

The Fimbrin and Alpha-Actinin Footprint on Actin

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A satisfying experience in science occurs when information from several areas converge to give a big picture about an important problem. In three papers (Holtzman et al., 1994; Honts et al., 1994; McGough et al., 1994) in this issue of *The Journal of Cell Biology*, the disciplines of genetics and structural biology have revealed that two actin cross-linking proteins, fimbrin and alpha-actinin, bind to the same region on actin. Because both proteins belong to a superfamily of actin cross-linking proteins, we have some confidence that other members of the superfamily, including filamin, spectrin, dystrophin, and ABP-120, also bind the same region of actin. This information about binding sites is an important step toward understanding how actin filaments are organized by cross-linking proteins into bundles and supramolecular networks.

This story is rooted in the classic biochemical studies of muscle proteins. Alpha-actinin was first purified from skeletal muscle (Maruyama and Ebashi, 1965) and characterized as a Z-line component; its rodlike shape was revealed by electron microscopy (see Meyer and Aebi, 1990). Later, spurred by cell biologists who demonstrated that cytoplasmic extracts of nonmuscle cells could undergo reversible sol-gel transformations, a generation of biologists isolated and characterized a number of actin gelation and bundling proteins, including ABP-280 and filamin, spectrin, fascin, and ABP-120, from amoeba (reviewed in Pollard and Cooper, 1986; Stossel et al., 1985; Weeds, 1982); alpha-actinin was rediscovered as a nonmuscle actin cross-linking protein (Burridge and Feramisco, 1981). During the late 1980's, cDNA sequencing showed that many of these gelation factors belonged to a superfamily that shares a common 27-kD NH₂-terminal domain (Baron et al., 1987) that binds actin filaments. Fimbrin bundles actin filaments in intestinal brush border microvilli, and it is also located where actin bundles terminate at membrane adhesion plaques (Bretscher and Weber, 1980). The sequence databases (de Arruda et al., 1990) showed that fimbrin was also involved in cell transformation (Lin et al., 1994), and that it was a target of phosphorylation when leukocytes are activated by growth factors and mitogens (Zu et al., 1990). Hence, fimbrin is also called plastin and pp70.

An important structural theme emerged from the sequences: cross-linking proteins in this superfamily are

modular, composed of a pair of actin-binding domains, a variable number of spacer domains, and sometimes a calmodulin-like calcium-binding domain (Matsudaira, 1991). The spacer domains come in two flavors, three-helix bundles, and cross-strand beta sheets, and the number of domains determines the length of the cross-link. Both motifs were first predicted by sequence analysis, and the helical nature of the repeats in spectrin was recently confirmed by x-ray crystallography (Yan et al., 1993). A crystal structure of the beta sheet motif has not yet been reported. Fimbrin lacks the spacer domains; its actin-binding domains lie in tandem. Electron microscopy of two-dimensional crystals of alpha-actinin revealed the modular organization (Taylor and Taylor, 1993). Consistent with earlier chemical cross-linking and antibody labeling studies, the 25-Å map shows that alpha-actinin is an antiparallel homodimer; its actin-binding domains lie at opposite ends of the rod-shaped molecule, and lateral interactions between the alpha-helical domains maintain the molecule as a dimer. The structure agrees with the functionality, an actin cross-linking protein must have two binding sites, one for each actin filament.

Given this understanding of the overall structure of cross-linking proteins, we would like to know how they bind actin. Because the actin binding domains in different proteins of this superfamily are homologous, do they bind identical sites on actin filaments? The answer from structural studies of alpha-actinin and genetic studies of yeast fimbrin is most likely yes. McGough et al. (1994) studied the structure of actin filaments decorated with the 27-kD actin-binding domain of alpha-actinin. Their helical reconstruction at 21 Å resolution shows that the domain is located primarily on the outside edge of F-actin. After fitting the crystal structure of the actin monomer into their model, McGough and colleagues saw that alpha-actinin interacts with actin subdomain two and a part of subdomain one. The 27-kD domain also contacts the bottom of subdomain one in the next actin subunit along the filament (Fig. 1). This observation is consistent with chemical cross-linking and protein-binding studies (Fabrizio et al., 1993; Lebart et al., 1993; Mimura and Asano, 1987) that had previously implicated both actin subdomains as binding sites for various members of this actin cross-linking superfamily.

Genetic studies on fimbrin in yeast also point to the same region on actin as a binding region. One advantage of a genetic approach is that a large number of mutated actin molecules can be examined, especially when the viability of

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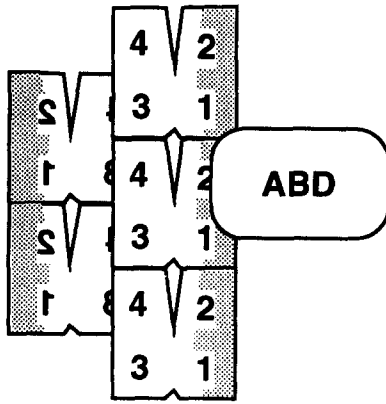


Figure 1. A schematic diagram showing the region of actin where actin-cross-linking proteins bind. The actin subunit is divided into four subdomains (1–4), and it is helically arranged to give front and back views of subunits on opposite strands in a filament. The actin-binding domain (ABD) of alpha-actinin overlays actin subdomain one and two of one subunit and subdomain one of the neighboring subunit. The shaded region shows where genetic and biochemical studies have identified an actin-binding region.

yeast is used as a biological readout. Fimbrin was discovered in yeast independently by Alison Adams as a gene (*SAC6*) that suppresses a temperature-sensitive mutation in Pro32 of actin and by David Drubin as a yeast actin-binding protein (ABP 67 or *Sac6p*) when they were in David Botstein's lab (Stanford University School of Medicine, Stanford, CA) (Adams et al., 1989). Both have now determined where fimbrin binds actin. Their studies were greatly aided by Ken Wertman, also from the Botstein lab. He created 36 charged-to-alanine actin mutants in which charged residues were replaced with alanine residues (Wertman et al., 1992). Honts et al. (1994) tested which of these alanine mutants, as well as other actin ts mutants, were suppressed by mutant alleles of yeast fimbrin (*SAC6*). In total, eight actin mutants were uncovered. Seven mutants were in actin subdomain two and one was in subdomain one. The same alanine mutants were used in a different approach by Holtzman et al. (1994). Based on the observation that *SAC6* is synthetically lethal with two yeast cytoskeletal genes, *ABP1* and *SLA2*. (Adams et al., 1993; Holtzman et al., 1993), they reasoned that synthetic lethality could be mimicked by actin mutations in a fimbrin-binding site. Holtzman and co-workers went on to identify alanine mutants of actin that were synthetically lethal with null mutants of *ABP1* and *SLA2*. Two mutants, one in subdomain one and the other in subdomain two, were isolated. The same mutants were also identified in the suppressor analysis study. Together, the two studies tested 25 different mutations that were distributed throughout the actin sequence. This large number of mutants would be difficult to characterize thoroughly in a biochemical study. The correspondence between genetic and structural studies is excellent, and their complementary findings point to subdomains one and two as an actin-binding region for this superfamily of proteins.

As we learn more about actin, we come to realize that the outer edge of actin is becoming quite crowded. Besides a binding site for fimbrin and alpha-actinin (and by homology spectrin, dystrophin, filamin, and ABP-120), it is also the

binding area for myosin (Rayment et al., 1993; Schroder et al., 1993), and probably a binding region for domain two of villin and gelsolin (Pope et al., 1994; Way et al., 1992). The overlap among binding sites indicates great potential for competitive binding, a bad situation for an enzyme but not for a structural protein. Fortunately, f-actin is a polymer, and any binding site is repeated along the length of a filament. These studies now uncover how little we know about the other half of the interaction, that is, where does actin bind to these proteins? Protein biochemistry has already started us down this path, and in the coming years, we would hope to learn about the detailed molecular interactions between this domain and actin, information that comes from x-ray crystallography. At another level of three-dimensional structure, we would like to see a cross-linking protein bridge a pair of actin filaments. But as we have experienced before, such studies will take time and, in the interim, we can certainly learn much by other approaches. The analysis of fimbrin mutants in yeasts should quickly fill in many of the details.

Received for publication 17 May 1994 and in revised form 1 June 1994.

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