ELECTRON MICROSCOPY IN BIOLOGY

VOLUME 2

Edited by
Jack D. Griffith

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The University of North Carolina
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SERIES PREFACE

Over the past 40 years, electron microscopy in biology has changed from a frontier of cell biology to a routine tool of fields as diverse as protein chemistry and chemical carcinogenesis. In the early days of electron microscopy most studies employed thin sectioning, and a microscopist could keep abreast of the major preparative procedures and advances in the field. Now, with so many diverse applications and subspecialties, few electron microscopists have more than a reading knowledge of the preparative techniques outside their own area of study. The past few years have also seen the cost of state-of-the-art electron microscopes rise beyond the usual resources of an individual laboratory. This has forced the growth of central EM facilities serving from a few research groups to one or more departments. Those with primary interests in biological electron microscopy are being called on to supervise these facilities, and to counsel colleagues who face research problems very different from their own. Following the literature in a single field is difficult enough; scanning journals for articles of possible interest to colleagues is impossible. Furthermore, the literature of biological electron microscopy appears primarily in journals dedicated to the area of application, making it even more likely that one would miss articles outside one's normal reading.

A yearly series of invited articles can help solve this dilemma. A well-executed series can bring together articles reviewing techniques and advances in all areas of biological electron microscopy. Properly written, each article should be useful to the expert as a brief refresher and resource of reference material, and to the novice as an introduction. A collection of these volumes spanning several years should provide a valuable resource for introducing colleagues to new applications of electron microscopy and to the expert as an on-hand reference library. The series also would provide a needed format for electron microscopists to communicate among themselves in ways that the journals cannot accommodate. Most journal articles focus on the end rather than the means, and often important details are omitted, simply because of the style dictated by the format of a journal article. A discussion of techniques can be very important in a technique-oriented field such as biological electron microscopy, and a critical review of techniques,

illustrated with successful applications, makes a valuable contribution to our colleagues.

JACK D. GRIFFITH

Chapel Hill, North Carolina
March, 1981

PREFACE

This volume contains essential reading for cell biologists and electron microscopists. Each chapter begins with a clear discussion of a particular technique at a level useful to novice and expert alike. The chapters then describe the application of these techniques with specific new examples. Immunocytochemistry and the visualization of the cytoskeleton and its components are emphasized because electron microscopy is providing the single most important contribution in these very timely topical areas. In addition, this volume contains new material and reviews in the visualization of DNA and viruses.

Volume 2 begins with a chapter by Pollard and Maupin that illustrates the use of electron microscopy to study actin and myosin. The authors warn us of deleterious preparative artifacts that should be considered by all electron microscopists. Immunoelectron microscopy of cell ultrastructure using new frozen sectioning methods is reviewed by Singer, Tokuyasu, Dutton, and Chen in Chapter 2. They demonstrate that this technology has come of age and will play an important role in the future. In Chapter 3, Childs provides a superb overview and detailed discussion of the use of immunocytochemistry in cell biology. Finally, in this group of chapters, Henderson and Weber prove just how powerful electron microscopy can be when used in combination with other techniques to dissect the cell cytoskeleton. In Chapter 5, Inman provides not only a hands-on discussion of DNA partial denaturation mapping, but shows how important this method has been and will continue to be in DNA studies. In the final two chapters, Breese and Miller supply needed reviews that demonstrate the use of electron microscopy for the identification and quantitation of viruses, either pure in solution or in tissues. Both contributions will be of great value.

JACK D. GRIFFITH

Chapel Hill, North Carolina
August, 1982

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ELECTRON MICROSCOPY OF ACTIN AND MYOSIN

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CONTENTS

Electron microscopy has provided some of the most important information on the structure and cellular localization of the contractile proteins actin and myosin. We review these contributions, with special emphasis on some of the technical difficulties that have limited these studies.

1 ACTIN

1.1 Molecular Structure

Actin is one of the two or three most abundant proteins in nature, but apart from its amino acid sequence (31), little was known about its structure until recently. The molecular weight is 43,000 daltons, and the amino acid sequences of actin from all parts of the phylogenetic tree are nearly identical (18, 69, 128). The molecule is generally considered to be globular and has usually been depicted as a sphere in models.

Currently the molecular structure of actin is being investigated by both X-ray diffraction of three-dimensional crystals and electron microscopy of crystalline sheets. Three-dimensional crystals of actin complexed with profilin (14) or DNase I (71) should eventually provide an atomic level map of the molecules. In the 0.6 nm resolution model of the actin-DNase I complex (116b), the boundary between the actin and the DNase I is not distinct. By localizing the divalent cation binding site of DNase I in the complex it was possible to identify a region of the complex as DNase I and to suggest tentatively that the actin molecule has dimensions of $6.7 \times 4.7 \times 3.3$ nm. The authors showed one way that an actin molecule with these dimensions might be packed in an actin filament, but this model of actin is considerably larger than expected from past work on actin filament structure (76, 131) and from the model derived from electron micrographs of actin sheets. It is presumed

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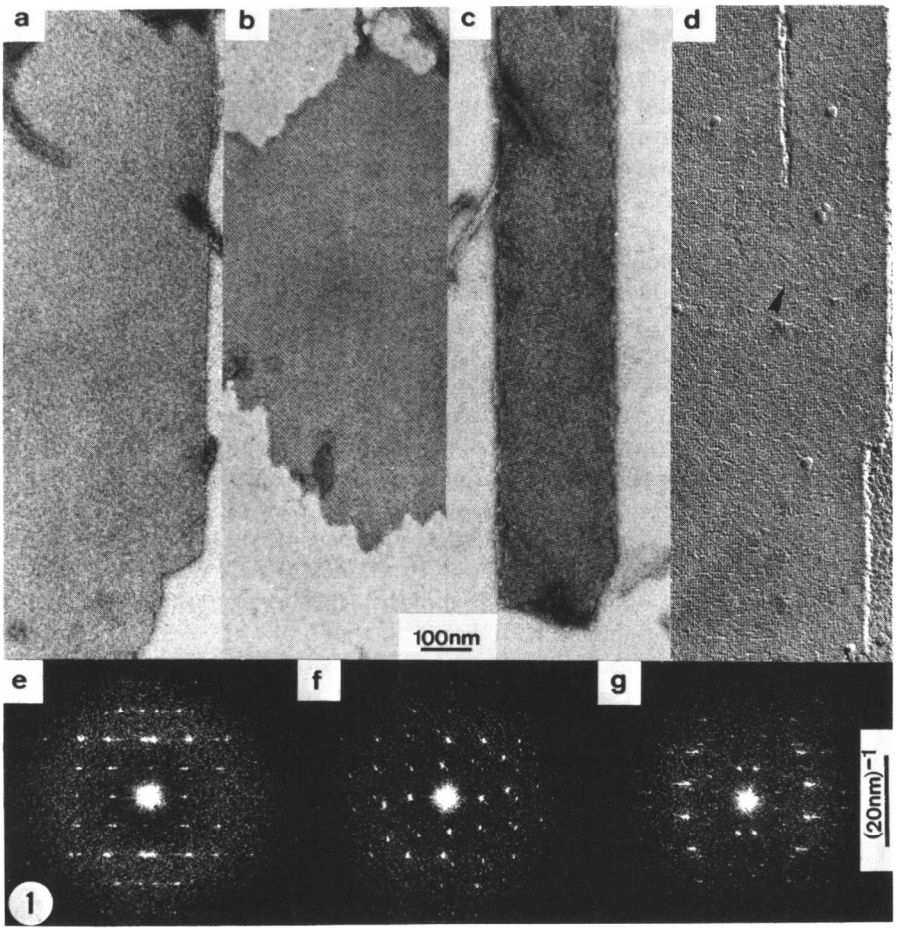

Figure 1 Crystalline *Acanthamoeba* actin arrays. Electron micrographs and optical diffraction patterns of negatively stained (*a–c*, *e–g*) and freeze-dried–metal-shadowed (*d*) samples. Negative stain: 0.75% uranyl formate, pH 4.25. The elevation angle for unidirectional platinum–carbon shadowing was 45°. Optical diffraction patterns were recorded from circular micrograph areas including about 1000 (5.6 × 6.5 nm^2) unit cells. (*a*), (*d*), and (*e*) Rectangular type of double-layered sheets. (*b*) and (*f*) Square type of double-layered sheet. (*c*) and (*g*) Flattened cylinder. [Courtesy of U. Aebi et al. (2).]

that these ambiguities will be clarified as soon as the polypeptide chains can be traced in higher resolution models from X-ray studies.

Two-dimensional crystals of pure muscle or *Acanthamoeba* actin suitable for image reconstruction can be induced by the trivalent lanthanide gadolinium (2, 3, 25). Depending on solution conditions, the crystalline sheets of *Acanthamoeba* actin have several forms (Figure 1). The basic sheet

is composed of a 5.6 × 6.6 × 4.5 nm unit cell containing two actin molecules side by side. The unit cell has a twofold axis of symmetry perpendicular to the sheet, which accounts for the rough irregular side and the smooth side with near-rectangular surface lattice of the basic sheets prepared by freeze drying–metal shadowing (2). These basic sheets can be rolled up to make cylinders with a helical arrangement of subunits (Figure 1*c*). In the cylinders the irregular surface is on the outside and the smooth regular surface is on the inside. Alternatively, two basic sheets can join back to back to form two different types of double layer sheet about 8 nm thick, with the rectangular surface lattice on the outside (Figure 1*d*). When the two layers are 85° to each other they form a "square-type" sheet (Figure 1*b*, *f*). When oriented parallel to each other they form a "rectangular-type" sheet (Figure 1*a*, *d*, *e*).

Optical diffraction analysis of electron micrographs of negatively stained sheets indicate preservation of structural detail to 1.5 nm resolution (Figure 1*e*, *f*, *g*). Preservation of detail to 1.1 nm is achieved by minimizing the electron dose received by these preparations (3). Computer filtration of actin sheets provided a detailed projection view of the actin molecule. It measures 5.6 × 3.3 nm and has a large and a small lobe separated by a shallow groove. From low electron dose micrographs of tilted sheets a 1.5 nm resolution, three dimensional model of the actin molecule has been computed (Figure 2). The molecule is pear-shaped with dimensions of 5.6 × 4.3 × 3.3 nm, and has a number of interesting surface features. This is the highest resolution three dimensional model of a protein molecule yet obtained from a negatively stained specimen. It may be possible to extend the resolution of this model down to 1 nm using unstained specimens, but further progress on internal details will probably depend on X-ray diffraction.

1.2 Filament Structure

Actin molecules polymerize under physiological conditions to form filaments 6.5 nm wide with double helical structure. The arrangement of molecules in these filaments has been studied by both X-ray diffraction of living muscles (55) and electron microscopy of negatively stained actin filaments (76). Micrographs of single filaments (Figure 3) can be used for this analysis, but paracrystals (Figure 4) induced by high concentrations of $MgCl_2$ (131) or by polylysine (32b) are more favorable for structural work. The paracrystals consist of sheets of filaments all in register with each other. Optical diffraction patterns of these paracrystals contain reflections on layer and row lines. The 5.5-nm longitudinal spacing of the subunits in the micrographs agrees closely with the 5.4-nm spacing measured in living muscle by X-ray diffraction (55) and the 5.6-nm length of the actin molecule measured in the actin sheets (2).

The molecules are arranged helically in the filament (Figures 3 and 4). Two "fundamental" or "genetic" helices can be described. One is left handed and repeats every 5.9 nm; the other is right handed and repeats every 5.1 nm (Figure 4). There is also a longer pitch, 2-start right-handed "struc-

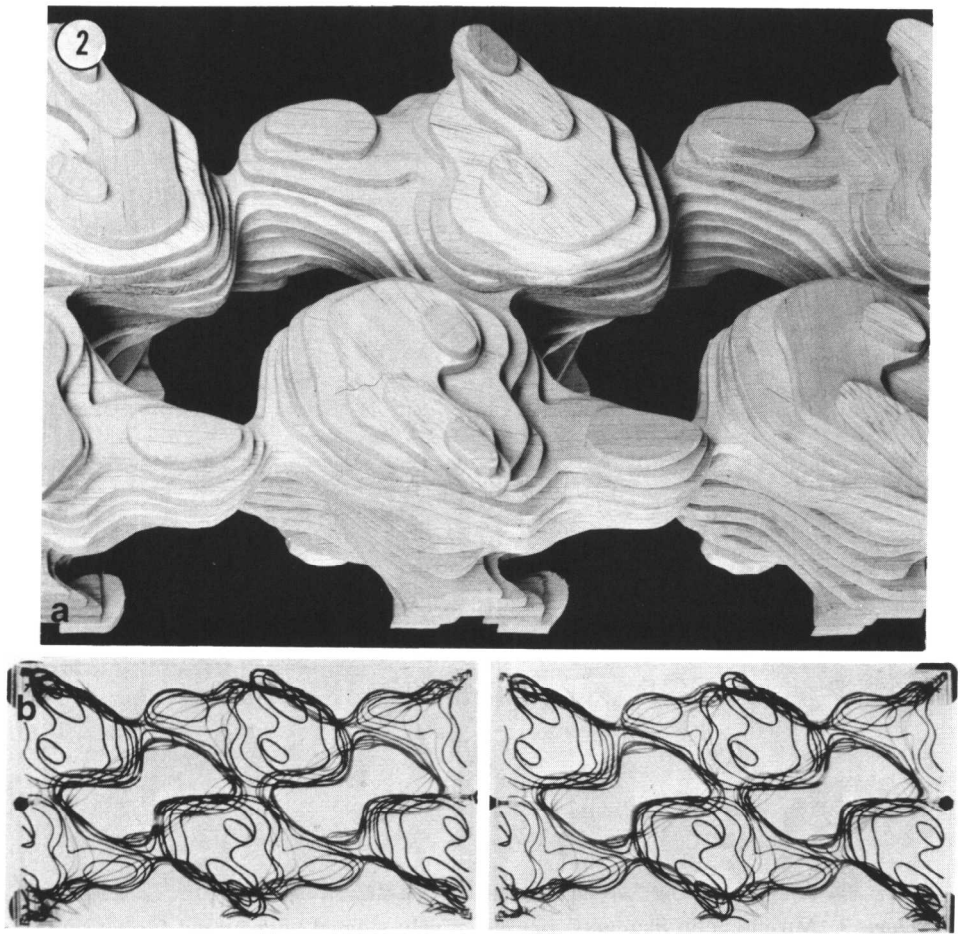

Figure 2 Three dimensional reconstruction of the actin molecule to 1.5 nm resolution by electron microscopy and image processing. (a) Three dimensional model of two unit cells of a negatively stained crystalline actin sheet, each of which contains two actin molecules. The molecules are bilobed, measure 5.5 × 6.6 × 4.5 nm and have an obvious polarity in the plane of the sheet. (b) Stereo presentation of the same data. [Courtesy of W. E. Fowler and U. Aebi (32b).]

tural'' helix that is more obvious in routine micrographs (Figure 3*a*). This helix repeats every 37.5 nm and contains a nonintegral ($\simeq$ 13.5) number of molecules. The width of the filaments obtained from direct measurement of negatively stained filaments (6.5 nm) and the spacing of filaments in paracrystals (6.5 nm) is consistent with the 3.3-nm width of the molecules in the actin sheets. Thus the long axis of the molecule is probably oriented along the filament. However the side-to-side intermolecular bonds in the sheets cannot be the same as those in the filaments, because the molecules in the

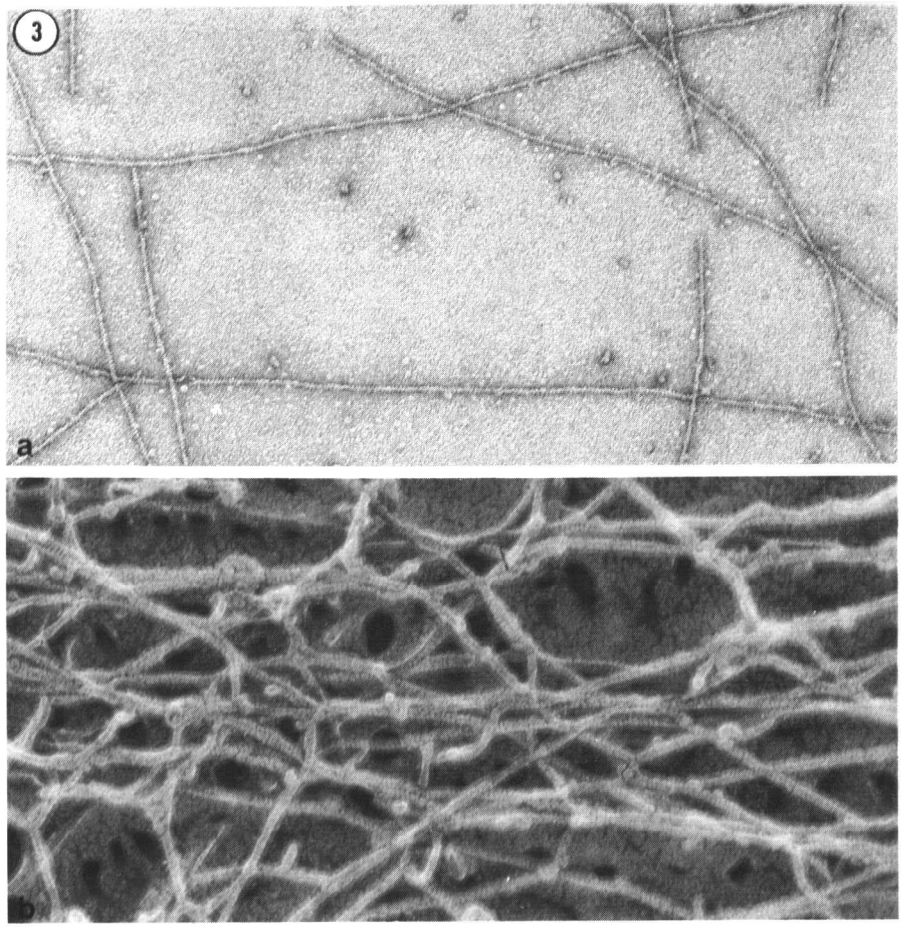

Figure 3 Muscle actin filaments. (*a*) Negatively stained with uranyl formate. (*b*) Quick-frozen in solution, etched, and rotary shadowed by the method Heuser and Kirschner (49). (Courtesy of J. Heuser and T. Pollard.) 92,000×.

sheets are in antiparallel pairs, whereas the molecules in the filaments are parallel and staggered by half their length, giving rise to a strong 2.7nm^{-1} meridional (longitudinal) reflection in the X-ray patterns of living muscle.

The original efforts to reconstruct the actin filament from electron micrographs of paracrystals used the first row line reflections $(6\text{ nm})^{-1}$ out to the $(5.1\text{ nm})^{-1}$ layer line and thus included only low resolution information (76). A new actin filament model (Figure 5*a*) has been computed from higher resolution micrographs of polylysine paracrystals (32b). This model is similar to one of Wakabayashi *et al.* (131) in that the lateral connectivity of the molecules is much stronger than the longitudinal connectivity. The three dimensional model of the actin molecule obtained from the crystalline sheets fits nicely into the filament model, as illustrated in figure 5*b*. This is a tenta-

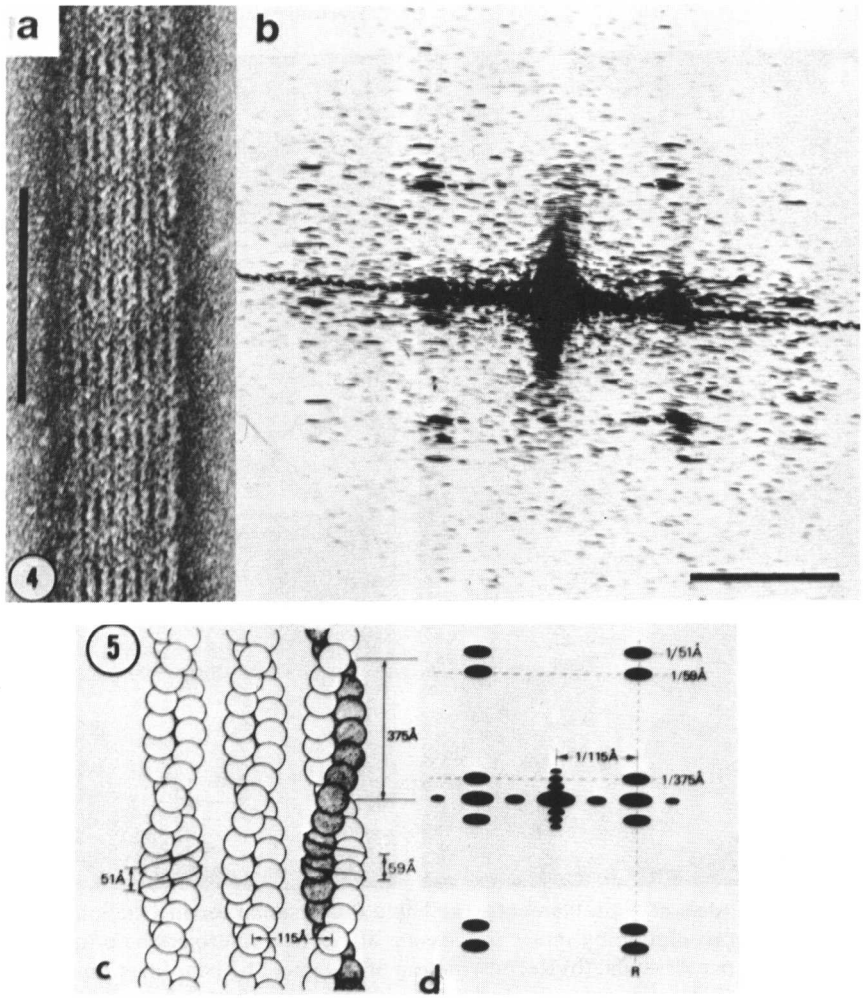

Figure 4 Muscle actin paracrystals. Electron micrograph (*a*) and optical diffraction pattern (*b*) of a muscle actin paracrystal formed in 50 m*M* $MgCl_2$. Negative stain: 0.75% uranyl formate; pH 4.25. Scale bars: (*a*) 100 nm, (*b*) $(5\ nm)^{-1}$. [Courtesy of G. Isenberg *et al.* (57).] (*c*) A diagram of a paracrystalline array of actin filaments with the crossover points of the helices in register. The circumferential lines indicate the genetic helices, one having a pitch of 59 Å; another of 51 Å, and the stippling indicates the structural helix with a periodicity of 375 Å. Adjacent filaments are separated by 115 Å. (*d*) A diagram of the expected diffraction of the array illustrated in (*c*). The pattern consists of a series of horizontal layer lines that arise from the helical symmetry of the actin. These layer lines occur at spacings of 1/375, 1/59, and 1/51 $Å^{-1}$. The strong spots on the different layers are lined up vertically in columns called row lines. The row lines spaced at 1/115 $Å^{-1}$ arise from the parallel arrangement of the adjacent filaments. [Courtesy of Tilney et al. (122).]

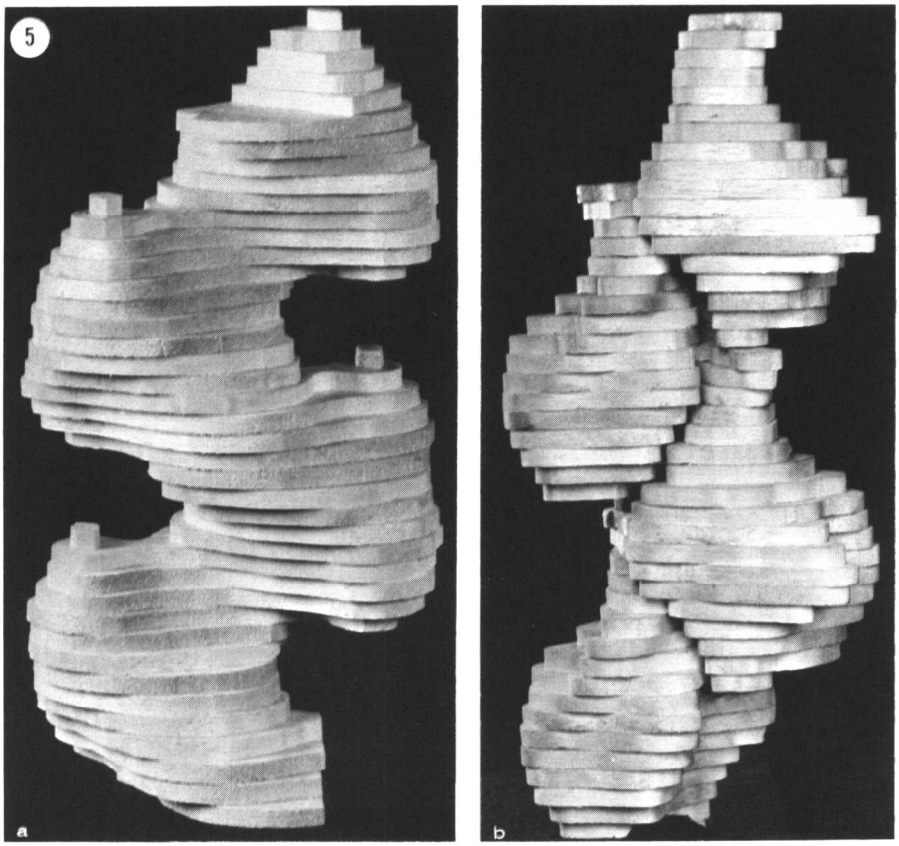

Figure 5 Models of actin filaments. (a) Three dimensional reconstruction of a filament to 3 nm resolution by image processing of electron micrographs of negatively stained actin paracrystals. (b) Reconstruction of a filament by best-fit assembly of the 1.5 nm resolution actin molecule models obtained as described in figure 2. [Courtesy of W. E. Fowler and U. Aebi (32b).]

tive placement of the molecule in the filament, because the resolution in the filament model is insufficient to distinguish molecular boundaries. There are complementary surfaces located on the molecular model at the presumed lateral contact sites which are oriented such that an actin helix is formed when a series of molecules are placed in contact.

A second new finding regarding the actin filament structure is that, compared with filaments in paracrystals, the helix of individual negatively stained filaments is rather disordered (27b). That is, there is some freedom in the way that adjacent molecules can be oriented relative to each other. It is not yet known whether this flexibility arises from inter- or intra-molecular motion. This suggests that the filament actually has considerable flexibility, thereby allowing formation of bonds to the wide variety of protein molecules which can bind to actin filaments.

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