

Actin

Straub, a young biochemist in Szent-Györgyi's laboratory, discovered actin in 1942. Previously Szent-Györgyi has shown that brief extraction of minced rabbit muscle with an alkaline 0.6 M KCl solution in the cold room yields a myosin with low viscosity (myosin A), whereas when the muscle mince was left in the 0.6 M KCl for a day a very viscous myosin solution was extracted (myosin B). Straub thought that the difference between myosin B and A is caused by the extraction of a new protein that makes the one-day extract viscous. Accordingly, he extracted myosin A from the muscle, then left the residue in the cold room for a day. The muscle residue was washed with distilled water to remove the KCl and remaining cytoplasmic proteins, and finally the residue was dried with acetone. The protein extracted from the acetone-dried residue formed a very viscous complex with myosin A, that was similar to myosin B, "it activated myosin" and hence it was named actin. In skeletal muscle, actin comprises about 15% of the total protein.

The two forms of actin: After an improved procedure for actin preparation, Straub has found that water extraction of the acetone-dried muscle residue yielded an actin solution with low viscosity, monomeric or globular (G) actin, that upon addition of salts (at physiological concentrations) polymerized to a highly viscous gel, filamentous or fibrous (F) actin (Fig. A).

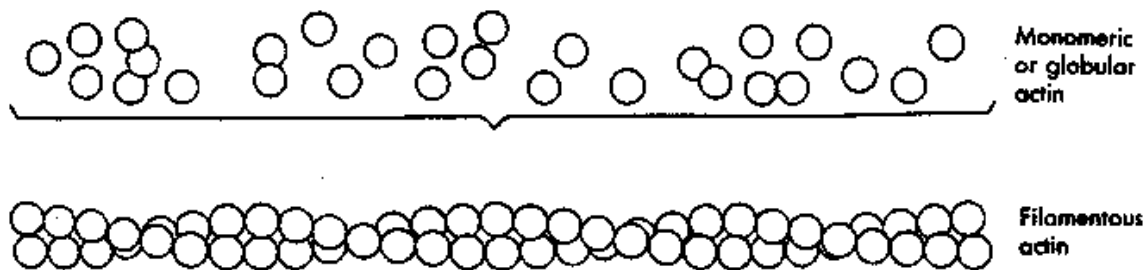


Fig. A. Illustration of the two forms of actin (Courtesy of Dr. Helen Rarick).

Straub followed the polymerization of actin by viscometry, shown in Fig. A1.

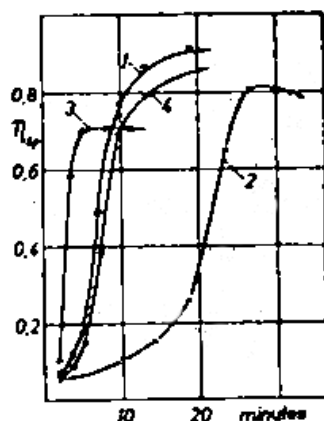


Fig. A1. Polymerization of actin in the presence of various ions. Curve 1) 110 mM NaCl, 3 mM KCl, 3 mM CaCl_2 , and 10 mM MgSO_4 ; Curve 2) same as 1 but without Mg^{2+} ; Curve 3) same as 1 but without Ca^{2+} ; Curve 4) same as 1 but without K^+ . Temperature 24°C (From Feuer et al., 1948).

Ionic strength, temperature, and pH affect polymerization. Optimal conditions are: 0.1 M salts concentration, 37° C, and pH 6.5 – 7.5.

In 1950 Straub and Feuer reported that G-actin contains bound ATP and during polymerization of actin the ATP is hydrolyzed to bound ADP and P_i . Straub postulated that the transformation of G-actin-ATP to F-actin-ADP plays a role in muscle contraction, but this was not found in skeletal muscle of live animals (Martonosi et al., 1960). However, the transformation of G-actin-ATP to F-actin-ADP was found in intact smooth muscle (Bárány et al., 2001) and in beating rat heart (Bárány and de Tombe, 2004), described in the Smooth Muscle and Heart Muscle chapters, respectively. Furthermore, actin polymerization with concomitant ATP hydrolysis takes place in non-muscle cells and provides the mechanochemistry for motility (see Cell Motility chapter).

Electron micrograph of fibrous actin filaments reveals that the structure consists of twin strings of actin globules wound around each other in a double helix. The subunit repeat is about 55 Å and the helical repeat is about 370 Å.



Fig. A2. Electron micrograph of actin filament (From Huxley, H.E., 1972). With permission from *The Structure and Function of Muscle* (G.H. Bourne, ed.), 1972, Academic Press.

Janmey et al. (1999) reviewed the characteristic properties of actin filaments.

Actin-myosin binding: F-actin combines with myosin to form actomyosin. In 0.6 M KCl actomyosin forms a viscous solution; upon addition of ATP, actomyosin dissociates into its components actin and myosin, with accompanying reduction of the viscosity. At physiological ionic strength actomyosin is insoluble, the same way as in the muscle; under these conditions actin activates the myosin ATPase 50-100-fold.

F-actin also combines with the proteolytic fragments of myosin, HMM or S1. The complex formed actoheavymyosin or actosubfragment 1 remains soluble at low ionic strength. When HMM or S1 is added to muscle thin filament it attaches to the actin component of the filament, forming a specific “arrow head” structure (Fig. A3). This suggests a structural polarity for the thin filament.

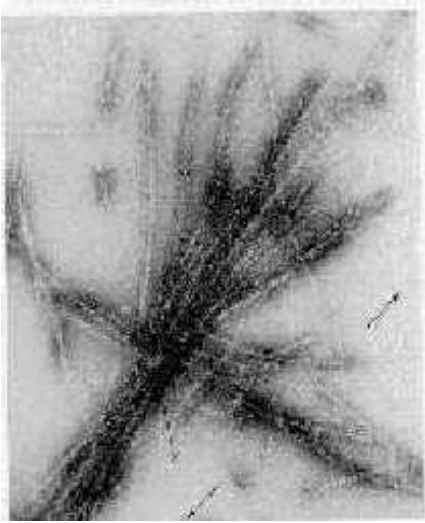


Fig. A3. Electron micrograph of thin filament decorated with HMM (From Huxley, H.E., 1972). With permission from *The Structure and Function of Muscle* (G.H. Bourne, ed.), 1972, Academic Press.

Based on this observation, H.E. Huxley postulated that the structural polarity of thin and thick filaments allows the sliding force to move the thin filaments toward the center of the sarcomere (Fig. A4).

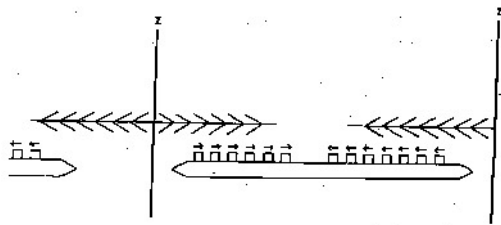


Fig. A4. Diagram for the structural polarity of thin and thick filaments (From Huxley, H.E., 1972). With permission from *The Structure and Function of Muscle* (G.H. Bourne, ed.), 1972, Academic Press.

Three-Dimensional Structure of Actin

Kabsch and collaborators (1990) were the first to crystallize G-actin and determine its structure (Fig. A5).

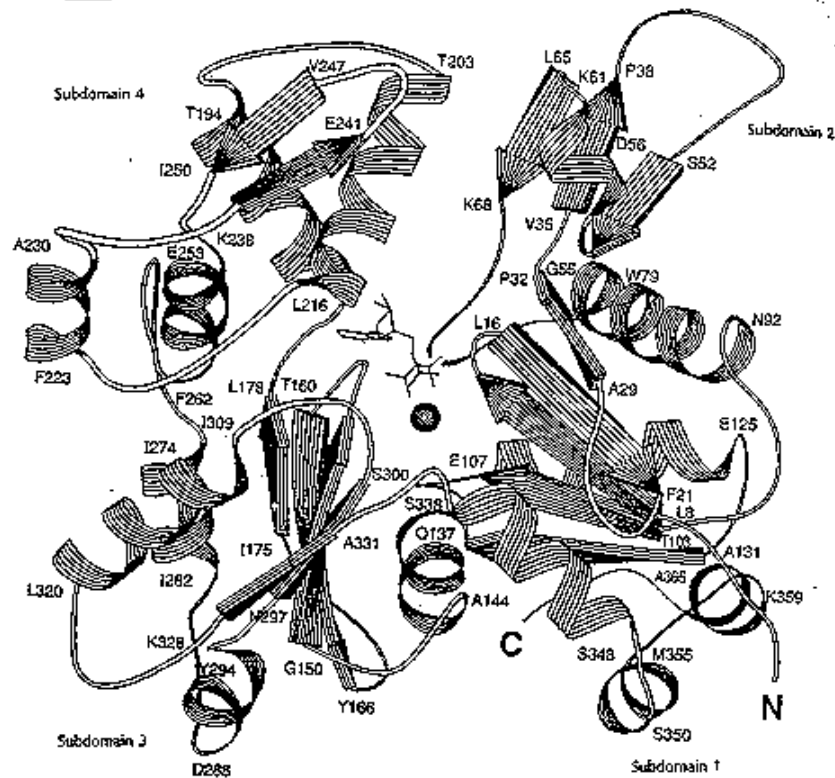


Fig. A5. Scheme for the structure of actin. (Reprinted from *Current Opinion in Structural Biology*, vol 1, Holmes and Kabsch, *Muscle Proteins: actin*, pp. 270-280, 1991, with permission from Elsevier Science).

Folding of the actin molecule is represented by ribbon tracing of the alpha-carbon atoms. N and C correspond to the amino- and carboxyl-terminals, respectively. The letters followed by numbers represent amino acids in the polypeptide chain. A hypothetical vertical line divides the actin molecule into two domains “large”, left side, and “small”, right side. ATP and Ca^{2+} are located between the two domains, called the cleft. These two domains can be subdivided further into two subdomains each, the small domain being composed of subdomains 1 and 2, and the large domain of subdomains 3 and 4. (Subdomain 2 has significantly less mass than the other three subdomains and this is the reason of dividing actin into small and large domains). The four subdomains are held together and stabilized mainly by salt bridges and hydrogen bonds to the phosphate groups of the bound ATP and to its associated Ca^{2+} localized in the center of the molecule. Because of the less mass in subdomain 2, the actin molecule is distinctly polar in the direction from subdomains 1 and 3, called the “barbed end”, toward subdomains 2 and 4, called the “pointed end”. This polarity defines the orientation of the actin molecule in the myosin HMM decoration pattern of the thin filament, shown in Fig. A3.

Recent studies on the structure of actin: Since the pioneer work of Kabsch et al., (1990), several actin crystal structures have been published, “but the structures are essentially the same” (Squire et al., 2005). Actin modified to prevent polymerization was crystallized,

and the structure was solved to 1.54-Å resolution. Compared with previous ATP-actin structures, monomeric ADP-actin was characterized by a marked conformational change in subdomain 2 (Otterbein et al., 2001). Continuing these studies on the structural basis of nucleotide-dependent actin dynamics, Graceffa and Dominguez (2003) determined the x-ray crystal structure of tetramethylrhodamine-5-maleimide-actin with bound AMPPNP, a non-hydrolyzable ATP analog, at 1.85-Å resolution. A comparison of this structure to that of tetramethylrhodamine-5-maleimide-actin with bound ADP revealed how the release of the nucleotide γ -phosphate sets in motion a sequence of events leading to a conformational change in subdomain 2. The side chain of Ser-14 in the catalytic site rotates upon P_i release, triggering the rearrangement of the loop containing the methylated His-73, referred to as a sensor loop. This in turn causes a transition in the DNase I-binding loop in subdomain 2 from a disordered loop in ATP-actin to an ordered α -helix in ADP-actin.

The structure of non-vertebrate actin (*Saccharomyces cerevisiae*, *Dictyostelium*, and *Caenorhabditis elegans*) was determined by Vorobiev et al., (2003). They visualized the network of eight water molecules that runs across the nucleotide binding cleft (Fig. A6). This network is stabilized by interactions with Gly-15, Asp-157, Arg-210, Glu-214, Lys-336, and Tyr-337, connecting subdomains 1,3, and 4, and may thus contribute to the overall dynamic and function of the actin monomer. This network is partially conserved in all of the non-vertebrate actin structures, and appears to be fully conserved in all of the mammalian ADP-actin structure.

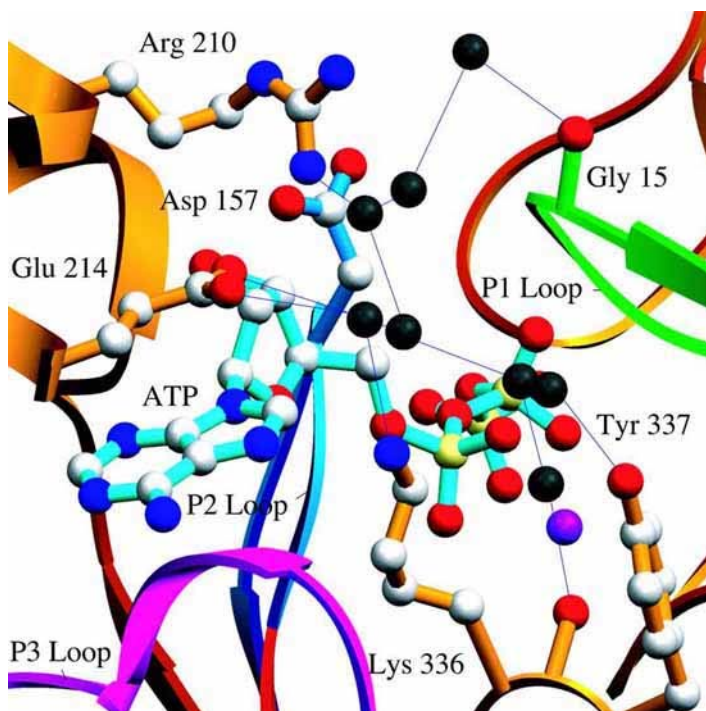


Fig. A6. Details of the water mediated hydrogen bonding network across the nucleotide-binding cleft. Eight solvent molecules (black) run across the nucleotide-binding cleft and may play a role in transducing changes in the nucleotide state into structural and dynamic changes relevant to polymerization and interactions with regulatory proteins. (Reprinted with permission from Vorobiev, S., Strokopytov, B., Drubin, D.G., Frieden, C., Ono, S., Condeelis, J., Rubenstein, P.A., and Almo, S.C., The structure of nonvertebrate actin: Implications for the ATP hydrolytic mechanism. Proc. Natl. Acad. Sci. USA, 100, 5760 -5765, 2003. Copyright, 2003, National Academy of Sciences, USA).

The intersubunit contacts in the F-actin filament: In helical structures, such as F-actin filament, two types of intersubunit contacts are possible in principle: those along and

those between the long-pitch helical strands. In the atomic model of the F-actin filament, 24 amino acid residues per subunit are involved in contacts along the long-pitch helical strands. By contrast, only 15 residues per subunit mediate the weaker contacts between the two strands. The crystal structure of the cross-linked actin-dimer (Kudryashov et al., 2005) suggests that the major interactions between subdomain 3 and subdomain 4 include the Lys-291 side chain forming both a salt bridge with Glu-205 and a hydrogen bond with the backbone carbonyl of Ser-199. An additional hydrogen bond is formed between the Asp-288 and Thr-203 side chains. In general, salt bridges, hydrogen bonds, and van der Waals contacts are holding together the actin monomers in F-actin.

Modification of the actin structure: Modified actin proteins, generated by mutation, or by blocking chemically reactive amino acid side chains (mainly cysteine) may provide new aspects of the actin-actin interaction and the interaction of actin with myosin or with tropomyosin-troponin. Interestingly, relocation of charged residues in subdomain 1 of actin (Wong et al., 1999) or placing a fluorescence probe on Cys10, located in subdomain 1 of actin, (Eli-Berchoer et al., 2000), had no effect on the functional properties of actin. On the other hand, mutations in actin subdomain 2 or subdomain 3 impaired thin filament regulation by troponin and tropomyosin (Korman and Tobacman, 1999; Korman et al., 2000). It was established that residue Glu93 in actin, located near the junction of subdomain 1 and subdomain 2, is involved in myosin binding (Razzaq et al., 1999). Fluorescence resonance energy transfer detected a conformational change around Cys374, at the C-terminal of actin, when F-actin combined with myosin (Moens and dos Remedios, 1997).

Localization of actin in the structure of muscle: Under the microscope, myosin extracted myofibrils exhibit the thin filaments, attached to the Z line. When 0.6 M KI solution, that dissolves F-actin, is added to such a myofibrillar ghost the structure disappears, indicating that the thin filaments are composed of actin. In the structure of muscle, the I band contains thin filaments whereas the A band contains both thick and thin filaments.

Structure of the thin filament: Fig. A7 shows the structure: actin molecules form two strings wound around each other, in the groove is the tropomyosin strand and at regular intervals troponin molecules are attached to tropomyosin.



Fig. A7. Model for the structure of the thin filament (From Huxley, H.E., 1972). With permission from *The Structure and Function of Muscle* (G.H. Bourne, ed.), 1972, Academic Press.

Mini-thin filaments: To study the cooperative mechanism of thin filaments, Gong et al., (2005) introduced this new preparation by isolating particles nearly matching the minimal structural repeat of thin filament: a double helix of actin subunits with each strand approximately seven actins long and spanned by a tropomyosin-troponin complex. One end of the particles was capped by a gelsolin (segment 1-3)-TN-T fusion protein, and the other was capped by tropomodulin. On average, the particles were 46 nm long, and one tropomyosin-troponin was attached to each strand of the two-stranded actin filament.

Myosin-S1 MgATPase activity was increased about 2.5-fold by the minifilament and it was Ca^{2+} sensitive.

Thin filaments were prepared by polymerizing G-actin in the presence of tropomyosin, tropomodulin, gelsolin-troponin and phalloidin and isolated by Sephacryl HR S-500 chromatography ((Fig. A8). The composition of the isolated minifilaments was determined by SDS/PAGE. The particles contained actin, tropomyosin, TN-C, TN-I, and TN-T-gelsolin in a 7:1:1:1 ratio. Thus, the composition of the minifilaments resembled that of the thin filaments.

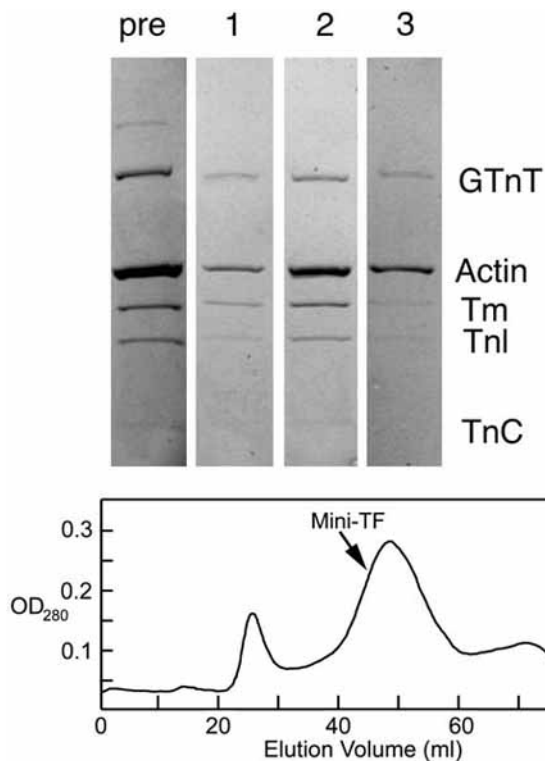


Fig. A8. Isolation of mini-thin filaments using size-exclusion HR S500 chromatography (Lower) and gradient SDS/PAGE analysis of the mini-thin filament (Upper). The mini-filament peak (Mini-TF) is indicated. Lanes 1-3 show column fractions of the leading edge, peak, and tail of the minifilament peak, respectively. “pre”, sample before column loading. GTnT, gelsolin-TN-T protein. (Reprinted with permission from Gong, H., Hatch, V., Ali, L., Lehman, W., Craig, R., and Tobacman, L.S., Mini-thin filaments regulated by troponin-tropomyosin: Proc. Natl. Acad. Sci. USA, 102, 656 -661, 2005. Copyright, 2005, National Academy of Sciences, USA).

After size exclusion chromatography, mini-thin filament structure was examined by negative-stain electronmicroscopy (Fig. A9). Minifilament width was relatively constant and was indistinguishable from normal F-actin-tropomyosin width. However, particle lengths were much shorter than those of normal thin filaments with a mean of 45.7 nm, corresponding to an F-actin filaments approximately seven to eight monomer long. Except for their shorter length, minifilaments appeared similar to reconstituted F-actin or native thin filaments (Part D of Fig. A9).

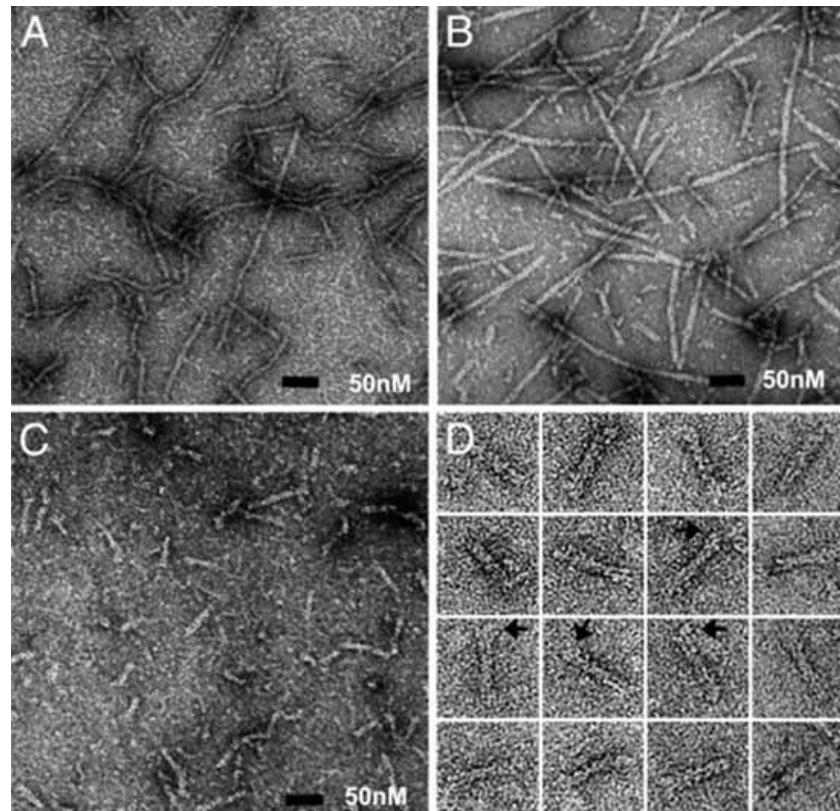


Fig. A9. Electron microscopy of mini-thin filaments. (A) Tropomyosin was omitted. (B) Tropomodulin was omitted. (C) All components were present. (D) Higher magnification of minifilaments prepared with all components present as in C and then chromatographed by using Sephacryl HR S500. . (Reprinted with permission from Gong, H., Hatch, V., Ali, L., Lehman, W., Craig, R., and Tobacman, L.S., Mini-thin filaments regulated by troponin- tropomyosin: Proc. Natl. Acad. Sci. USA, 102, 656 -661, 2005. Copyright, 2005, National Academy of Sciences, USA).

Mini-thin filaments may help in a better understanding the mechanism of thin filament regulation.

Suggested readings: Graceffa and Dominguez, 2003; Vorobiev et al., 2003; Gong et al., (2005); Kudryashov et al., 2005

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