Schistosome Surface Spines Are "Crystals" of Actin

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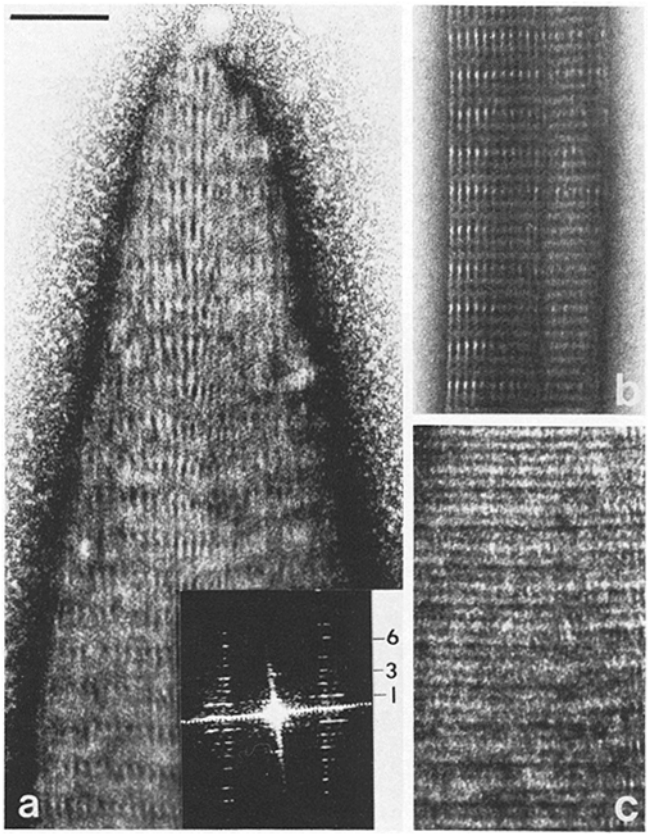
ABSTRACT The characteristic spines on the surface of the schistosome flatworm tegument consist of hexagonally packed actin filaments. Electron microscopy of isolated spines shows that their structure is very similar to that of other bundles of actin cross-linked with an additional protein.

One of the most severe and widespread of human diseases is caused by the parasitic schistosome flatworm. The life cycle of this blood fluke involves asexual larval reproduction in an intermediate snail host, giving rise to the infectious free-swimming cercarial form. After penetrating the human tissue, the worms develop into adults that become situated in the gut or bladder veins; the female generally lodges—in permanent copulation—in the ventral groove of the male worm. The surface of the male is studded with prominent rigid intracellular spines that may be critical in attachment.



Our attention was drawn to these structures by the report that they consist of a "crystalline protein" (sic) (1). The size of the male worm (1–1.5 cm long and 1-mm thick) and the size of the spines (~2.5–3 μm in length) make it feasible to examine these structures by negative staining in the electron microscope. Scraping the surface with a sharp blade readily produces spines—often, however, in low yield. When negatively stained with uranyl acetate, these structures are seen to be comprised of actin filaments aligned in register axially and spaced laterally in a regular array. The axial period is 385 Å, and is divided into three bands (Fig. 1 *a*, *c*). This image is, in fact, very similar to that given by other actin bundles in a variety of systems.

FIGURE 1 Comparison of electron micrographs of spines (*a*, *c*) isolated from tegument of male *Schistosoma mansoni* and (*b*) "needles" of F-actin cross-linked with protein fascin from oocyte of Hawaiian sea urchin (*Tripneustes gratella*)[2]. The axial period is 385 Å and is divided into three bands. The "needle" on the left in (*b*) and the lower (left) portion of the spine in (*a*) show a [1,0] view of the hexagonal lattice; that on the right in (*b*) a [1,1] view as in the spine in (*c*). In the [1,0] view, the axial repeat shows two weak stain-excluding stripes due to protein cross-bridges between actin filaments, and one bright node (where the bridge is obscured by a filament lying directly over it). In the [1,1] view, three equivalent stripes are seen in each axial repeat (see reference 3 for details). The projected lateral repeat in the spine (*a*) is ~100 Å corresponding to a spacing between filaments of $100(2/\sqrt{3}) = 116$ Å. Insert in *a* shows the optical diffraction pattern of the lower portion of the spine in *a*. The pattern shows off meridional reflections on the 1st, 6th, and 7th layer lines characteristic of the actin helix. An additional meridional reflection is present on the 3rd layer line due to the cross-bridges. Note that additional actin layer lines (generally not seen) are present. They are due to the contribution of the cross-bridges. Other weak reflections midway between layer lines indicate a longer true repeat of the structure. The actin bundles in the spines could be preserved by freezing the worms in liquid nitrogen in a mixture of 50% glycerol, 50% actin buffer (5 mM $NaPO_4$, pH 7.4, 50 mM NaCl, 0.5 mM ATP·Na_2, 0.5 mM $CaCl_2$, 3 mM NaN_3). Specimens negatively stained with 1% uranyl acetate. Needles in (*b*) from unpublished photograph provided by R. E. Kane of the University of Hawaii. Bar, 0.1 μm. × 130,000.

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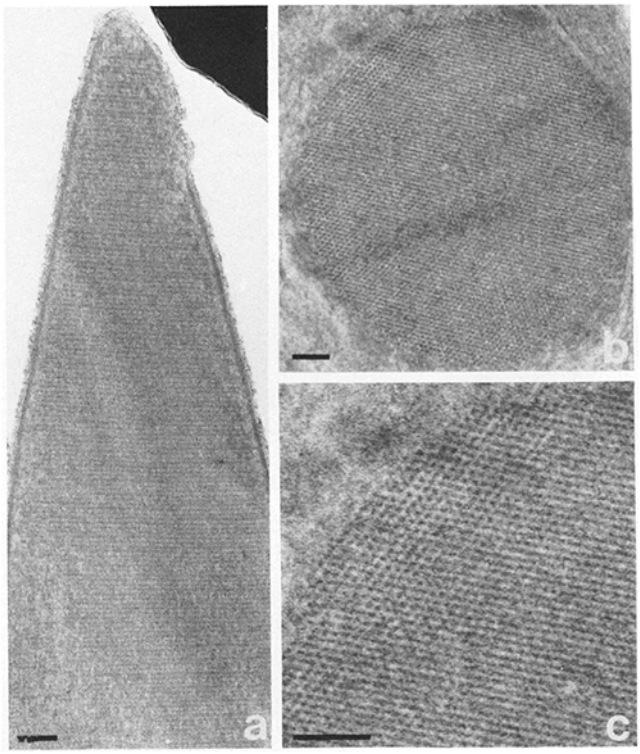

FIGURE 2 Electron micrographs of thin sections of spines. The axial period seen in longitudinal section is 380 Å and is divided into three bands (*a*); the lateral spacing of the filaments shown in the transverse section is 110 Å (*b*, *c*). Live male worms were embedded in Epon 812 after fixation in 2% glutaraldehyde, postfixation in 2% osmium tetroxide, and acetone dehydration. Thin sections were cut on an LKB-Huxley Ultramicrotome with a glass knife and stained with 4% uranyl acetate and 1% lead citrate. *a* and *b*, bar, 0.1 μm; × 50,000. *c*, Bar, 0.1 μm. × 100,000.

Optical diffraction patterns of selected areas of the spines show strong reflections lying on a set of layer lines characteristic of the helical symmetry of actin. These maxima are also sampled by row lines that arise from the packing of the filaments in a hexagonal lattice. Characteristic features of the images in Fig. 1 (and of corresponding diffraction patterns) indicate that the view of Fig. 1 *a* (lower left) is along the [1,0] and Fig. 1 *c* (upper left) along the [1,1] axis of the hexagonal lattice. The axial spacing of 130 Å can be attributed to another protein cross-linking the actin filaments. Similar features have been described in a large variety of actin bundles (2, 3). A typical example is shown in Fig. 1 *b*. Thin sections of the spines (Fig. 2) confirm the results of negative staining and demonstrate directly the hexagonal packing of the actin filaments (Fig. 2 *b*, *c*). Similar images of sectioned spines have been reported previously, but the axial repeat was reported to be 450 Å and the protein not identified (4).

The surface spines of schistosomes appear to be similar to those of other parasitic flukes (trematodes)—such as *Fasciola hepatica*—which have closely related tegument morphology and development. There is immense variety, however, in the form and arrangements of these structures: they appear as bullet-shaped projections in neat arrays in the adult *Transversotrema patialense* or as serrated spines in *Microphallus* (5). The evolutionary relations of these structures are also intriguing: it is an interesting fact that another major group of parasitic flatworms, the tapeworms (cestodes), are covered with very small, slender, flexible microvilli (microtriches) that also consist of hexagonally packed actin filaments (6). Many of the attachment or ornamental spines or hooks so characteristic of parasites of widely different species (e.g. *Acanthocephala* or thorny-headed worms) may turn out as well to be comprised of actin. Such spines may provide useful new systems for the analysis of how actin packs into bundles.

These actin spines are but one vivid example of the great variety of intracellular structures formed by actin filaments (3). They illustrate as well the fact that parasites use proteins common to the host. Surface proteins of parasites including those that extend outside the membrane may also be related to proteins whose design is well known. For example, α-helical spikes are common extensions on the surface of microorganisms (7, 8). Failures in host immunological defense against the major parasitic diseases (9) may well be related to this similarity between parasitic surface proteins and those of the hosts' own cells.

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REFERENCES

1. Schmidt, G. D., and L. S. Roberts. 1981. Subclass digenea: form, function, biology, and classification. *In* Foundations of Parasitology, 2nd edition. D. L. Bowen, Editor. C. V. Mosby Co., St. Louis. 242.
2. DeRosier, D., E. Mandelkow, A. Silliman, L. Tilney, and R. Kane. 1977. Structure of actin-containing filaments from two types of nonmuscle cells. *J. Mol. Biol.* 113:679–695.
3. DeRosier, D. J., and L. G. Tilney. 1982. How actin filaments pack into bundles. *Cold Spring Harbor Symp. Quant. Biol.* 46:525–540.
4. Smith, J. H., E. S. Reynolds, and F. von Lichtenberg. 1969. The integument of *Schistosoma mansoni*. *Am. J. Tropical Med. and Hygiene.* 18:28–49.
5. Davies, C. 1976. Development and *In Vitro* Culture of Some Digenea. PhD. thesis. University of London. Quoted in Whitfield, P. J. 1979. The Biology of Parasitism. University Park Press, Baltimore, MD. 60–61.
6. Lumsden, R. D., and R. Specian. 1980. The morphology, histology, and fine structure of the adult stage of the cyclophyllidean tapeworm *Hymenolepis diminuta*. *In* Biology of the Tapeworm Hymenolepis diminuta. H. P. Arai, editor. Academic Press, Inc., New York. 157–280.
7. Cohen, C., and G. N. Phillips, Jr. 1981. Spikes and fimbriae: α-helical proteins form surface projections on microorganisms. *Proc. Natl. Acad. Sci. U. S. A.* 78:5303–5304.
8. Phillips, G. N., Jr., P. F. Flicker, C. Cohen, B. N. Manjula, and V. A. Fischetti. 1981. Streptococcal M protein: α-helical coiled-coil structure and arrangement on the cell surface. *Proc. Natl. Acad. Sci. U. S. A.* 78:4689–4693.
9. Ottesen, E. A. 1979. Modulation of the host response in human schistosomiasis. I. Adherent suppressor cells that inhibit lymphocyte proliferative responses to parasite antigens. *J. Immunol.* 123:1639–1644.