Actin Constitution: Guaranteeing the Right to Assemble

Kenneth F. Wertman* and David G. Drubin†

Actin is a highly exploited protein. Its polymeric filaments can be tailored to serve many needs. For example, the actin filaments that underlie skeletal muscle contraction are long-lived and of uniform length, and are organized into nearly crystalline arrays. In contrast, the actin filaments that underlie the movement of amoeba continuously assemble and disassemble, are variable in length, and are arranged in apparently disordered meshworks. The recent description of the atomic structure of actin (*1*), and the use of these data to develop a detailed model of the actin filament (*2*), provide for the first time a structural basis for understanding these types of functional differences. To this end, we present here a synthesis of functional comparisons of naturally occurring actin variants and genetically engineered actin mutants.

Actin is composed of two peanut-shaped domains of approximately equal size, lying side-by-side and connected at one end by two short crossovers of the peptide backbone. The result is a hinged molecule with a deep cleft. Within this cleft reside the binding sites for actin's essential cofactors: an adenosine nucleotide [adenosine triphosphate (ATP) or adenosine diphosphate (ADP)] and a divalent metal ion. The nucleotide and metal ion make extensive contacts with both domains, greatly increasing connectivity between them. This explains the stabilizing effect of these cofactors on the actin monomer in solution and might provide the structural basis for the greater positive effect of ATP than ADP on assembly (*3*); perhaps ATP more optimally positions the two domains for assembly than does ADP.

The primary structure of actin is highly conserved (yeast and rabbit skeletal muscle actin are 88% identical in amino acid sequence), implying a large biological constraint on structure. An approach we (*4*) and others (*5*) have employed to identify structural aspects of actin that are important for function is to make targeted mutations in actin and determine the effects of the mutations in vivo. Studies of this sort are most informative when the functions of the mutant actin can be tested in a biochemical as well as biological context. *Drosophila* flight muscle and budding yeast each express a single actin, and for each organism it is possible to replace the wild-type actin with a genetically engineered copy. At present, only yeast (*6*) and *Dictyostelium* (*7*) provide opportunities for the isolation of biochemically useful amounts of pure mutant actin.

Figure 1 shows the positions and phenotypic effects of a set of mutations made in yeast actin by replacement of charged residues with alanine (*4*). The mutations are mapped onto the atomic structure of rabbit actin. Substitution of different amino acids in actin resulted in various phenotypes: recessive lethal (red), dominant lethal (blue), temperature sensitive for growth (yellow), and wild type (green). This strategy effectively enriched for replacements that are on the surface of the monomer and so are unlikely to affect monomer folding. Many positions on the surface of actin are important for function, but they vary considerably in the severity of the phenotypes imparted by their disruption. The 7 of the 36 alleles that did not show a mutant phenotype identify regions of the molecule that do not make essential interactions.

What portions of the protein can be changed without affecting the fundamental properties of actin? The protozoan *Tetrahymena* has helped to answer this question. *Tetrahymena* actin is among the most divergent of actins, showing only 75% identity in amino acid sequence to rabbit muscle actin (*8*). In Fig. 2, the residues that are replaced in *Tetrahymena* by nonconserved amino acids are colored orange on rabbit actin. Comparison of Fig. 1 to Fig. 2 reveals that residues essentials for function in yeast are conserved in *Tetrahymena*.

Because actin is only known to function

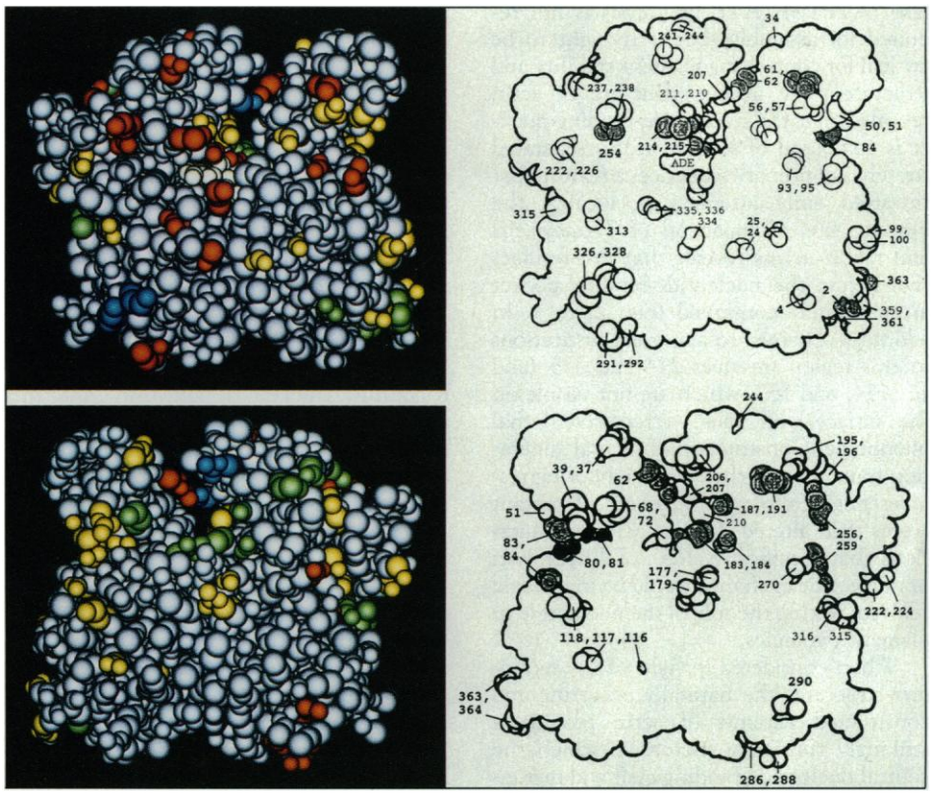

Fig. 1. Actin and the effects of mutations. The front (top) and back (bottom) of rabbit actin (*1*) are shown. Colors of residues (identified at right) indicate the growth phenotype of yeast cells expressing an actin isotype generated by mutating that residue (*4*). Red, recessive lethal; yellow, temperature-sensitive; green, wild type; blue, dominant lethal. Numbers separated by commas indicate residues that were changed simultaneously to yield single alleles (*4*). Shading is used to distinguish closely juxtaposed atoms of different amino acids. ADE, the base portion of the adenine nucleotide.

The authors are in the Department of Molecular and Cell Biology, University of California, Berkeley, CA 94720.

*Present address: Selectide Corporation, Tucson, AZ 85737.
†To whom correspondence should be addressed.

as a polymer, the most fundamental interaction that actin makes is arguably with other monomers within a filament. The actin filament can be described as a two-stranded helix, with the strongest contacts occurring along each of the two strands. At its present resolution, the filament model predicts the monomer-monomer interfaces that make contributions to filament stability. The strong contacts are thought to be made along the top and bottom of the monomers as oriented in the diagrams. The residues implicated as participating in polymerization (*2*) are not divergently substituted in *Tetrahymena* actin. Indeed, *Tetrahymena* and rabbit actin can coassemble into hybrid filaments (*9*). In addition, mutations in yeast actin (residues 37, 39, 61, 62, 205, 244, 286, and 288) that affect these contact surfaces all seriously compromise actin function (*4*). Together, the above observations confirm the importance of the regions that the filament model implicates in making monomer-monomer contacts. Biochemical and ultrastructural analysis of actin filaments formed from the mutant actins will test the contributions of specific residues to filament stability and structure.

Actin is both a structural protein and an assembly-stimulated adenosine triphosphatase (ATPase). ATP hydrolysis is not required for assembly, but is thought to be critical for controlling filament stability and structure. The atomic structure of actin revealed that the nucleotide-binding pocket is composed of several widely separated stretches of primary sequence, a feature that thwarted early attempts to localize the binding site. Comparison of *Tetrahymena* and rabbit actins reveals that the residues that define the nucleotide-binding pocket are absolutely conserved (Fig. 2, top). In addition, charged-to-alanine substitutions in this region [residues 214 and 335 (and 11, 154, and 157, which are not visible on the surface)] all cause a recessive lethal phenotype. Construction of second generation mutations in these or neighboring residues might allow the recovery of mutant actins with altered ATPase activity. Mutations that specifically alter ATP hydrolysis or nucleotide exchange would be invaluable tools for testing the role of the nucleotide in filament dynamics.

When considered in light of the monomer structure, the naturally occurring and constructed variants of actin provide a strikingly consistent picture in which the central nucleotide-binding cleft and regions involved in monomer-monomer contacts are conserved and immutable. What is less clear, but is of central importance to understanding how actin functions in many different processes, is an appreciation of where and how actin-binding proteins, which are necessary for many of the functions of actin, exert their effects.

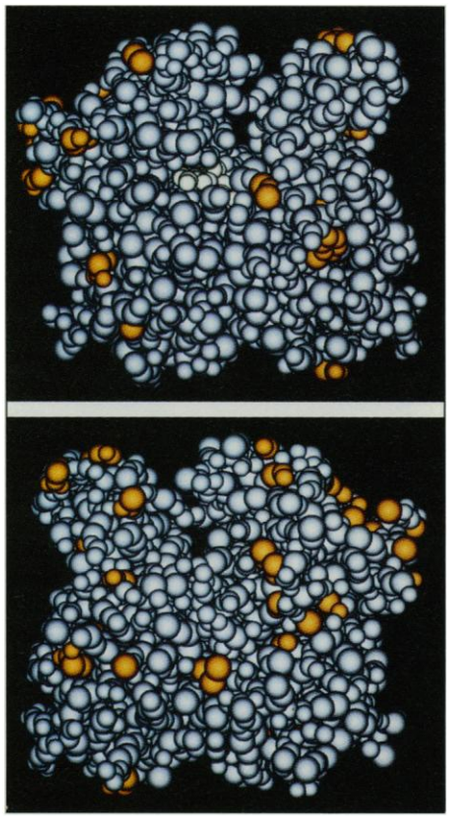

Fig. 2. Nonessential residues in actin. Rabbit actin structure (*1*) with orange residues at sites in which *Tetrahymena* actin contains nonconservative substitutions.

Tetrahymena actin retains the essential features of ATP hydrolysis and assembly, but it is defective in interactions with rabbit α-actinin, chicken tropomyosin, and the actin-binding drug phalloidin (*9*). Although some information about the interactions of binding proteins and drugs with actin can be gained from examination of *Tetrahymena* actin and other naturally occurring actin variants, this type of analysis will be especially powerful when applied to the study of engineered mutations that allow selection of the precise position and chemical properties of the amino acid replacements. For example, several charged-to-alanine mutations change residues within the myosin footprint (residues 2, 4, 24, 25, 99, 100, 358, 361, and 363) as identified by various chemical crosslinking and electron microscopic studies (*2*). Residues that are functionally important for the actin-myosin interaction can now be identified by analyzing the ability of myosin to translocate filaments assembled from these mutant actins (*6*). In addition, mutant filaments that fail to move or that demonstrate altered translocation kinetics would be invaluable in dissecting the molecular mechanics of contraction. Because functional assays exist for most of the actin-binding proteins, this approach can be used to create a detailed functional map of the filament surface.

A functional map of the filament will allow the development of predictive models for the mechanisms of actin-binding protein function and will serve as a powerful tool for examining the in vivo roles of binding proteins. For example, genetic selection could be used to identify ways in which cells can compensate for a mutation that eliminates the binding of tropomyosin. Finding that such a mutant could be rescued by inactivation of a filament-severing protein or reduction of actin's intrinsic ATPase would provide important clues about tropomyosin's in vivo function and identify functional relations between binding proteins. Actin's functional repertoire cannot, however, be fully probed while working within the context of a single cell type. Thus, it is fortunate that complementary studies employing different organisms—*Drosophila* and *Dictyostelium*—are in progress. From these studies we are likely to learn a great deal about how protein interactions in the actin cytoskeleton can be tuned to accomplish such varied objectives as determination of cell shape, organelle motility, and cell locomotion.

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