PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Direct Demonstration of the Helical Nature of Paramyosin Filaments

A. Elliott

Phil. Trans. R. Soc. Lond. B 1971 **261**, 197-199
doi: 10.1098/rstb.1971.0050

Phil. Trans. Roy. Soc. Lond. B. **261**, 197–199 (1971) [197]
Printed in Great Britain

Direct demonstration of the helical nature of paramyosin filaments

By A. Elliott
King's College Department of Biophysics, 26–29 Drury Lane, London, W.C. 2

[Plate 35]

A method is described by which the front and rear sides of a paramyosin filament can both be shadowed with a platinum–palladium alloy. An electron micrograph of such a filament shows the structure on both sides. A symmetrical cross, indicative of a helical arrangement is seen in the optical diffraction photograph made from the micrograph.

Introduction

The adductor muscles of some molluscs, which hold the two sides of the bivalve together, contain large numbers of filaments whose diameters are up to about 160 nm and whose lengths are perhaps 50 μm or more. These filaments (known as paramyosin filaments), when suitably stained or shadowed and examined in the electron microscope show a two-dimensional pattern (figure 1*a*, plate 35). Since the first observation of this pattern by Hall, Jakus & Schmitt (1945) it has been realized that the pattern might indicate an arrangement of parallel, flat layers (as in a crystal) or alternatively a many-stranded cable or rope, in which case the structural units would be arranged in a helical or more generally in a helicoidal manner. Most authors have tended to favour the flat layer structure (Hall *et al.* 1945; Bear & Selby 1956; G. F. Elliott 1964; Lanzavecchia 1966). Recently, Elliott & Lowy (1969, 1970) have produced evidence for a helicoidal model, based on X-ray diffraction and quantitative assessment of electron micrographs. Their most direct evidence was the observation that the 'hand' of the pattern, though not always the same on all filaments, was retained within one filament after many bends, when it might be expected that different aspects of the filament would be seen, and also that occasionally a filament was seen partly unfolded into a wide ribbon. They proposed a model showing how paramyosin molecules might be arranged to give a structure whose surface when shadowed or stained would resemble electron micrographs of paramyosin filaments, and whose internal structure would be consistent with observations of the X-ray diffraction pattern from molluscan muscle. The present paper describes the simultaneous observation of the front and back surfaces of a filament in an electron microscope, on which the patterns are found to be of opposite hand, which clearly demonstrates the helicoidal nature of the structure.

Experimental

The use of shadowing rather than staining to reveal structural details has the advantage that it is known with certainty which part of the structure is seen, i.e. the surface. It was decided, therefore, to attempt to shadow both sides of a filament so that their structures could be recorded simultaneously.

Specimen preparation

In order to obtain clean preparations of filaments, it is desirable to remove as much as possible of the glycogen, actin and soluble proteins from the muscle, leaving only the

paramyosin filaments. The white adductor muscle of the scallop *Pecten maximus* was immersed in 50 % aqueous glycerol buffered at pH 7.0 for several weeks at a temperature of −20 °C. Portions were then cut up with scissors and washed in several changes of dilute ammonium acetate solution (0.01 mol l^{-1}) and relaxed by immersion in a solution buffered to pH 7 containing (mmol l^{-1}): 2 ethylene glycol bis-(2-aminoethyl) tetra-acetic acid, 2 magnesium chloride, 6 potassium phosphate, 100 potassium chloride, to which adenosine triphosphate (5 mmol l^{-1}) was subsequently added (this procedure is essentially that described by Huxley 1963). The pieces of muscle were then washed in distilled water (in which they swell greatly) and blended in the same material. This gives (for the same mild blending conditions) an enormously greater yield of filaments than blending in the relaxing medium or in ammonium acetate solution. To separate the filaments from the other material, the suspension is diluted about a hundredfold, either with distilled water or with ammonium acetate solution (0.1 mol l^{-1} has been used) and centrifuged in a tube at the bottom of which is a grid covered with a plain collodion film. Most of the liquid is then decanted off and the grid is washed several times with ammonium acetate solution (0.6 mol l^{-1}), which probably causes the swollen filaments to shrink *before* drying. An alternative procedure is to pipette a layer of 0.6 mol l^{-1} ammonium acetate solution between the grid and the suspension, so that the filaments must pass through a zone of salt solution before being deposited on the grid. In this way the heavy filaments are separated from the smaller structures.

The dried grid is shadowed with platinum–paladium alloy (80:20) in the usual way so that the free surface of the filaments is coated, and a thin layer of carbon is evaporated on to the metal-coated surface. The grid is held in forceps and washed thoroughly in two changes of acetone to remove the collodion (Bradley 1965) after which it is shadowed with Pt–Pd on the side now free, which previously was in contact with the collodion film. It seems advantageous to choose the two directions of shadowing mutually at right angles. After the second shadowing the grid is ready for examination in the electron microscope.

Results

An electron micrograph of a twice-shadowed paramyosin filament is shown in figure 1*b*, plate 35. The filaments shows some diagonal markings, and the central part looks as though *two* sets of markings equally inclined on either side of the long axis of the filament might be present.

It is readily seen that two sets of shadows are present. The irregular particle casts a shadow upwards, whereas the shadow cast by the filament must be caused by a source at the left-hand side of the photograph. An optical diffraction pattern of a transparency made from the electron micrograph shows that the two sets of markings have the same axial period and are equally inclined to the axis. Figure 1*c*, plate 35, shows the optical diffraction pattern; the symmetry shows that the shadowed patterns are related by mirror-image symmetry, exactly as the strands on opposite sides of a transparent helix.

Conclusion

A paramyosin filament from the adductor muscle of *Pecten maximus* has been found to have a surface pattern of the kind associated with a helical arrangement.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

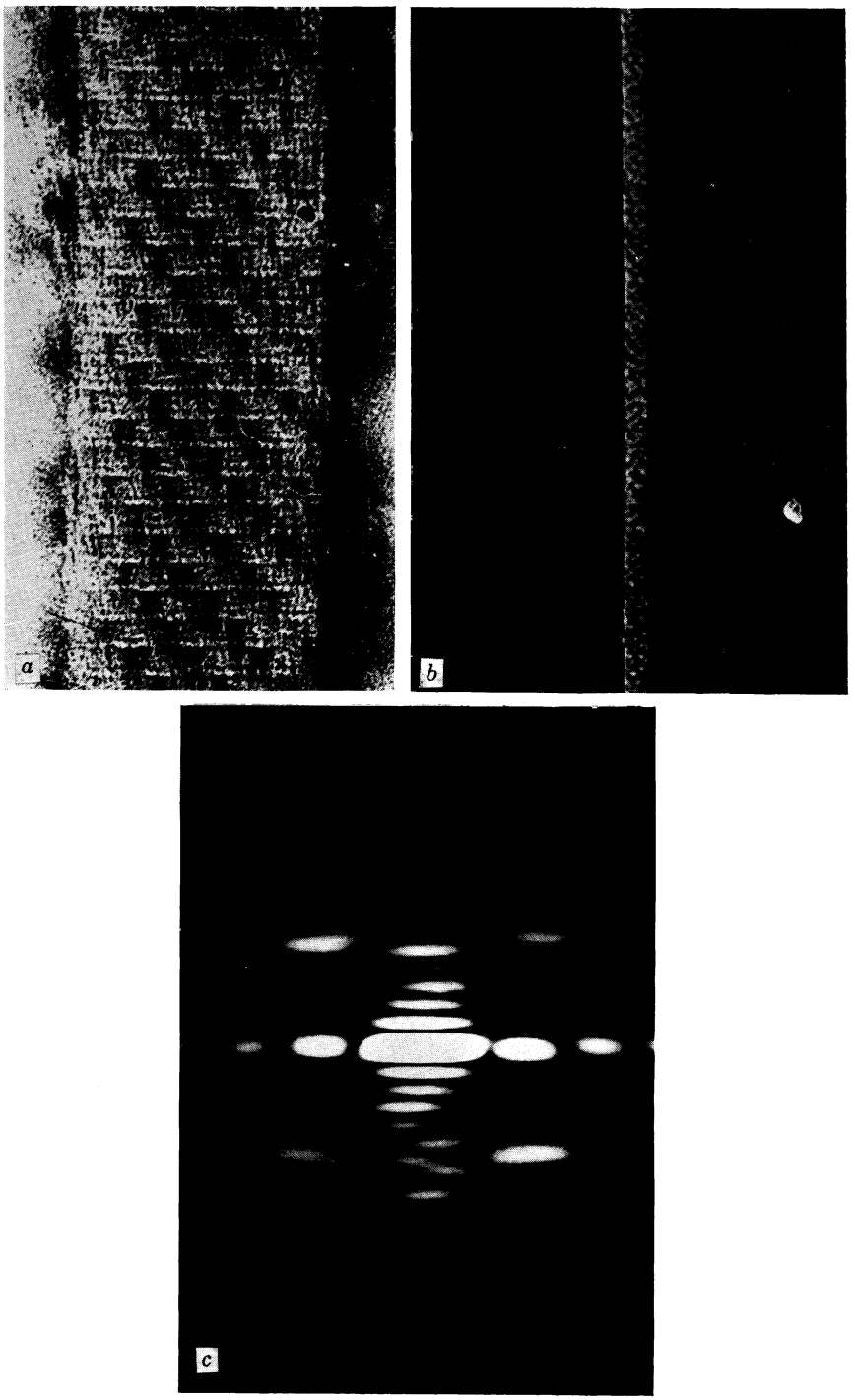

FIGURE 1. (*a*) Electron micrograph of a negatively stained paramyosin filament from the adductor muscle of *Crassostrea angulata*, showing a two-dimensional net pattern (Hanson & Lowy 1964). (*b*) Electron micrograph of a paramyosin filament from the adductor muscle of *Pecten maximus*, shadowed on both sides with platinum-palladium alloy (80:20). (*c*) Optical diffraction pattern from (*b*) showing a pattern symmetrical about the vertical (axis of filament) indicating a helical pattern on the paramyosin filament.

(*Facing p.* 198)

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

The author wishes to express his indebtedness to Sir John Randall, F.R.S., for the facilities of the laboratory, and to Mr A. Fasoli who has made many special pieces of apparatus required during the investigation. Thanks are also due to Professor J. Hanson, F.R.S., and Professor J. Lowy for permission to use figure 1*a*.

References (Elliott)

Bear, R. S. & Selby, C. C. 1956 *J. biophys. biochem. Cytol.* **2**, 55.
Bradley, D. E. 1965 In *Techniques for electron microscopy* (ed. D. H. Kay, p. 70). London: Blackwell.
Elliott, A. & Lowy, J. 1969 *Nature, Lond.* **224**, 1105.
Elliott, A. & Lowy, J. 1970 *J. molec. Biol.* **53**, 181.
Elliott, G. F. 1964 *J. molec. Biol.* **10**, 89.
Hall, C. E., Jakus, M. A. & Schmitt, F. O. 1945 *J. appl. Phys.* **16**, 459.
Hanson, J. & Lowy, J. 1964 *Biochemistry of muscle contraction* (ed. J. Gergely.) Boston: Retina Foundation.
Huxley, H. E. 1963 *J. molec. Biol.* **7**, 281.
Lanzavecchia, G. 1966 *Atti Accad. naz. Lincei Rc.* **41**, 334.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

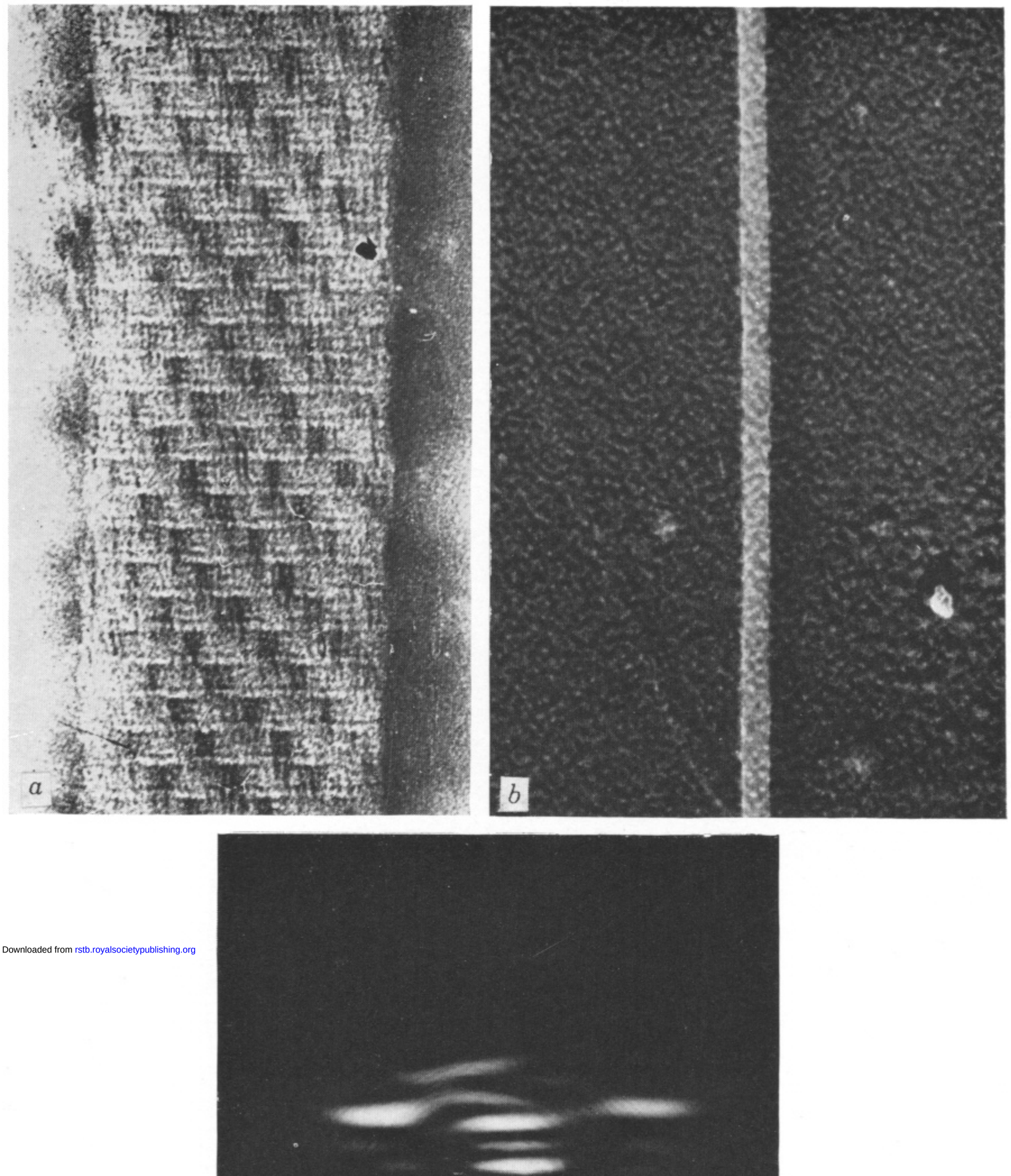

FIGURE 1. (*a*) Electron micrograph of a negatively stained paramyosin filament from the adductor muscle of *Crassostrea angulata*, showing a two-dimensional net pattern (Hanson & Lowy 1964). (*b*) Electron micrograph of a paramyosin filament from the adductor muscle of *Pecten maximus*, shadowed on both sides with platinum-palladium alloy (80:20). (*c*) Optical diffraction pattern from (*b*) showing a pattern symmetrical about the vertical (axis of filament) indicating a helical pattern on the paramyosin filament.