

Myosin Filament Structure in Vertebrate Smooth Muscle

Jun-Qing Xu, Beatrice A. Harder, Pedro Uman, and Roger Craig

Department of Cell Biology, University of Massachusetts Medical School, Worcester, MA 01655

Abstract. The *in vivo* structure of the myosin filaments in vertebrate smooth muscle is unknown. Evidence from purified smooth muscle myosin and from some studies of intact smooth muscle suggests that they may have a nonhelical, side-polar arrangement of crossbridges. However, the bipolar, helical structure characteristic of myosin filaments in striated muscle has not been disproved for smooth muscle. We have used EM to investigate this question in a functionally diverse group of smooth muscles (from the vascular, gastrointestinal, reproductive, and visual systems) from mammalian, amphibian, and avian species. Intact muscle under physiological conditions, rapidly frozen and then freeze substituted, shows many myosin filaments with a square backbone in transverse profile. Transverse sections of fixed, chemically skinned muscles also

show square backbones and, in addition, reveal projections (crossbridges) on only two opposite sides of the square. Filaments gently isolated from skinned smooth muscles and observed by negative staining show crossbridges with a 14.5-nm repeat projecting in opposite directions on opposite sides of the filament. Such filaments subjected to low ionic strength conditions show bare filament ends and an antiparallel arrangement of myosin tails along the length of the filament. All of these observations are consistent with a side-polar structure and argue against a bipolar, helical crossbridge arrangement. We conclude that myosin filaments in all smooth muscles, regardless of function, are likely to be side-polar. Such a structure could be an important factor in the ability of smooth muscles to contract by large amounts.

THE three-dimensional arrangement of the crossbridges on the myosin filaments of vertebrate smooth muscle is unknown. This arrangement, however, will have a critical impact on the shortening capability and length-tension relationship of smooth muscle (cf. Gordon et al., 1966). It is known, for example, that some smooth muscles can contract *in situ* to one quarter of their initial length, far more than skeletal muscles, but the structural basis of this extreme shortening is not well understood (Gabella, 1984). Two possible arrangements of crossbridges have been proposed (Fig. 1). The first is a bipolar, helical arrangement similar to that occurring in striated muscles (Huxley, 1963; Huxley and Brown, 1967; Wray et al., 1975). In this structure, the crossbridges all have the same polarity in one half of the filament, and the opposite polarity in the opposite half. They lie on helical paths, and there is an axial spacing of 14.5-nm between one level of crossbridges and the next. The myosin tails in the filament backbone undergo antiparallel interactions with each other at the midpoint of the filament, where the polarity reverses (the bare zone), and parallel interactions

in the remainder of each half of the filament. The backbone has a circular or polygonal cross-sectional profile, and crossbridges are evenly distributed around the circumference. Fourier transforms of such filaments (observed as x-ray diffraction patterns of muscle or optical diffraction patterns of electron micrographs) show a series of layer lines indexing on the helical repeat and a strong, symmetrical, meridional reflection coming from the axial 14.5-nm spacing of the crossbridges (Huxley and Brown, 1967; Wray et al., 1975; Stewart and Kensler, 1986; Craig et al., 1992). In the second proposed structure, the crossbridges have a nonhelical, side-polar arrangement (Craig and Megerman, 1977, 1979; Cross et al., 1991; cf. Small and Squire, 1972). Here the crossbridges are also arranged with a 14.5-nm repeat, but their polarity is the same along the entire length of one side of the filament and the opposite on the opposite side. In the filament backbone, the myosin tails have antiparallel overlaps with each other along the entire filament length, and there is no region with pure parallel interactions. The filament has no central bare zone of polarity reversal, but instead it has asymmetrically tapered bare ends. Its backbone has a square cross-sectional profile in which crossbridges protrude only from two opposite sides of the square. Fourier transforms of such filaments show an asymmetric 14.5-nm meridional reflection (Small and Squire, 1972; Craig and Megerman, 1977) but no lower-angle helical layer lines.

Previous evidence for the two types of structure comes

Address all correspondence to Roger Craig, Department of Cell Biology, University of Massachusetts Medical School, 55 Lake Avenue North, Worcester, MA 01655. Tel.: (508) 856-2474. Fax: (508) 856-6361. e-mail: roger.craig@ummed.edu.

B.A. Harder's present address is Institute of Pharmacology, University of Zürich, CH-8057 Zürich, Switzerland.

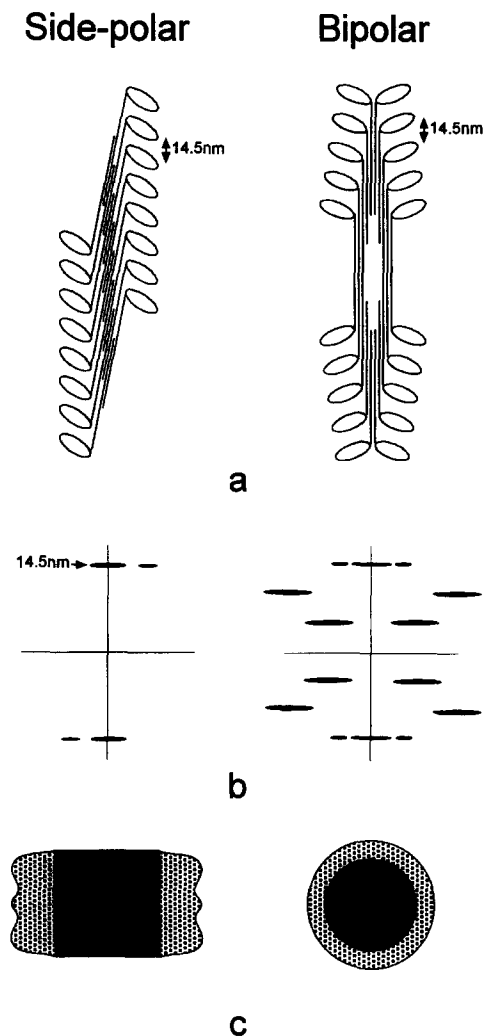


Figure 1. Schematic comparison of nonhelical, side-polar filaments with helical, bipolar filaments. (a) The two structures seen in longitudinal view; (b) Fourier transforms of longitudinal views; (c) transverse views seen in thick section (black, backbone; stipple, crossbridges).

mainly from EM of sectioned smooth muscle and negative staining of purified smooth muscle myosin (Table I). The bipolar, helical structure has been suggested on the basis of ultrastructural observations of chemically fixed, vascular smooth muscle (Ashton et al., 1975). However, the detail observed was not sufficient to establish this structure unequivocally. Evidence for the side-polar structure comes from EM studies of: (a) purified smooth muscle myosin; (b) filaments isolated from intact smooth muscle; and (c) sections of chemically fixed smooth muscle. Purified smooth muscle myosin *in vitro* assembles preferentially into side-polar rather than bipolar filaments (Craig and Megerman, 1977; Cross et al., 1991; Trybus and Lowey, 1987; cf. Sobieszek, 1972; Hinssen et al., 1978). However, the applicability of such observations to the *in vivo* structure is uncertain. Filaments isolated directly from some smooth muscles appear to have a side-polar structure (Cooke et al., 1987, 1989), but because of the lability of smooth muscle myosin filaments, their *in vivo* structure

again remains uncertain. Some longitudinal sections of smooth muscle have suggested a side-polar type of arrangement (Small and Squire, 1972), but these observations were made on smooth muscle under nonphysiological conditions, and their significance is therefore also unknown (Somlyo et al., 1971; Shoenberg and Haselgrove, 1974; Small, 1977; cf. Squire, 1975). A recent study on rapidly frozen, freeze-substituted muscle concluded that the myosin filaments are side-polar (Hodgkinson et al., 1995). However, the main evidence cited was the orientation of crossbridges in permeabilized rigor muscle, in which major rearrangements of the contractile apparatus can occur. The findings are again, therefore, not conclusive.

The *in vivo* structure of smooth muscle myosin filaments thus remains unknown. An added complication arises from the fact that smooth muscles vary greatly in physiological function. It is therefore possible that myosin filament structure is different in different smooth muscles. For example, fast contracting smooth muscles in principle might have bipolar filaments, like (fast contracting) skeletal muscle, and slowly contracting muscles might have side-polar filaments.

Our goal in this paper was to observe the *in vivo* polarity of crossbridges in a functionally diverse group of smooth muscles by rapidly freezing the specimens under physiological conditions, and then freeze substituting and observing them in thin sections. This approach provides close to *in vivo* preservation of cell molecular structure, which is not achieved by conventional chemical fixation (See Discussion). To obtain additional insights into the molecular structure, we complemented this approach with a variety of other EM techniques. We find evidence for a side-polar arrangement of crossbridges in all of the muscles we have studied.

Materials and Methods

Muscle Preparations

Smooth muscles were obtained from guinea pigs (taenia coli and vas deferens), rabbits (rectococcygeus, iris, and portal mesenteric vein), toads (stomach muscle from *Bufo marinus*), and chickens (gizzard). Animals were killed by decapitation (rabbits by cervical dislocation). Muscle strips ~2 mm wide and 20 mm long were carefully dissected from freshly killed animals. In the case of toad stomach and chicken gizzard, strips were obtained by cutting with a razor blade. The strips were gently stretched to body length and incubated in the appropriate physiological solution for 30 min at room temperature. When isolated cells were required, they were prepared from freshly isolated muscles as described by Small (1977) for guinea pig taenia coli and vas deferens, and by Kargacin and Fay (1987) for toad stomach.

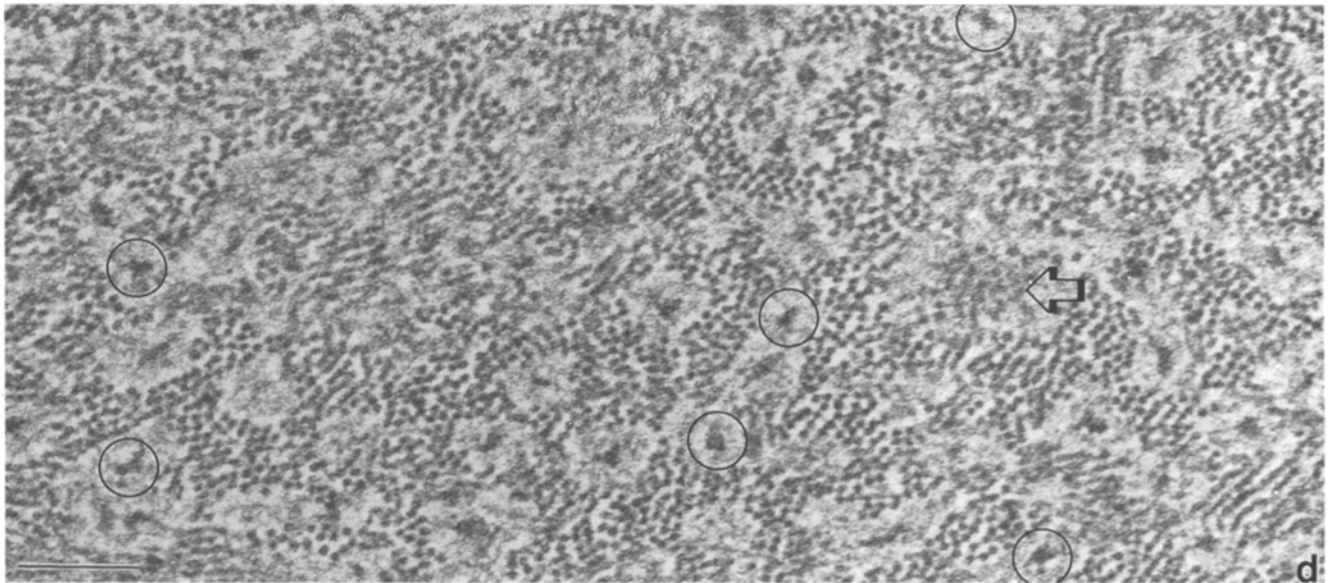
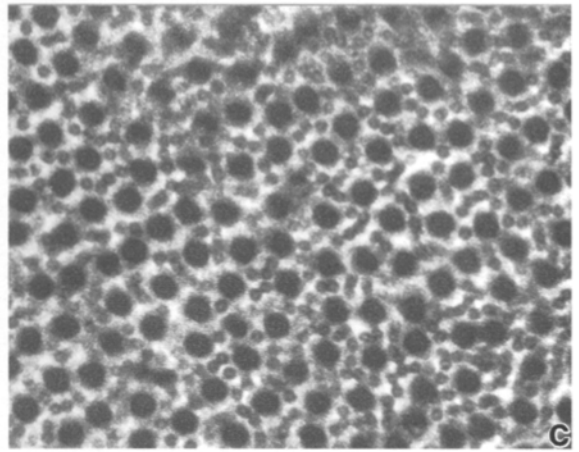
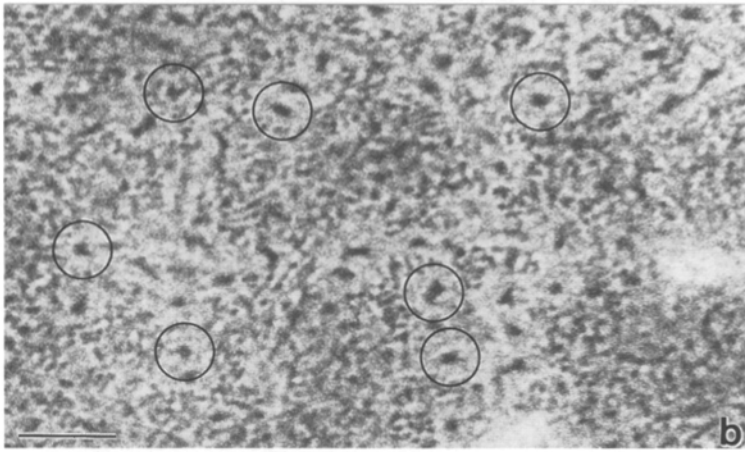
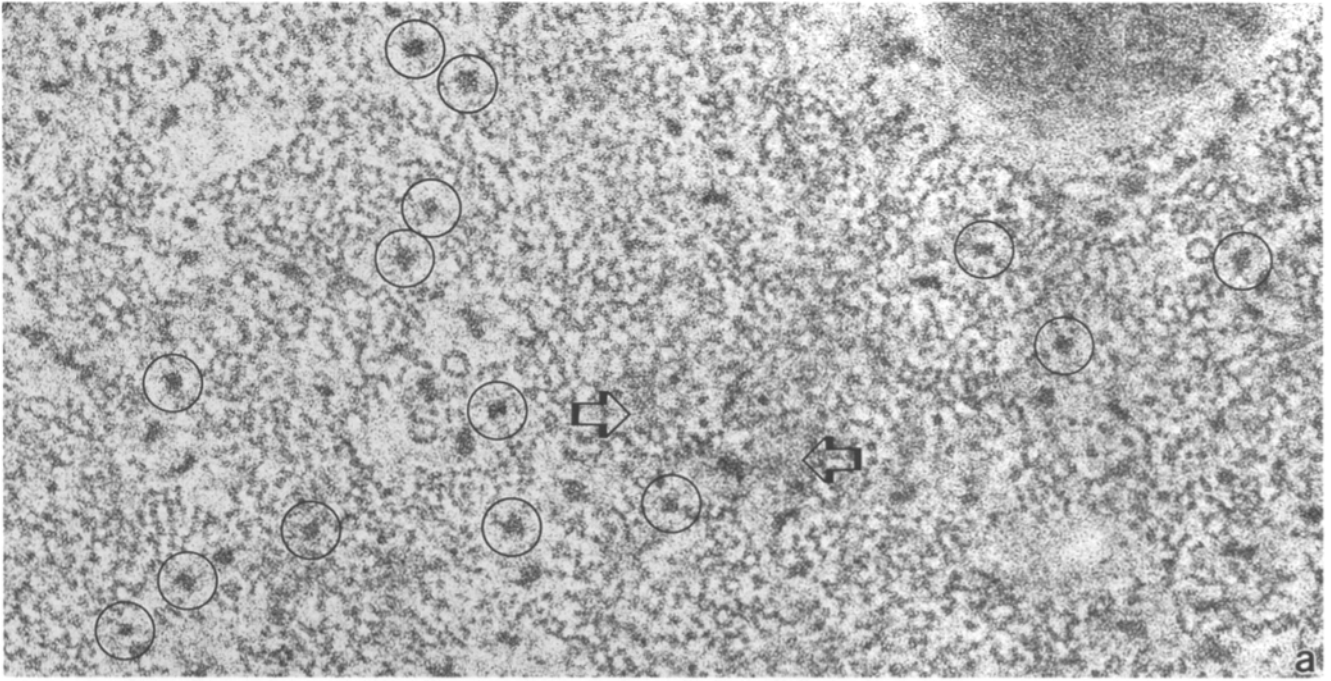
Solutions

Mammalian Ringer solution (Bülbring and Golenhofen, 1967) was used for physiological incubation of guinea pig and rabbit muscles. It consisted of 121.5 mM NaCl, 15.5 mM NaHCO₃, 4.7 mM KCl, 2.5 mM CaCl₂, 1.2 mM KH₂PO₄, 1.2 mM MgCl₂, and 11.5 mM glucose, pH 7.4, and was continuously aerated with 95% O₂/5% CO₂. Amphibian Ringer solution (Fay et al., 1982), used for incubation of toad stomach muscle, contained 108 mM NaCl, 3 mM KCl, 0.14 mM NaH₂PO₄, 0.55 mM Na₂HPO₄, 20 mM NaHCO₃, 0.98 mM MgCl₂, 1.8 mM CaCl₂, and 10 mM glucose, pH 7.4, and was aerated as above. Avian Ringer solution, used for chicken gizzard experiments, contained 137 mM NaCl, 5 mM KCl, 1.1 mM Na₂HPO₄, 0.4 mM KH₂PO₄, 4 mM NaHCO₃, 5.5 mM glucose, 2 mM MgCl₂, 2 mM EGTA, and 10 mg/l streptomycin, pH 6.1 (Small et al., 1990), and was also aerated as above. Rigor solution, used for skinning of muscles and isola-

Table 1. Previous Evidence for Smooth Muscle Myosin Filament Polarity

Paper	Muscle	Preparation/Conditions	Technique	Findings	Conclusion	Criticisms
Small and Squire (1972)	Guinea pig taenia coli and vas deferens	Live muscle/hypertonic, cold conditions	EM, conventional fixation and sectioning	LS: OD shows asymmetric 14.5 nm meridional	Face-polar	Nonphysiological conditions; filaments form ribbons
Sobieszek (1972)	Guinea pig taenia coli, vas deferens; chicken gizzard	Purified myosin	EM, negative stain	Heads along entire length of filament, terminal bare zones	Side-polar (our interpretation)*	Synthetic filaments; interpreted as helical by author
Ashton et al. (1975)	Rabbit portal vein	Live muscle, physiological conditions	EM, conventional fixation and sectioning	LS and TS: head distribution consistent with bipolar, helical structure	Bipolar, helical	Image detail insufficient to be certain of crossbridge distribution
Craig and Megerman (1977)	Calf aorta	Purified myosin	EM, negative stain and sectioning	Heads along full length; terminal bare zones; OD: asymmetric 14.5 nm meridional; TS: square backbone, heads on two opposite sides	Side-polar	Synthetic filaments; nonphysiological conditions
Hinssen et al. (1978)	Chicken gizzard; guinea pig taenia coli, vas deferens	Isolated filaments; purified myosin	EM, negative stain	Heads along full length; terminal bare zones; opposite polarity on opposite sides	Side-polar (our interpretation)*	Structure interpreted by authors as "mixed polarity" (see Cooke et al., 1989); isolated filaments really native?
Trybus and Lowey (1987)	Calf aorta, turkey gizzard	Purified myosin	EM, negative stain	Heads along full length; terminal bare zones	Side-polar	Synthetic filaments; nonphysiological conditions
Cooke et al. (1989)	Toad stomach	Isolated filaments	EM, negative stain	Heads, on 2 sides only, along full length; no central bare zone	Side-polar	Isolated filaments really native?
Cross et al. (1991)	Chicken gizzard	Purified myosin; low ionic strength	EM, negative stain	Heads along entire length, terminal bare zones; heads and tails emerge with opposite polarity on opposite sides	Side-polar	Synthetic filaments, nonphysiological conditions
Arner et al. (1991)	Rabbit rectococcygeus	Live and permeabilized muscles, physiological conditions	XRD	Strong 14.5-nm meridional reflection; no low-angle layer lines	Not helical	Layer lines may have been too weak to see
Hodgkinson et al. (1995)	Guinea pig taenia coli	Permeabilized muscle in rigor	EM, rapid freezing, freeze-substitution	Heads on opposite sides of myosin filament attach to actin filaments of opposite polarity	Side-polar	Nonphysiological (rigor) conditions; actin defines head polarity
This paper	Guinea pig taenia coli, vas deferens; rabbit portal vein, rectococcygeus, iris; chicken gizzard; toad stomach	Live muscles, physiological conditions; permeabilized, relaxed muscles; isolated filaments, low ionic strength	EM, rapid freezing, freeze-substitution; conventional fixation; negative staining	Square backbone; heads on only two sides; heads along full length; terminal bare zones; heads and tails have opposite polarity on opposite sides	Side-polar	14.5-nm repeat not seen in LS, precluding a definitive conclusion on <i>in vivo</i> polarity

*In some cases, the authors made a different interpretation of their data from us (see Criticisms); the interpretation shown is ours. LS, longitudinal section; TS, transverse section; OD, optical diffraction; XRD, x-ray diffraction.



tion of filaments, consisted of 135 mM KOH, 10 mM MOPS, 2 mM EGTA, 2.5 mM MgCl₂, titrated to pH 6.5 with propionic acid (Cooke et al., 1989) or 80 mM KCl, 3 mM MgCl₂, 2 mM EGTA, 1 mM cysteine, and 10 mM Pipes, pH 6.5 (Small, 1977). Relaxing solution consisted of rigor solution plus either 1 mM MgATP (Cooke et al., 1989) or 2 mM NaATP (Small, 1977). Skinning solution was rigor solution containing either saponin (Cooke et al., 1989) or Triton X-100 (Small, 1977). Low ionic strength solutions, used to fray isolated myosin filaments, consisted of (a) 5 mM Tris-HCl, 2 mM sodium phosphate, 1.5–2.0 mM MgCl₂, with or without 1 mM MgATP, pH 7.8–9.1 (based on Cross et al., 1991) or (b) 2 mM potassium phosphate or 2 mM imidazole chloride, 1.85 mM MgCl₂, pH 8.0 (Trinick, 1981).

Chemical Skinning of Smooth Muscles

Muscle strips or isolated smooth muscle cells were used for skinning. The methods were based on Cooke et al (1989), Kargacin and Fay (1987), and Small (1977). (a) Muscle strips, tied to plastic sheets to maintain their original body length, were skinned overnight at 4°C in rigor solution containing Triton X-100 (0.03% for guinea pig taenia coli; 0.1% for rabbit rectococcygeus, guinea pig vas deferens, toad stomach, and chicken gizzard; and 0.5% for rabbit portal vein and iris). (b) Isolated toad stomach smooth muscle cells were skinned for 12–15 min in rigor solution (Cooke, 1989) containing 50 µg/ml saponin at 4°C, and then rinsed twice with rigor solution by centrifugation at 1,500 rpm for 5 min. Guinea pig taenia coli and vas deferens cells were skinned in rigor solution containing 0.2% Triton X-100 for 20 min at 4°C as described by Small (1977).

Chemical Fixation

Skinned muscle strips tied to plastic sheets were exposed to relaxing solution (Cooke et al., 1989; Small, 1977) for 45–60 s at 4°C, and then fixed with 2.5% glutaraldehyde in the same medium for 2 h at 4°C. They were washed overnight in rigor solution, postfixed in 1% OsO₄, rinsed with rigor solution, and then treated with rigor solution containing 0.2% tannic acid for 1.5 h (Ashton et al., 1975). They were then washed again in rigor solution, stained en bloc for 1.5 h in saturated aqueous uranyl acetate, dehydrated in ethanol, and embedded in Polybed (Polysciences, Inc., Warrington, PA). Thin sections (30–100 nm) obtained with a diamond knife were collected on naked 400-mesh copper grids, stained with saturated uranyl acetate and lead citrate, and coated lightly with carbon.

Rapid Freezing and Freeze-substitution

Strips of freshly dissected guinea pig taenia coli and toad stomach were stretched to body length and incubated in the appropriate Ringer solution for 30 min to allow recovery from dissection. Rapid freezing was carried out as described by Padrón et al. (1988). Muscle strips were mounted on a slab of fixed lung (5 mm × 5 mm × 0.8 mm) on the freezing disk to make sure that the specimen made good contact with the copper block during freezing (Heuser et al., 1979). Specimens were relaxed with 10⁻⁶ M isoproterenol for 1–5 min before freezing. Excess solution was removed with filter paper without touching the muscle surface, and the freezing head was immediately covered with a humidity chamber (a small beaker containing saturated filter paper) to prevent air-drying of the specimen during transfer to the freezing machine. Specimens were frozen on a polished copper block using a liquid helium-cooled Cryopress rapid freezing device (MedVac, St. Louis, MO).

Specimens were freeze substituted in acetone containing molecular sieves at –80°C for 48 h, warmed slowly to –20°C, fixed in 5% OsO₄ in acetone at –20°C for 4–6 h, warmed slowly to 4°C, rinsed with acetone at 4°C for 2 h, and then warmed slowly to room temperature. Alternatively, the specimens were freeze substituted by progressive warming of acetone containing 2% OsO₄ from –90°C to –10°C over 36 h, rinsed with acetone at –10°C, and then warmed to room temperature over 2 h (Padrón et al.,

1988). They were then rinsed three times with ethanol (including en bloc staining with 1% uranyl acetate for 1 h) and infiltrated with Polybed. Sections were cut as above.

Negative Staining of Isolated Filaments

Filaments were prepared according to Cooke et al. (1989) or Small (1977). Rigor bundles of actin and myosin filaments were made by homogenization of skinned cells in concentrated suspension in rigor solution. The bundles were washed by centrifugation and kept on ice. The homogenate was diluted with rigor solution, and a 5–10-µl drop was applied to a thin carbon film on an EM grid. After 20 s the grid was rinsed with relaxing solution, and then immediately negatively stained with ice-cold 1% uranyl acetate.

In the case of myosin filaments treated with low ionic strength solution, carbon films exposed to UV irradiation for 10–20 min were used (Knight and Trinick, 1984). After relaxing the rigor bundles on the grid, the specimen was rinsed with five drops of low ionic strength solution and negatively stained.

EM and Image Processing

Grids were examined at 80 kV in an electron microscope (100CX; JEOL USA, Peabody, MA) (thick sections at 120 kV in an electron microscope [CM120; Philips Electronic Instruments, Inc., Mahwah, NJ]). Image digitization, Fourier transformation, and filtering were carried out essentially as described by Craig et al. (1992).

Results

Transverse Sections of Rapidly Frozen, Freeze-substituted, Live Smooth Muscles

Transverse sections of mammalian (taenia coli) and amphibian (toad stomach) smooth muscle, rapidly frozen under physiological conditions and then freeze substituted, showed the well-known features of smooth muscle obtained with conventional fixation (Fig. 2). Irregular bundles of thin filaments (actin) were interspersed with thick filaments (myosin). Cytoplasmic “dense” bodies were also observed, although their electron density was much less than in conventionally fixed muscles. The backbone profiles of the myosin filaments had a variety of appearances. They were frequently approximately square (i.e., they had clearly defined straight edges of similar length and ~90° corners; sometimes they looked slightly rectangular, but broad ribbons were not seen). Other poorly defined or irregular profiles were also observed, the variability presumably arising from variations in fixation, the orientation of the filament with respect to the section, and the deposition of sarcoplasmic proteins onto the filaments. Of the filaments having a well-defined, regular shape, ~75% were approximately square. The edge of the square had a length of 16 nm (taenia coli) and 12 nm (toad stomach; see Table II). Profiles were frequently approximately square in thick as well as thin sections, suggesting the absence of helical twisting along the filament length (Fig. 2 *b*; cf. Fig. 2 *c*). Material (crossbridges) projecting from the backbone of

Figure 2. Transverse sections of rapidly frozen, freeze-substituted live muscles. (a) Guinea pig taenia coli muscle, 50-nm section. Myosin filaments with approximately square profiles are circled; arrows point to cytoplasmic dense bodies (which are not very dense in these preparations). (b) Thick section (300 nm) of guinea pig taenia coli (myosin filaments with approximately square profiles are circled). (c) 300-nm section of tarantula striated muscle. This is a control for *b*, and it shows that helical filaments, even those with fourfold rotational symmetry (Padrón et al., 1993), have a roughly circular backbone in thick section. (d) Toad stomach smooth muscle, 50-nm section. The circled myosin filaments show roughly square backbones, with hints of material projecting preferentially from two opposite sides; arrow points to dense body. Bars, 100 nm. (*a–c* all at same magnification).

Table II. Myosin Filament Backbone Dimensions (nm)

	Rapidly frozen, live	Chemically fixed, skinned	Negatively stained, isolated
Toad stomach	12.4 ± 3.0 (n = 90)	20.7 ± 3.5 (n = 32)	12 (9–14)
Guinea pig taenia coli	15.7 ± 2.6 (n = 32)	12.2 ± 2.1 (n = 28)	15 (12–21)
Guinea pig vas deferens		19.5 ± 2.4 (n = 30)	25 (19–27)
Rabbit portal vein		17.3 ± 2.1 (n = 20)	
Rabbit rectococcygeus		19.3 ± 1.6 (n = 54)	
Rabbit iris		13.8 ± 2.6 (n = 24)	
Chicken gizzard		17.8 ± 3.7 (n = 36)	

Measurements shown are means ± SD. In the case of negatively stained filaments, ranges rather than statistics are given, due to the small number of measurements possible. Backbone widths are measured along the two sides of the filament from transverse sections of muscle or as seen in edge view of negatively stained isolated filaments.

the filaments was poorly defined, and it was generally not possible to describe its distribution around the filament with any precision. In a few cases, the definition was better, and it appeared that the material was not uniformly distributed but tended to occur on two opposite sides of the square backbone (Fig. 2 d).

Longitudinal sections of rapidly frozen taenia coli and toad stomach muscle were also cut with the goal of observing the *in vivo* polarity of the 14.5-nm repeat. However, for reasons that we do not understand, the repeat was not convincingly demonstrated except on rare occasions when the muscles had been incubated at 4°C in hypertonic salt–nonphysiological conditions in which filaments aggregate into ribbons with more stable crossbridge arrays (Small and Squire, 1972; Shoenberg and Haselgrove, 1974; see Discussion).

Transverse Sections of Chemically Fixed, Skinned Smooth Muscles

To observe the structure of the myosin filaments more clearly, muscles were first chemically skinned to remove the background of cytosolic proteins and to allow the filament lattice to swell. They were then chemically fixed and sectioned. Smooth muscles from the gastrointestinal, reproductive, visual (not shown), and vascular systems, including mammalian, amphibian and avian species, were all observed in this way. In all cases, clearly defined, approximately square backbone profiles were common in transverse sections (Fig. 3, a and b), the side of the square having a length varying from 12 to 21 nm depending on the muscle (Table II). In the skinned muscles, it was much easier to distinguish the distribution of material (crossbridges) projecting from the backbone, especially when the filament lattice had swollen. In many cases, the projecting material clearly came from only two opposite sides of the square. This was best demonstrated in the case of the taenia coli (Fig. 3, c and d), vas deferens, and rectococcygeus muscles, but examples could be found in all of the muscles examined (Fig. 4). In some cases, presumably when the crossbridges were not as well ordered, the projecting material was not so well defined.

Negative Staining of Isolated Myosin Filaments

To observe the structure of the myosin filaments in more detail and in longitudinal view, filaments were isolated directly from some skinned smooth muscle cells and observed by negative staining under relaxing conditions (Cooke et al., 1989).

In the case of mammalian (taenia coli and vas deferens) and avian (chicken gizzard) muscles, the myosin filaments tended to form regular bundles in which the filaments ran parallel to each other. Although the bundles were very densely stained, many showed regions with an ~14.5-nm repeat of crossbridges, but the distribution of mass at each crossbridge level within any one filament was difficult to define unequivocally because of the proximity of neighboring filaments within the bundle. Bundling could be minimized, and individual filaments thus observed, by reducing the Mg²⁺ concentration in the rigor and relaxing solutions from 2 to 0.5 mM. However, this also had the effect of reducing the ordering of the 14.5-nm crossbridge repeat, so that it remained difficult to define the crossbridge arrangement. On the few occasions when the crossbridges were sufficiently well ordered, they showed two appearances, one in which the 14.5-nm repeat appeared as projections, at ~90° to the filament axis, on the two sides of the filament, without superposition on the filament backbone (Fig. 5 a), and the other in which the repeat was superimposed on, but did not project from, the backbone (Fig. 5 d). These two appearances are consistent with “edge” and “face” views, respectively, of a side-polar crossbridge arrangement (Cooke et al., 1989; see Discussion). Computed Fourier transforms of these views both showed a clear 14.5-nm layer line, symmetrical about the meridian (Fig. 5, b and e), but neither showed lower-angle layer lines that would have been expected of a helical structure (e.g., Kensler and Levine, 1982). Filtered images revealed more clearly the two appearances (edge and face views) of the filaments (Fig. 5, c and f).

Myosin filaments prepared in the same way from toad stomach cells showed much less bundling, and single filaments with a well-ordered 14.5-nm repeat were readily observed (Fig. 6). The face and edge views described above were again seen, and the angle of the projections in the edge view was again ~90°. As before, Fourier transforms of face and edge views showed symmetrical 14.5-nm layer lines.

Although the above observations suggested that the myosin filaments had a side-polar structure, the observed ~90° angle of the projections meant that any underlying asymmetry in the crossbridge arrangement was not readily apparent. The evidence for side polarity would have been more conclusive if the crossbridges projected with equal but opposite angles on opposite sides of the filament when seen in edge view. In an attempt to alter the 90° angle of the crossbridges, we therefore subjected toad stomach myosin filaments to a variety of ionic conditions, by rinsing

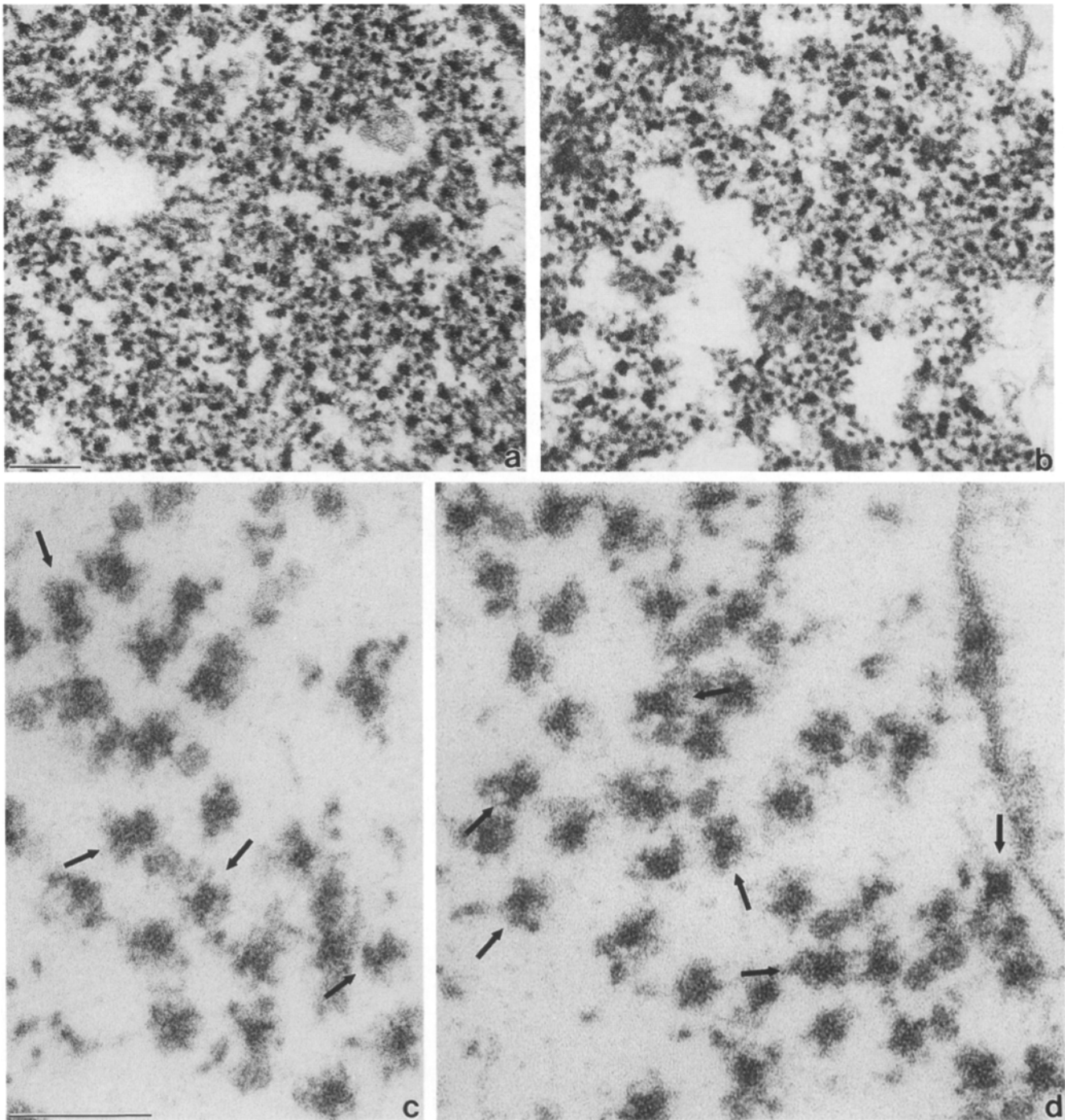


Figure 3. Transverse sections (50 nm) of skinned, relaxed, conventionally fixed smooth muscles. (*a* and *b*) Low power micrographs showing many myosin filaments with clearly defined square backbones (*a*, guinea pig vas deferens; *b*, rabbit rectococcygeus). (*c* and *d*) High power micrographs of guinea pig taenia coli muscle showing myosin filaments with square backbones, and, in addition, material projecting preferentially from two opposite sides of the square (indicated with arrows pointing parallel to the direction of the projections). Bars, 100 nm (*a* and *b* at same magnification; *c* and *d* at same magnification).

them on the grid before staining. Most conditions tested had little or no effect on the crossbridge angle. However, when filaments were rinsed with low ionic strength solution (see next section), some myosin filaments were observed to have tilted crossbridges. This change in tilt was

revealed as an asymmetric 14.5-nm layer line in the Fourier transform of such filaments, the asymmetry appearing either as a 14.5-nm meridional reflection with an off-meridional satellite reflection on only one side of the meridian (Fig. 7 *b*), or as a displacement of the primary 14.5-nm re-

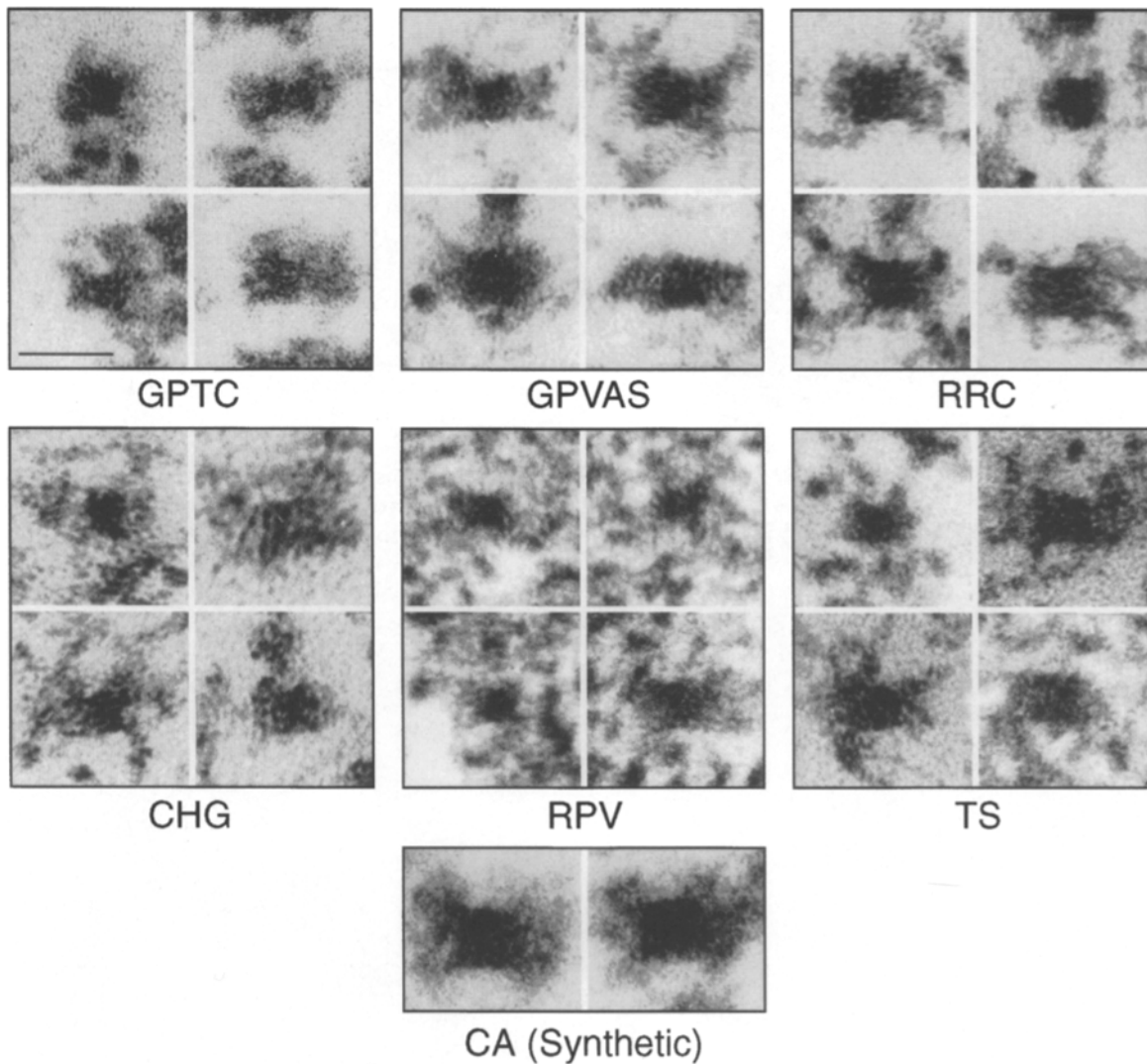


Figure 4. Gallery of selected filaments in skinned, relaxed muscles prepared by conventional fixation, showing square backbones with material projecting from two opposite sides of the square (50-nm transverse sections). Known side-polar filaments formed in vitro from purified calf aorta myosin (Craig and Megerman, 1977), also chemically fixed, are shown for comparison. *GPTC*, guinea pig taenia coli; *GPVAS*, guinea pig vas deferens; *RRC*, rabbit rectococcygeus; *CHG*, chicken gizzard; *RPV*, rabbit portal vein; *TS*, toad stomach; *CA*, synthetic filaments from calf aorta myosin. Bar, 50 nm.

flection away from the meridian. The tilt was clearly apparent in filtered images (Figs. 7 *c* and 8) and was in opposite directions on opposite sides of the filament.

Negative Staining of Myosin Filaments Treated with Low Ionic Strength Solution

Isolated myosin filaments were briefly exposed to low ionic strength solution to open up the filament structure and thus reveal more clearly the arrangement of myosin tails in the filament backbone (Maw and Rowe, 1980; Trinick, 1981; Cross et al., 1991). This treatment had varying disruptive effects on the myosin filaments, which depended on the free Mg^{2+} concentration and pH. In the absence of Mg^{2+} , there were no myosin filaments. As the free $[Mg^{2+}]$ increased, the pH necessary to disrupt the structure also increased. When the $[Mg^{2+}]$ was higher than 2.0 mM and the pH ≤ 7.9 , many longer myosin filaments

with a regular 14.5-nm crossbridge repeat were observed (these were the conditions used in the previous section to observe tilted crossbridges). At the same pH, but when the $[Mg^{2+}]$ was ≤ 2.0 mM, the crossbridges became disordered and the S2 portion of the myosin tail peeled away from the filament backbone (Fig. 9). The opposite polarity of the S2 regions of the myosin tails on opposite sides of the filament backbone was clearly revealed by this procedure, and asymmetric bare zones, parallel to each other on opposite sides of the filament at opposite ends, were seen to occur at the filament tips. These backbone features are consistent with a nonhelical, side-polar structure and inconsistent with a helical, bipolar filament.

Discussion

The polarity of the myosin filaments of vertebrate smooth

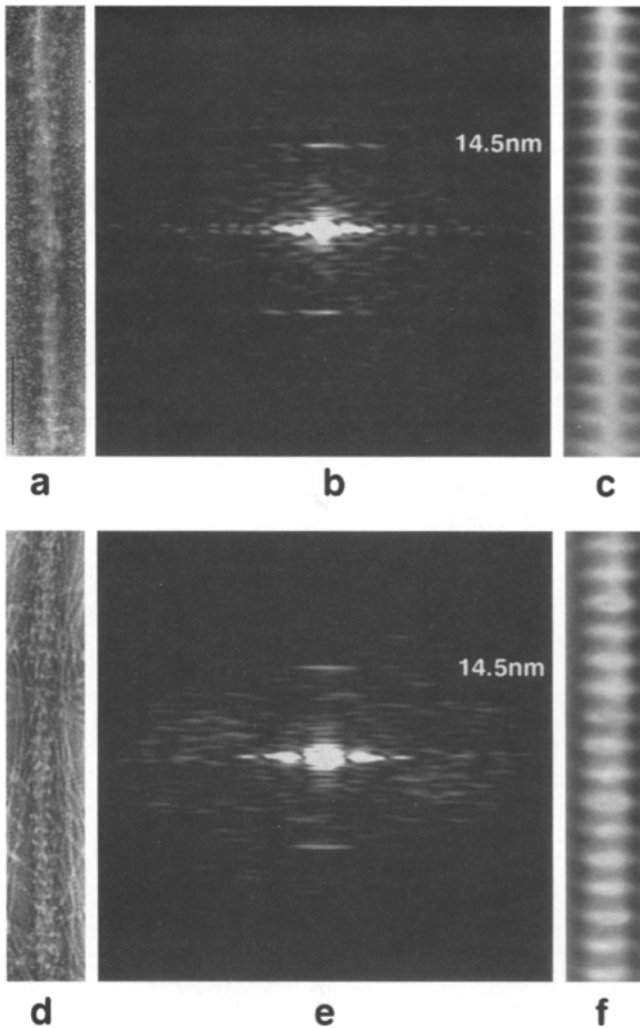


Figure 5. Negatively stained, isolated myosin filaments from guinea pig taenia coli (*a* and *d*), together with their Fourier transforms (*b* and *e*) and filtered images (*c* and *f*). (*a-c*) Edge view; (*d-f*) face view. Filtered images (using the equator extending radially to $1/6.5 \text{ nm}^{-1}$ and the 14.5- and 7.2-nm layer lines extending to $1/9 \text{ nm}^{-1}$ on either side of the meridian) are enlarged relative to originals and show clearly the 14.5-nm repeat of crossbridges, projecting from the backbone at $\sim 90^\circ$ in edge view (*c*) and superimposed on the backbone in face view (*f*). The crossbridge order was normally poor in isolated mammalian filaments: the filaments shown here are among the best ordered that we have seen. Bar (*a* and *d*), 100 nm.

muscle in vivo has proven difficult to determine unequivocally (Table I). This is because the filaments are labile (e.g., Suzuki et al., 1978), so that conclusions based on in vitro studies and on isolated filaments cannot be definitive, and because the crossbridge arrangement has not been preserved in sectioned muscle under in vivo conditions. It is also possible that filaments from functionally diverse smooth muscles may have differing structures, leading to conflicting findings by different workers. In this paper, we have attempted to elucidate the structure of smooth muscle myosin filaments by applying a variety of complementary EM techniques to filaments from a functionally diverse group of muscles in as controlled a way as

possible. For technical reasons, it was not possible to apply all of our techniques to every muscle. However, all of the muscles showed many examples of square thick filament backbones, with projections on opposite sides of the square, in transverse sections of fixed, skinned specimens (Fig. 4). Detailed studies of certain of these muscles (Figs. 2 and 5–9) showed that a side-polar structure was the basis of this appearance in these cases, and we reason that it is the most likely basis in the other muscles as well. Taking all the results together, we conclude that myosin filaments in all smooth muscles are likely to have a side-polar arrangement of myosin molecules.

Our initial goal was to observe the in vivo arrangement of crossbridges by rapidly freezing living smooth muscle under physiological conditions, and then freeze substituting the frozen specimens. Longitudinal sections of striated muscles prepared in this way show close to in vivo preservation of the helical crossbridge arrangement at the molecular level, which is not achieved by conventional chemical fixation (Craig et al., 1992). This approach seemed especially appropriate in light of the known lability of smooth muscle myosin filaments: because rapid freezing physically arrests molecular motions within a millisecond (Heuser et al., 1979; Sitte, 1987), it should minimize any structural changes that might normally occur during conventional chemical fixation, which is orders of magnitude slower (cf. Shoenberg and Needham, 1976). So far, however, we have not been able to preserve the 14.5-nm crossbridge periodicity by these methods. Other rapid freezing studies of mammalian muscle also do not report preservation of the 14.5-nm repeat (Somlyo et al., 1981; Tsukita et al., 1982; Hodgkinson et al., 1995). This could mean that in the case of smooth muscle, the techniques are inadequate to preserve the ordered array of crossbridges, or that the crossbridges in relaxed, living smooth muscle are not well ordered. The latter possibility seems more likely considering the difficulty that has been experienced in obtaining a strong 14.5-nm reflection in x-ray diffraction patterns of living smooth muscle under physiological conditions (Shoenberg and Haselgrove, 1974; Watanabe et al., 1993).

Despite its failure to reveal the 14.5-nm crossbridge repeat, rapid freezing of live smooth muscle under physiological conditions does reveal other features of the myosin filaments that suggest a side-polar structure. The first is the observation that many filaments have an approximately square backbone in thin transverse section (Fig. 2). This shape is similar to that of known side-polar myosin filaments assembled in vitro from purified smooth muscle myosin (Fig. 4) (Craig and Megerman, 1977) and quite distinct from the roughly circular or polygonal profiles of myosin filaments in vertebrate striated muscle (e.g., Huxley, 1968; Craig et al., 1992). However, bipolar, helical filaments with fourfold rotational symmetry (e.g., tarantula) also show square cross-sectional profiles in thin section (Padrón et al., 1993), and it is necessary to study thick sections to distinguish a fourfold helical structure from a side-polar one. Because side-polar filaments are not helical, they would be expected to maintain the same azimuthal orientation along their length and therefore retain their square appearance in thick as well as thin sections. In myosin filaments with helical symmetry, the profile would be expected to rotate along the helix, so that thicker sections

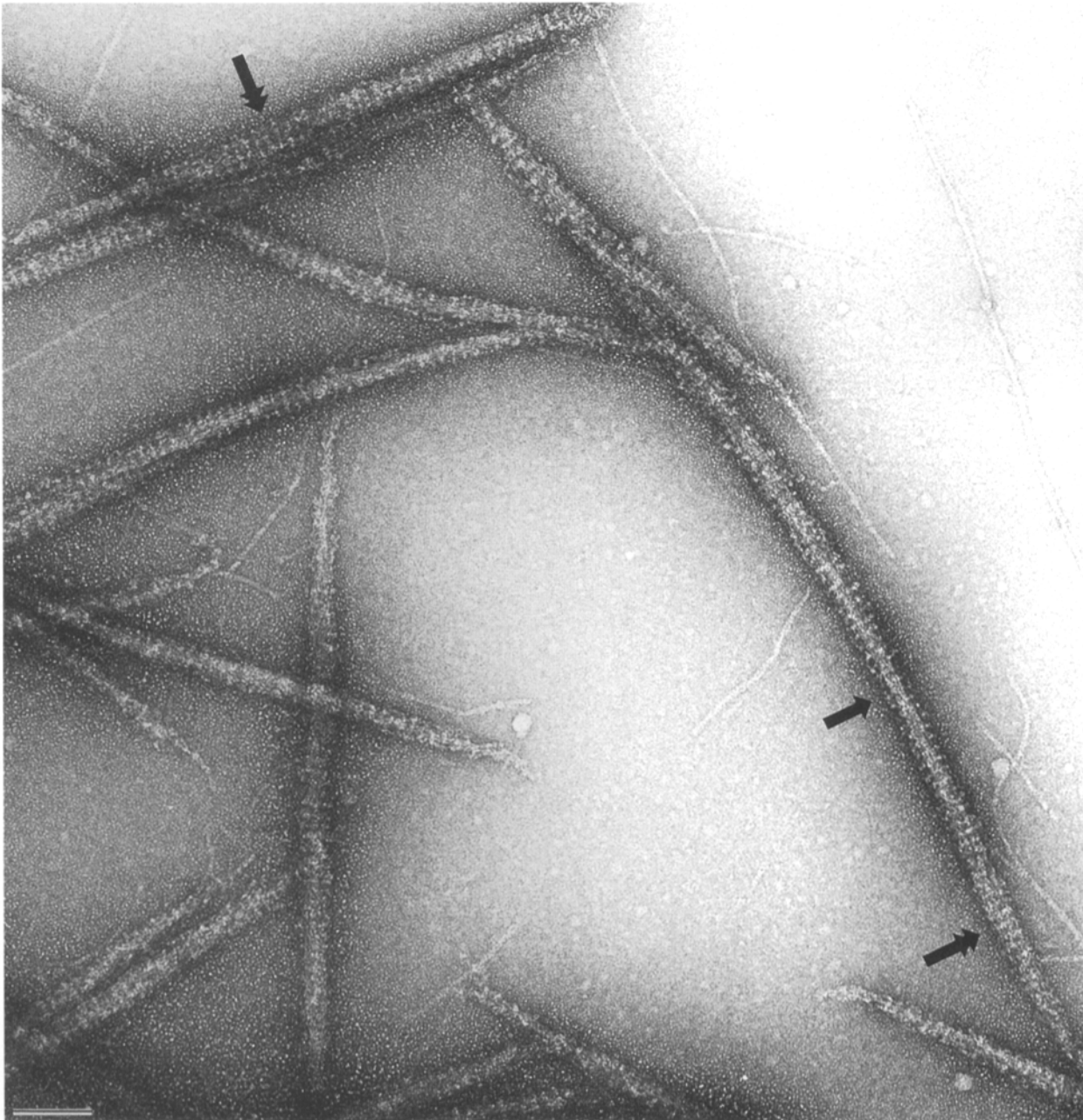


Figure 6. Negatively stained toad stomach myosin filaments with 14.5-nm repeat seen in edge (arrow), face (double arrows), and intermediate views. Bar, 100 nm.

containing several crossbridge levels would be close to circular in profile. We compared the myosin filament profiles in 300-nm-thick sections of one of the smooth muscles (taenia coli) and tarantula striated muscle, which would contain ~ 20 levels of crossbridges. The approximately square appearance was retained in the smooth muscle, but converted to a circular appearance in the striated muscle (Fig. 2, *b* and *c*), arguing for a side-polar and against a helical structure in smooth muscle.

In most rapidly frozen, freeze-substituted live muscles we have observed, it has been difficult to distinguish the crossbridges at the surface of the myosin filaments, possibly because the good preservation and staining of the

background cytosolic proteins makes them similar in density to the crossbridges. In some cases, however, the projections did appear to be more localized to two opposite sides of the square (Fig. 2 *d*). This agrees with the side-polar structure but not with a helical structure, which would predict a circularly symmetric distribution of crossbridges in transverse section (Fig. 1).

To observe the crossbridge distribution in transverse section more clearly, the soluble proteins must first be removed. This was achieved by chemically skinning the muscles before fixation. To minimize any structural change in the myosin filaments during this preparation, the muscles were skinned in rigor solution (absence of ATP), in which

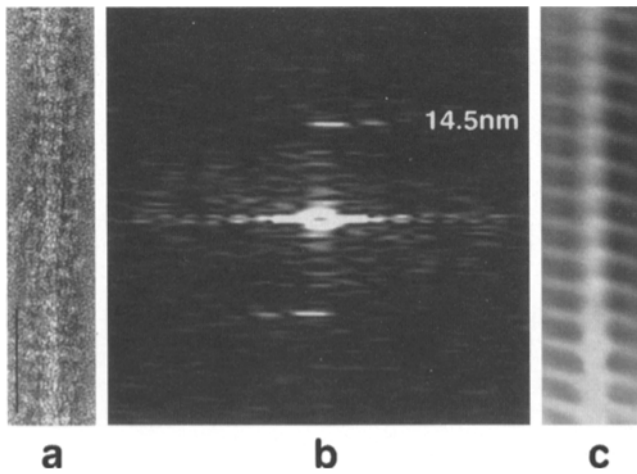


Figure 7. Edge view of negatively stained toad stomach myosin filament (a), its Fourier transform (b), and filtered image (c). Filament was rinsed with 5 mM Tris-HCl, 2 mM sodium phosphate, 2.0 mM MgCl₂, and 1 mM MgATP, pH 7.9, before negative staining, inducing a slight tilt in the crossbridges and an asymmetric 14.5-nm meridional reflection. Filtered image is enlarged relative to original and shows that crossbridges are tilted in opposite directions on opposite sides of the filament. Filtering parameters were similar to those in Fig. 5. Bar, 100 nm.

the filaments are stable (Cooke et al., 1989). They were then only briefly exposed to relaxing solution (to allow the crossbridges to detach from the actin filaments and take up their relaxed positions on the thick filament) before fixation. The filament lattice swelling that sometimes occurred in skinned muscles helped to make the details of myosin filament structure clearer. Square, well-defined backbone profiles were common in all muscles studied, and in many cases, the projections could now be clearly observed on only two opposite sides of the square, in agreement with the side-polar structure (Figs. 3 and 4). This included muscles from three of the major classes of vertebrates (mammals, amphibia, and birds) and from the reproductive, vascular, gastrointestinal, and visual systems. Muscles included both phasic and tonic types and ranged in contraction speed from very slow to very rapid. These observations of backbone shape and crossbridge distribution suggest that myosin filaments are likely to have a side-polar structure in all smooth muscles.

The finest details of myosin filament structure were observed in negatively stained preparations of isolated filaments. This approach carries with it the possibility that the filament structure could change during isolation, and the preparative procedure was therefore chosen, as above, to minimize this possibility (Cooke et al., 1989). In the mammalian muscles that we studied, the 14.5-nm crossbridge repeat was clearest when the filaments aggregated into bundles and was very weak in single filaments. This correlates with the observation that in intact taenia coli muscle, the 14.5-nm repeat is clear when the filaments occur in bundles (as occurs under cold, hypertonic conditions; Small and Squire, 1972; Shoenberg and Haselgrove, 1974), but weak when they exist as single filaments, as in the case under physiological conditions (Shoenberg and Hasel-

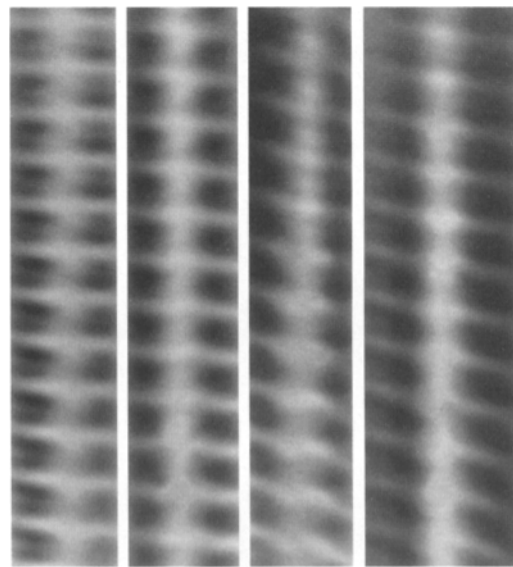


Figure 8. Gallery of filtered images (edge views) of toad stomach myosin filaments showing oppositely tilted crossbridges on opposite sides. The first image is one of the few that showed tilting under normal ionic conditions. The other three were obtained by rinsing the filaments at low ionic strength as described in Fig. 7. The original images, Fourier transforms, and filtering parameters from which these filtered images were obtained were similar to those in Fig. 7.

grove, 1974; Watanabe et al., 1993). These observations point to the possibility that under physiological conditions, in the relaxed state when the myosin of smooth muscle is in the form of (nonaggregated) filaments, the crossbridges of mammalian smooth muscle may be poorly ordered. This may explain our experience, mentioned earlier, that we were unable to observe a 14.5-nm repeat in rapidly frozen, freeze-substituted taenia coli. Lateral (nonphysiological) association with other filaments may help to stabilize the array, as seen in our experiments and in x-ray and sectioning studies of whole smooth muscle (Small and Squire, 1972; Shoenberg and Haselgrove, 1974).

In the isolated filaments, two distinct appearances of the crossbridge array were observed with both mammalian and amphibian muscle. These two appearances were previously shown to be simply orthogonal views (face and edge views) of a side-polar structure (Cooke et al., 1989) and are inconsistent with a helical arrangement of crossbridges. Although the $\sim 90^\circ$ angle of the projections seen in edge views of filaments prepared in normal ionic strength does little to reveal the underlying polarity (Fig. 5, a and c), the equal but opposite tilting of crossbridges on opposite sides of the filament seen under certain low ionic strength conditions strongly supports the side-polar model (Figs. 7 and 8).

Further evidence for the filament polarity comes from the arrangement of the myosin tails in the filament backbone. In the isolated filaments we have examined, the backbone is compact and details of its structure are not revealed under normal ionic conditions. However, brief treatment of isolated filaments with low ionic strength solution ($Mg^{2+} < 2$ mM) before staining caused disruption of

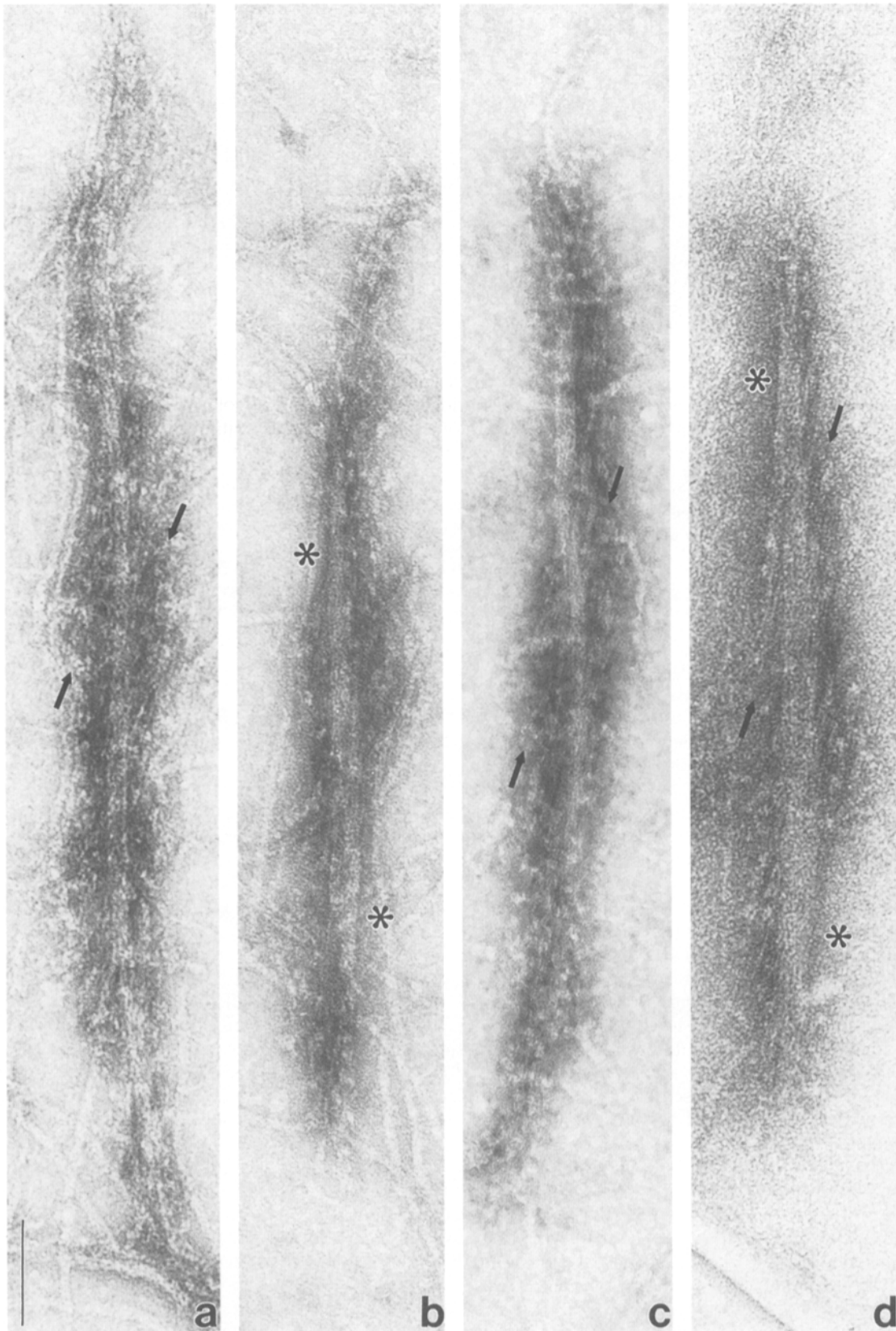


Figure 9. Negatively stained toad stomach myosin filaments rinsed briefly on the grid with low ionic strength solution (5 mM Tris-HCl, 2 mM sodium phosphate, 1.5 mM MgCl₂, and 1 mM MgATP, pH 7.9). S2 portions of myosin tails pointing in opposite directions on opposite sides of the filament are seen in the densely stained fringes on the two sides of the filaments (*arrows*). Asymmetric bare zones are seen on opposite sides of the filaments at opposite ends (***). Bar, 100 nm.

the crossbridge array and some opening up of the backbone. It was then possible to see that the S2 regions of the myosin tails extended from the backbone in opposite directions on opposite sides, and in some cases, it was clear that the filaments had bare, asymmetrically tapered ends (Fig. 9). All of these backbone features are those predicted by the side-polar model (Craig and Megerman, 1977) and are inconsistent with a helical, bipolar structure.

Based on all of the approaches we have used and on the diverse group of filaments we have studied, we conclude that myosin filaments in all vertebrate smooth muscles are likely to be side-polar. X-ray observations on living smooth muscle are also consistent with a side-polar structure, showing a 14.5-nm reflection on the meridian of the pattern, but not the lower-angle off-meridional layer lines that are known to characterize diffraction patterns of striated muscle (Arner et al., 1991; cf. Fig. 1). Our conclusions are also consistent with the strong tendency of purified smooth muscle myosin to assemble into side-polar filaments *in vitro* (Craig and Megerman, 1977; Cross et al., 1991; cf. Hinssen et al., 1978) and with earlier observations on filaments isolated directly from smooth muscle (Cooke et al., 1989; cf. Hinssen et al., 1978) and on skinned smooth muscle in rigor (Hodgkinson et al., 1995). Nonmuscle myosin can also form side-polar filaments *in vitro* (Hinssen et al., 1978; Smith et al., 1983; Stewart and Spudich, 1979), so that our observations on smooth muscle filaments may also be relevant to motility in nonmuscle cells.

Based on dry mass measurements of synthetic side-polar filaments examined by scanning transmission EM, it has been suggested that side-polar filaments are built from stacked monolayers of myosin molecules having a side-polar arrangement (Cross and Engel, 1991; Cross et al., 1991). We have attempted to incorporate this model into our observations of native filaments. With the antiparallel overlap of 14 nm between myosin molecules from opposite sides of the filament inferred by Cross et al., the bare ends of the filaments would be ~ 285 nm long, consistent with our images in Fig. 9. However, the width of the monolayer in the Cross structure is 40 nm, much greater than the ~ 15 -nm backbone we have measured in native filaments. To accommodate all of the tails in one monolayer within a 15-nm backbone width, the monolayer would have to be compressed laterally by a factor of 2–3, by stacking tails on top of each other forming a layer two to three tails (5 nm) thick. Three such 5-nm layers placed on top of each other would build a filament that was ~ 15 nm thick, as measured. On this model, three crossbridges would project from each side of the filament at each 14.5-nm level. The same number is also arrived at based on the number of tails (each occupying a volume of 522 nm^3 , assuming hexagonal close packing) that can be accommodated in one 14.5-nm repeat of the filament backbone (volume $3,262 \text{ nm}^3$). The amount of variability in the backbone measurements is quite large, for reasons previously discussed. It is thus possible that this value for the number of myosin molecules at each level might vary between filaments.

These calculations would suggest that there is ~ 5 nm laterally on the filament surface for each pair of myosin heads. Since each head is ~ 5 nm wide at its widest, this would suggest that the two heads lie adjacent to each other

longitudinally rather than laterally. This kind of packing of myosin heads on the filament surface is tighter than that in helical filaments where pairs of heads are distributed evenly around the filament circumference, with ~ 12 nm available azimuthally for each pair (cf. Squire, 1981). The height of the projections above the filament surface was difficult to measure, but it is probably most accurately represented by measurements from edge views of negatively stained specimens. This height (~ 9 nm in mammalian and ~ 14 nm in toad filaments) appears to be substantially less than the length of the myosin head, suggesting that the heads are tilted azimuthally away from the normal to the filament axis.

Side-polar filaments could, in principle, help to explain the ability of smooth muscles to shorten by large amounts. Actin filaments, probably several times as long as myosin filaments *in vivo* (Small et al., 1990), would interact with crossbridges of appropriate polarity along the entire length of a side-polar filament without encountering crossbridges with the opposite polarity. In the case of the bipolar filaments of striated muscle, actin filaments encounter crossbridges with the wrong polarity when they reach the middle of the sarcomere, and further shortening does not result in increased tension (Gordon et al., 1966; Yanagida and Ishijima, 1995). How side-polar filaments actually interact with actin filaments *in vivo* will remain unknown until the detailed three-dimensional arrangement of actin and myosin filaments *in vivo*, in particular the relative polarities of actin filaments in the vicinity of a myosin filament, is understood.

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References

- Arner, A., U. Malmqvist, and J.S. Wray. 1991. X-ray diffraction and electron microscopy of living and skinned rectococcygeus muscles of the rabbit. *In* Sarcomeric and Non-sarcomeric Muscles. U. Carraro, editor. Unipress, Padova, Italy. 699–704.
- Ashton, F.T., A.V. Somlyo, and A.P. Somlyo. 1975. The contractile apparatus of vascular smooth muscle: intermediate high voltage stereo electron microscopy. *J. Mol. Biol.* 98:17–29.
- Bülbring, E., and K. Golenhofen. 1967. Oxygen consumption by the isolated smooth muscle of guinea-pig taenia coli. *J. Physiol.* 193:213–224.
- Cooke, P.H., G. Kargacin, R. Craig, K. Fogarty, F.S. Fay, and S. Hagen. 1987. Molecular structure and organization of filaments in single skinned smooth muscle cells. *In* Regulation and Contraction of Smooth Muscle. M. Siegman, N. Stephens, and A. Somlyo, editors. Alan R. Liss, Inc., New York. 1–25.
- Cooke, P.H., F.S. Fay, and R. Craig. 1989. Myosin filaments isolated from skinned amphibian smooth muscle cells are side-polar. *J. Muscle Res. Cell Motil.* 10:206–220.
- Craig, R., and J. Megerman. 1977. Assembly of smooth muscle myosin into side-polar filaments. *J. Cell Biol.* 75:990–996.
- Craig, R., and J. Megerman. 1979. Electron microscope studies on muscle thick filaments. *In* Motility in Cell Function. F.A. Pepe, J.W. Sanger, and V.T. Nachmias, editors. Academic Press, New York. 91–102.
- Craig, R., L. Alamo, and R. Padrón. 1992. Structure of the myosin filaments of relaxed and rigor vertebrate striated muscle studied by rapid freezing electron microscopy. *J. Mol. Biol.* 228:474–487.
- Cross, R.A., and A. Engel. 1991. Scanning transmission electron microscopic mass determination of *in vitro* self-assembled smooth muscle myosin fila-

- ments. *J. Mol. Biol.* 222:455–458.
- Cross, R.A., M.A. Geeves, and J. Kendrick-Jones. 1991. A nucleation-elongation mechanism for the self-assembly of side-polar sheets of smooth muscle myosin. *EMBO (Eur. Mol. Biol. Organ.) J.* 10:747–756.
- Fay, F.S., R. Hoffman, S. Leclair, and P. Merriam. 1982. Preparation of individual smooth muscle cells from the stomach of *Bufo marinus*. *Methods Enzymol.* 85:284–292.
- Gabella, G. 1984. Structural apparatus for force transmission in smooth muscles. *Physiol. Rev.* 64:455–477.
- Gordon, A.M., A.F. Huxley, and F.J. Julian. 1966. The variation in isometric tension with sarcomere length in vertebrate muscle fibres. *J. Physiol.* 184:170–192.
- Heuser, J.E., T.S. Reese, M.J. Dennis, Y. Jan, and L. Jan. 1979. Synaptic vesicle exocytosis captured by quick freezing and correlated with quantal transmitter release. *J. Cell Biol.* 81:275–300.
- Hinssen, H., J. d'Haese, J.V. Small, and A. Sobieszek. 1978. Mode of filament assembly of myosin from muscle and non-muscle cells. *J. Ultrastruct. Res.* 64:282–302.
- Hodgkinson, J.L., T.M. Newman, S.B. Marston, and N.J. Severs. 1995. The structure of the contractile apparatus in ultrarapidly frozen smooth muscle: freeze-fracture, deep-etch, and freeze-substitution studies. *J. Struct. Biol.* 114:93–104.
- Huxley, H.E. 1963. Electron microscope studies on the structure of natural and synthetic protein filaments from striated muscle. *J. Mol. Biol.* 7:281–308.
- Huxley, H.E. 1968. Structural difference between resting and rigor muscle; evidence from intensity changes in the low-angle equatorial x-ray diagram. *J. Mol. Biol.* 37:507–520.
- Huxley, H.E., and W. Brown. 1967. The low angle x-ray diagram of vertebrate striated muscle and its behavior during contraction and rigor. *J. Mol. Biol.* 30:383–434.
- Kargacin, G.J., and F.S. Fay. 1987. Physiological and structural properties of saponin-skinned single smooth muscle cells. *J. Gen. Physiol.* 90:49–73.
- Kensler, R.W., and R.J. Levine. 1982. An electron microscopic and optical diffraction analysis of the structure of *Limulus* telson muscle thick filaments. *J. Cell Biol.* 92:443–451.
- Knight, P., and J. Trinick. 1984. Structure of myosin projections on native thick filaments from vertebrate striated muscle. *J. Mol. Biol.* 177:461–482.
- Maw, M.C., and A.J. Rowe. 1980. Fraying of A-filaments into three subfilaments. *Nature (Lond.)* 286:412–414.
- Padrón, R., L. Alamo, R. Craig, and C. Caputo. 1988. A method for quick-freezing live muscles at known instants during contraction with simultaneous recording of mechanical tension. *J. Microsc.* 151:81–102.
- Padrón, R., J.R. Guerrero, L. Alamo, M. Granados, N. Gherbesi, and R. Craig. 1993. Direct visualization of myosin filament symmetry in tarantula striated muscle by electron microscopy. *J. Struct. Biol.* 111:17–21.
- Shoenberg, C.F., and J.C. Haselgrove. 1974. Filaments and ribbons in vertebrate smooth muscle. *Nature (Lond.)* 249:152–154.
- Shoenberg, C.F., and D.M. Needham. 1976. A study of the mechanism of contraction in vertebrate smooth muscle. *Biol. Rev.* 51:53–104.
- Sitte, H., L. Edelmann, and K. Neumann. 1987. Cryofixation without pretreatment at ambient pressure. In *Cryotechniques in Biological Electron Microscopy*. R.A. Steinbrecht and K. Zierold, editors. Springer-Verlag, Berlin. 87–113.
- Small, J.V. 1977. Study on isolated smooth muscle cells: the contractile apparatus. *J. Cell Sci.* 24:327–349.
- Small, J.V., and J.M. Squire. 1972. Structural basis of contraction in vertebrate smooth muscle. *J. Mol. Biol.* 67:117–149.
- Small, J.V., M. Herzog, M. Barth, and A. Draeger. 1990. Supercontracted state of vertebrate smooth muscle cell fragments reveals myofilament lengths. *J. Cell Biol.* 111:2451–2461.
- Smith, R.C., W.Z. Cande, R. Craig, P.J. Tooth, J. M. Scholey, and J. Kendrick-Jones. 1983. Regulation of myosin filament assembly by light-chain phosphorylation. *Philos. Trans. R. Soc. Lond. Ser. Biol. Sci.* 302:73–82.
- Sobieszek, A. 1972. Cross-bridges on self-assembled smooth muscle myosin filaments. *J. Mol. Biol.* 70:741–744.
- Somlyo, A.P., A.V. Somlyo, C.E. Devine, and R.V. Rice. 1971. Aggregation of thick filaments into ribbons in mammalian smooth muscle. *Nat. New Biol.* 231:243–246.
- Somlyo, A.V., T.M. Butler, M. Bond, and A.P. Somlyo. 1981. Myosin filaments have non-phosphorylated light chains in relaxed smooth muscle. *Nature (Lond.)* 294:567–570.
- Squire, J.M. 1975. Muscle filament structure and muscle contraction. *Annu. Rev. Biophys. Bioeng.* 4:137–163.
- Squire, J. 1981. *The Structural Basis of Muscular Contraction*. Plenum Press, New York. 698 pp.
- Stewart, M., and R.W. Kensler. 1986. Arrangement of myosin heads in relaxed thick filaments from frog skeletal muscle. *J. Mol. Biol.* 192:831–851.
- Stewart, P.R., and J.A. Spudich. 1979. Structural states of Dictyostelium myosin. *J. Supramol. Struct.* 12:1–14.
- Suzuki, H., H. Onishi, K. Takahashi, and S. Watanabe. 1978. Structure and function of chicken gizzard myosin. *J. Biochem. (Tokyo)* 84:1529–1542.
- Trinick, J.A. 1981. End-filaments: a new structural element of vertebrate skeletal muscle thick filaments. *J. Mol. Biol.* 151:309–314.
- Trybus, K.M., and S. Lowey. 1987. Assembly of smooth muscle myosin minifilaments: effects of phosphorylation and nucleotide binding. *J. Cell Biol.* 105:3007–3019.
- Tsukita, S., S. Tsukita, J. Usukura, and H. Ishikawa. 1982. Myosin filaments in smooth muscle cells of the guinea pig taenia coli: a freeze-substitution study. *Eur. J. Cell Biol.* 28:195–201.
- Watanabe, M., S. Takemori, and N. Yagi. 1993. X-ray diffraction study on mammalian visceral smooth muscles in resting and activated states. *J. Muscle Res. Cell Motil.* 14:469–475.
- Wray, J.S., P.J. Vibert, and C. Cohen. 1975. Diversity of crossbridge arrangements in invertebrate muscles. *Nature (Lond.)* 257:561–564.
- Yanagida, T., and A. Ishijima. 1995. Forces and steps generated by single myosin molecules. *Biophys. J.* 68:312s–320s.