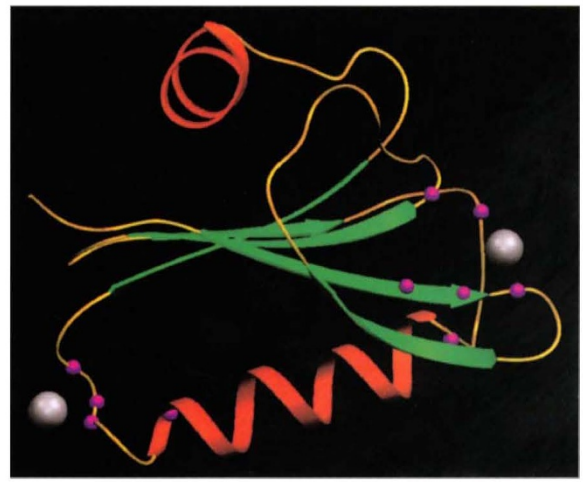
Interacting with actin



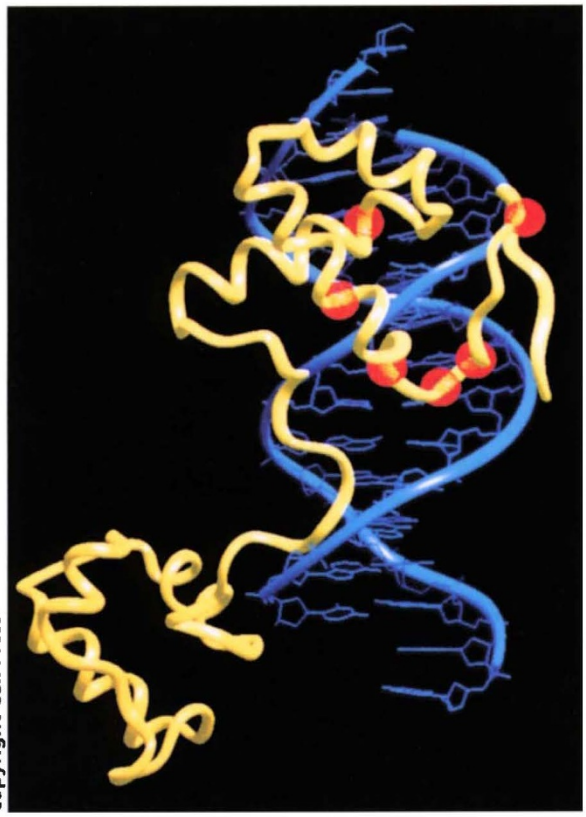
The dynamics of the actin cytoskeleton, crucial for cell motility, shape determination, cytokinesis etc, are controlled by a collection of actin-binding proteins. With the determination by NMR of the structure of part of severin, a protein which binds to and fragments actin filaments (Schnuchel, A., Wiltscheck, R., Eichinger, L., Schleicher, M. & Holak, T.A., *J.molec.Biol* **in the press**), structural principles unifying these proteins is beginning to emerge.

Severin is a member of a family of actin binding proteins which consist of a tandem arrangement of homologous domains which have developed slightly modified activities. Severin has three such domains, as does fragmin, while gelsolin and villin have six. Although the first domain of severin can cap the ends of actin filaments the second domain is required to sever them. It is therefore assumed that the second domain binds to the side of the actin filaments while the first is forced into the filament disrupting it. Calcium has been implicated in the regulation of this activity and calcium-induced changes in severin's NMR spectrum has identified residues (purple in figure) which may bind calcium ions (grey).

Comparison of the structures of severin-like proteins and the non-homologous profilins show that these two groups share a common three-layered motif: a β-sheet sandwiched between two α-helical portions, one of which varies from protein to protein. How these elements are arranged in the amino-acid sequence and the number of strands in the β-sheet is not, however, the same. It therefore seems likely that a range of unrelated actin-binding proteins will share a common molecular architecture.

Pax and paired

The Pax proteins play a vital role in the development of vertebrates; mutations in the *PAX* genes of humans cause genetic diseases such as Waardenburgs's syndrome and aniridia. The Pax proteins are sequence-specific transcription factors; they share a common DNA-binding motif, the so-called paired domain (prd: also found in a number of homeobox- proteins; the *Drosophila paired* and *gooseberry* proteins being archetypal examples). The structure of the prd domain (from *paired*: Xu, W. *et al. Cell* **80**, 639-650, (1995)) now provides a structural framework for understanding the known Pax developmental mutants.



The X-ray crystal structure reveals that it is the highly conserved N-terminal subdomain of prd (upper part of figure) which latches to the *in vitro* optimized 15 base-pair DNA binding site. An N-terminal β-sheet interacts with the phosphate backbone and is followed by a β turn which fits neatly into the minor groove, making a number of base-specific contacts. The adjacent helix-turn-helix (HTH) motif consists of three helices and is similar to the homeobox and Hin recombinase HTH motifs; helices 1 and 2 makes extensive phosphate backbone contacts and helix 3 (the 'recognition' helix) nestles deep in the major groove, making a series of base-specific contacts. The C-terminal tail of the N-terminal subdomain also binds in the minor groove, adjacent to the β turn. Beyond this, the less well conserved C-terminal subdomain does not interact with the DNA in this structure (lower left), even though it also bears a strong resemblance to the HTH motif of both the homeodomain and the Hin recombinase.

The figure indicates the locations (red spheres) of missense mutations in the Pax proteins of both mice and man: all map to the interface between DNA and the N-terminal sub-domain of the prd domain; three of the mutations are found in the β turn, a region which is conserved among Pax proteins. While the C-terminal subdomain seems to have little or no function in DNA binding in the Drosophila *prd* protein the domain does seem to have an important role in DNA binding in other prd domain-containing proteins, such as mouse Pax-5 and the human PAX-6 proteins.