ULTRASTRUCTURAL ORGANIZATION
OF OBLIQUELY STRIATED MUSCLE FIBERS
IN ASCARIS LUMBRICOIDES

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ABSTRACT

The somatic musculature of the nematode, Ascaris, is currently thought to consist of smooth muscle fibers, which contain intracellular supporting fibrils arranged in a regular pattern. Electron microscopic examination shows that the muscle fibers are, in fact, comparable to the striated muscles of vertebrates in that they contain interdigitating arrays of thick and thin myofilaments which form H, A, and I bands. In the A bands each thick filament is surrounded by about 10 to 12 thin filaments. The earlier confusion about the classification of this muscle probably arose from the fact that in one longitudinal plane the myofilaments are markedly staggered and, as a result, the striations in that plane of section are not transverse but oblique, forming an angle of only about 6° with the filament axis. The apparent direction of the striations changes with the plane of the section and may vary all the way from radial to longitudinal. A three-dimensional model is proposed which accounts for the appearance of this muscle in various planes. Z lines as such are absent but are replaced by smaller, less orderly, counterpart "Z bundles" to which thin filaments attach. These bundles are closely associated with fibrillar dense bodies and with deep infoldings of the plasma membrane. The invaginations of the plasma membrane together with intracellular, flattened, membranous cisternae form dyads and triads. It is suggested that these complexes, which also occur at the cell surface, may constitute strategically located, low-impedance patches through which local currents are channeled selectively.

INTRODUCTION

In describing the muscles of vertebrates one differentiates at the outset between two basic histological subdivisions, smooth and striated, which are readily identifiable and distinguishable from each other and which overlap only very little. When one considers invertebrate muscles, however, this primary distinction is not always so easy to make (13). Forms occur which do not fit comfortably into either of the two classical categories and which have been the source of much controversy. A case in point is the somatic musculature of the parasitic nematode Ascaris.

Although these were among the earliest muscle cells to be studied, probably because of their large size and the ready availability of the worms, their structural organization has never been entirely clear, and discussion about whether they should be considered smooth or striated appears repeatedly in the literature. The conflict was most sharply delineated by Roskin (40) and Plenk (26). Plenk contended that these cells had striations which were entirely comparable to those of vertebrate skeletal muscles, and that the apparent deviations from the vertebrate pattern were the
FIGURE 1 Transverse section through the whole animal. The worm is surrounded by cuticle (C), beneath which lies the thin, dense hypodermis (H). The latter expands to form the two lateral lines (L) between which lies the intestine (I). Nearly all other structures visible are parts of somatic muscle cells. The radial structures subjacent to the hypodermis are the fibers (F). In several instances these are seen to be continuous with the balloon-like muscle bellies (B). The slender arms (A) converge on a nerve cord (N). × 60.

result of artifact. Roskin, on the other hand, stated flatly that these were smooth muscle cells and that the regular patterns within them were produced by an intracellular skeleton of "supporting fibrils." Roskin's view is the currently accepted one (15, 30).

In the present investigation, electron microscopy has been employed to study this muscle again. Although some aspects of its structural organization have still not been elucidated entirely, it is at least clear that this muscle corresponds closely to vertebrate striated muscle with respect to the arrangement of both myofilaments and sarcoplasmic reticulum. It differs in several particulars, however, of which one—the obliquity of the striations—undoubtedly underlies most of the earlier confusion.

MATERIALS AND METHODS
Specimens of *Ascaris lumbricoides* were obtained from the intestines of freshly slaughtered pigs and transferred immediately to warm Kronecker's solution (see reference 7) for transport back to the laboratory. Some worms were fixed immediately and others

\[ \text{FIGURE 2 Patterns of striation in light micrographs of muscle fibers. Figs. 2 a to 2 d, oblique and longitudinal sections. The apparent direction of striation varies from radial to horizontal and includes herringbone patterns. In Fig. 2 b the thin dense bands look beaded, and the broad pale bands are divided into sub-bands. A thin line bisects the middle sub-band in several places (arrows). The equivalent thin line is seen more clearly in Fig. 2 c (arrows). Fig. 2 e, transverse section showing radial striations. The pale bands are biseected by thin lines in this plane also (arrows). S, sarcoplasmic core of fibers; IS, interstitial space between fibers. Fig. 2 a, × 400; Figs. 2 b to 2 d, × 1000; Fig. 2 e, × 1200.} \]
after 1 to 6 days at 37°C in the same solution. The
most successful method of fixation consisted of ampu-
tating the anterior 1 to 2 mm of the worm, then
introducing a syringe needle through the cuticle into
the body cavity and perfusing fixative through the
worm—the efflux occurring at the cut anterior end.
By this means the impervious cuticle was circum-
vented. After about 15 minutes of perfusion with an
efflux rate of about 1 drop per second, the anterior
part of the worm was moderately stiff and noticeably
darker in color than the unperfused portion posterior
to the needle. At this time the fixed part of the
animal was cut into cylinders and these were either
slit along one lateral line or left intact and immersed
for an additional hour in chilled fixative.

The fixative consisted of 1 per cent OsO4 in sodium-
acetate—sodium-Veronal buffer (final concentration,
0.028 M for each salt; pH adjusted to 7.4 to 7.5 with
0.1 N HCl) with the following salts added:

<table>
<thead>
<tr>
<th>Salt</th>
<th>Concentration (mg/ml)</th>
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<tr>
<td>NaCl</td>
<td>4.2</td>
</tr>
<tr>
<td>KCl</td>
<td>1.8</td>
</tr>
<tr>
<td>CaCl2</td>
<td>0.65</td>
</tr>
<tr>
<td>MgCl2·6H2O</td>
<td>1.00</td>
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The concentration of ions in the final fixative
solution (including the contribution from the buffer)
was approximately that which normally occurs in
the body fluid of the animal (29).

The fixed specimens were cut into still smaller
cylinders after the total time for both perfusion and
immersion; they were then rinsed in isotonic saline,
derhydrated in methanol, and embedded in Epon 812
(23). Sections were cut on a Porter-Blum microtome
and mounted on uncoated grids or on grids coated
with either carbon and Formvar or carbon alone.

They were stained with aqueous saturated uranyl
acetate (45) or Karnovsky’s lead stain A (20), and
examined with an RCA EMU 3G or 3E electron
microscope at 50 kv or with a Philips EM 200 at
60 or 80 kv.

Thick sections from the same blocks were stained
with toluidine blue according to the method of
Richardson et al. (32) and studied by light micros-

OBSERVATIONS

The body musculature of Ascaris consists of a single
layer of large, longitudinally-oriented cells, each of
which is composed of three structurally distinct
parts (Fig. 1): (a) the ribbon-like contractile part,
or fiber,1 situated most peripherally, (b) the balloon-
like perinuclear part, or belly, more centrally, and (c)
the slender, elongated innervation process, or
arm, which extends from the belly to either the
dorsal or ventral nerve cord. (See reference 6 for
most recent descriptions and bibliography.) This
paper is concerned only with the first of these com-
ponents, the fiber; the latter two will be treated
separately (39).

LIGHT MICROSCOPY: In transverse sections
(Fig. 1) the fibers have U-shaped profiles, which
are oriented radially with respect to the circular
profile of the worm itself (cf. Fig. 3). Peripherally,
at the bend of the U, each fiber is attached to the
hypodermis, a cytoplasmic layer underlying the
acellular cuticle which invests the whole animal.
Within the fiber two separate regions can be dis-
tinguished, a sarcoplasmic core which occupies
the hollow of the U and is continuous with the muscle
belly, and a cortex which forms the walls of the U
and contains the contractile apparatus of the fiber.
In the transverse plane of section (Fig. 2 e) the
cortex exhibits distinct striations, which are regu-
lar in pattern and which are roughly perpendicular
to the surface of the fiber. Two repeating com-
ponents can be distinguished easily. One consists
of thin, dense bands which may appear beaded or
discontinuous. They always reach the surface of the
fiber but do not always extend all the way to the
core. These bands sometimes converge and join one
another. The second repeating component con-
stitutes the broad pale bands that intervene be-
tween successive dense bands. Some of the pale
bands are divided into three sub-bands, of which
the middle one may in addition be bisected by a
thin line of moderate density.

In contrast to the relatively simple pattern seen
in transverse sections, a very complex pattern of
stripations appears in oblique and longitudinal sec-

1 Usually called the muscle spindle, a term which is
 misleading because of its other connotations.

FIGURE 3. Transverse section. The fibers are U-shaped with the bend of the U against
the dense hypodermis (H) at the left. Each fiber has a striated cortex consisting of broad,
pale bands (M) alternating with thin, dense bands (D). The latter have a heterogeneous
content. The sarcoplasmic core (S) contains numerous mitochondria. IS, interstitial
space. X 4000.
tions through the worm (Figs. 2 a to 2 d). The fibers exhibit very irregular, ever-changing patterns of bands in which not only the orientation but also the width, spacing, and density of the bands vary continuously along each fiber. The same sub-bands that occur in transverse section appear more clearly here (Fig. 2 b). The orientation of the bands and sub-bands may change gradually from radial to longitudinal, with all degrees of obliquity in between, and in some segments the bands disappear altogether. The appearance of the striations in these sections is reminiscent of the grain in a plank of wood. In addition to these continuous variations, there are also abrupt transitions, producing zig-zag and herringbone patterns (Fig. 2 c).

**Contractile Apparatus, Transverse Sections**

In electron micrographs, as in light micrographs, the most consistently regular patterns of striation are seen in transverse sections through the worm. Sections in this plane were therefore studied and analyzed most extensively.

At low magnification (Fig. 3), electron micrographs of transverse sections display the same pattern of bands seen in light micrographs. However, it is at once plain that the thin dense bands are not comparable to the broad pale bands. The former are complex and irregular in structure, consisting of several different constituents, while the latter are composed of regular arrays of dots, which look like cross-sections through myofilaments. At first it appears that the pale bands are cross-sectioned myofibrils comparable to the myofibrils of vertebrate striated muscles (17), and the dense bands merely extensions of the sarcoplasmic core of the fiber.

With closer scrutiny and at higher magnification (Fig. 4), however, a very interesting and important difference appears between these cross-sections and those through vertebrate striated muscle. In the latter, each myofibril is ordinarily sectioned across one band only and the organization of the myofilaments in the plane of the section is characteristic of that one band. A very slightly oblique section may occasionally pass through two adjacent bands in the same fibril and rarely through three. In the cross-sections through *Ascaris* muscle fibers—sections in which the individual myofilaments are cut transversely—a regular succession of five bands appears in every one of the “myofibrils.” In the center, running perpendicularly to the cell surface, is a narrow strip containing cross-sections through thick filaments only (the H zone); on either side is a wider strip containing cross-sections through both thick and thin filaments (the A zone), and on the lateral side of each A zone is another narrow strip containing cross-sections through thin filaments alone (the I zone). Thus transverse sections through these “myofibrils” exhibit the same sequence of bands normally seen in longitudinal sections of vertebrate striated myofibrils, except that typical Z lines are absent. The beaded dense bands between the myofibrils are in just the location where one would expect to find Z lines and, as will be shown later, they contain Z line counterparts as well as interfibrillar structures. They will be referred to here simply as “dense bands.”

At high magnification (Figs. 5 and 6) the structure of the various bands is revealed in more detail. The H zone consists of thick filaments surrounded by amorphous and thready material of medium density. The diameter of the thick filaments here is ~230 Å, slightly greater than their diameter in the adjacent A zones. There appears to be a gradient in the width of these filaments from the center of the H zone to the A-I junction (17). The thick filaments are not entirely homogeneous in structure, appearing rather as braids of subunits ~40 to 50 Å in diameter (Fig. 6); some of the thick filaments have low density cores (Figs. 5 and 6). Small bundles of supporting fibrils (see below)
and elements of the sarcoplasmic reticulum also occur in the H zone (Figs. 4 and 11), but these apparently have no structural connection with the thick filaments themselves.

In the A zone each of the thick filaments is surrounded by about 10 to 12 thin filaments (cf. references 14, 44) which are separated from the thick filament by \( \sim 120 \text{ Å} \) (Figs. 5 and 6). It is sometimes possible to discern delicate cross-links connecting the thick filaments to the thin (Fig. 6). Several such bridges may appear to spring from one thick filament in the same plane, but in all probability these bridges are spaced along the length of the thick filament and appear superimposed only because of the relative thickness of the section (14). The total width of the H zone and the two A zones flanking it is \( \sim 0.6 \mu \).

The I zones are composed exclusively of thin filaments which, like the thick filaments, sometimes have cores of low density. The thin filaments are \( \sim 80 \text{ Å} \) in diameter and are spaced about equally from one another. At the lateral edges of the I zones, the thin filaments become clumped together into small, sheaf-like aggregates, the “Z bundles,” in which the individual filaments appear to be linked together (Fig. 6). These bundles are apparently the counterparts of Z lines, but differ in that they are not obviously interconnected or lined up with one another. They occur not only at the edges of the I zones, but are also distributed throughout the width of the dense bands. Thus myofibrillar components are not confined to the pale bands referred to here as “myofibrils” but occur in the dense bands as well.

Contractile Apparatus, Longitudinal and Oblique Sections

Unlike transverse sections, longitudinal and oblique sections through the muscle fibers exhibit

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**Figure 5** Transverse section of a “myofibril” at high magnification. H, A, and I zones are indicated. Dense bands (D) containing Z bundles flank the I zones. Thick filaments in the H zone are thicker than those near the A-I junction. In the A zone, thick filaments are surrounded by 10 to 12 thin filaments. \( \times 125,000 \).

**Figure 6** Transverse section of dense band and adjacent “myofibrils.” The dense band contains membrane-limited cisternae of the sarcoplasmic reticulum (C), a fibrillar “dense body” (F), and Z bundles (Z) cut in various planes. Some of the thick filaments can be resolved into subunits (arrows). Cross-links between thick and thin filaments are visible (circles). H zones are indicated (H). \( \times 181,000 \).
a great variety of structural patterns, which are all the more complex because of the marked undulation of the myofilaments (Fig. 7). The diverse patterns that occur appear to be intermediates between two extreme pictures: At one extreme the myofilaments are approximately parallel to the plasma membrane and the dense bands radial to it (Fig. 7, top); at the other, both the dense bands and the myofilaments follow a course roughly parallel to the plasma membrane (Fig. 7, bottom). Actually, at higher magnification, it is clear that in the latter case the dense bands are slightly oblique to the axis of the myofilaments (Fig. 8). It can also be seen in occasional fortunate sections that the thick filaments are very long, each extending for approximately 6 μ (Fig. 8). In pictures intermediate between the extremes, the myofilaments and the dense bands may run at any angle from perpendicular to parallel with respect to the plasma membrane. Sometimes it is possible to find instances in both longitudinal and oblique sections in which thin filaments are continuous across I bands from A bands to Z bundles (Fig. 9 b) and others in which thick filaments are continuous across H bands (Fig. 9 a).

An incidental observation made in longitudinal sections is that muscle fibers are sometimes connected by stout protoplasmic bridges which also

FIGURE 7 Longitudinal section. In the upper fiber the myofilaments are parallel to the fiber surface and the dense bands radial. In the lower fiber myofilaments and dense bands undulate but are both roughly parallel to the fiber surface. Inset, coiled bundle of supporting fibrils. × 24,000; Inset, × 34,000.
contain myofilaments. Such anastomoses between apparently separate muscle cells have been re-reported by previous workers (e.g., reference 4) who saw them not only between the fiber portions of the muscle cells but also between muscle bellies.

Plasma Membrane Invaginations and Sarcoplasmic and Supporting Components

In addition to the Z bundles, which are myofibrillar components, the dense bands also contain "dense" bodies (reference 14, and Figs. 4 and 6), which account for the beaded character of these bands in light micrographs of 1 μ sections (Figs. 2 b and 2 c), deep plasma membrane invaginations associated with membranous cisternae (Figs. 10 to 12), glycogen (Fig. 10), components of the sarcoplasmic reticulum (Figs. 11 and 12), and occasional mitochondria. All of these elements are closely interwoven and to some extent interconnected.²

The plasma membrane invaginations are roughly perpendicular to the surface of the fiber and they appear to be tubular. In occasional fortunate sections the whole length of an invagination and its continuity with the plasma membrane are visible (Fig. 10). More commonly the plane of section is not quite parallel to the invagination and as a result only a short length of the invagination is seen (Fig. 11). The invaginations may be regarded as finger-like intrusions of the plasma membrane into the striated cortex, extending almost to the core of the fiber. In effect, they bring the surface of the fiber into close proximity to its depths.

² The term "Z bands" could be substituted for the non-committal term "dense bands" since these bands are in just the location where Z lines would be expected (see Discussion). "Z bands" would not be entirely accurate, however, for the bands contain not only Z line counterparts, but also structures which, in classical striated muscles, are interfibrillar in location. The term "interfibrillar bands" would not be applicable because, except for the sarcoplasmic core of the fibers, there is, in the Ascaris muscle, no counterpart to the interfibrillar regions of classical striated muscles (see Discussion).
These invaginations are analogous to the central components of the triads of sarcoplasmic reticulum in vertebrate striated muscles (28). They differ from the latter in several respects, however. They are relatively wide (~1000 A) and they display no tendency to fragment into chains of discontinuous vesicles. The bands of extracellular matrix which surround the fiber extend into the invaginations and can often be identified as such even when the connection between the invagination and the fiber surface is not present in the plane of the section. Thus there is no question that the invaginations are merely extensions of the plasma membrane and that even at their deepest extremity they separate sarcoplasm at one surface from extracellular matrix at the other.

Two separate structures are intimately associated with the plasma membrane invaginations, dense bodies (Figs. 10 and 12), and flattened membranous cisternae, which correspond to the lateral elements of the dyads and triads of vertebrate muscles (Figs. 10 to 12, and 14). In the Ascaris muscle the two lateral elements of one triad sometimes appear to be parts of the same flattened cisterna wrapped around a finger-like invagination and sectioned twice (Fig. 14). Flattened cisternae...
Figs. 12 to 14 show association of intracellular cisternae with the sarcolemma.

**Figure 12** Plasma membrane invagination. Unit membrane structure is visible in both the cisternal and sarcolemmal membranes (at arrow). A fibrillar "dense body" (F) is very closely associated with the sarcolemma on one side and with a cisterna of the sarcoplasmic reticulum on the other. Z bundles (Z) abut against the invaginated plasma membrane. × 87,000.

**Figure 13** Flattened cisterna (C) applied to the sarcolemma (P) at the cell surface. Unit membrane structure is visible in all the membranes, and an intermediate line (I) is present between the cisterna and the plasma membrane. This complex is comparable to the association of subsurface cisterns with the plasma membrane in neurons. × 189,000.

**Figure 14** Plasma membrane invagination cut transversely. The lateral components of the triad are parts of one cisterna. At the arrow an intermediate density can be seen within the cisterna and between it and the sarcolemma. Unit membrane structure is visible in all the membranes, but the unit of the sarcolemma is not symmetrical. × 117,000.
ternae also occur in close apposition to the plasma membrane at the surface of the fiber (Figs. 4 and 13). In either location the light zone separating such a cisterna from the plasma membrane is ~120 Å and it contains an intermediate line (Figs. 13 and 14). Both the cisternal membrane and the plasmalemma exhibit unit membrane structure but the respective units are not identical. The cisternal unit membrane is symmetrical whereas the cytoplasmic surface of the plasma membrane is denser than its outer lamina (Fig. 14).

The lumina of the cisternae are ordinarily very shallow but in specimens where the fibers as a whole look swollen, they too appear swollen. Comparable membranous cisternae occur at the surface of vertebrate striated muscle cells (2, 28) and smooth muscle cells (33) and have also been noted in other invertebrate muscles (14). Equivalent structures occur in nerve cells (35) and certain sensory cells (41) as well. Probably the variations in luminal size of these cisternae in various locations depend on the method of preparation of the tissue.

The second major component of the dense bands is more difficult to delineate. It consists of bundles of fibrils which in cross-section appear as tightly packed ovoid dense bodies measuring about 0.1 by 0.2 μ. Several may occur in each dense band at various distances from the surface of the fiber (Fig. 3). They are located just in the middle of the band and are frequently surrounded by Z bundles from the adjacent I bands on both sides (Figs. 4 and 6). Thin filaments sometimes seem to join the dense bodies and indeed at high magnification the dense bodies can be resolved into subunits which resemble cross-sections through tightly packed thin filaments (Fig. 6). At the surface of the fiber, dense bodies are often closely applied to the plasma membrane (Figs. 8 and 12) and, in a corresponding manner, at deeper levels they may be attached to invaginations of the plasma membrane (Figs. 4 and 10). Smaller fibrillar bundles sometimes occur in the H zones.

In longitudinal and oblique sections, these fibrillar bundles appear fusiform in shape. Some extend from the dense bands to the sarcoplasmic core of the fiber (Figs. 4 and 7) and occasionally they exhibit coiling within the plane of the section (Fig. 7, inset). Coiling is very pronounced in standard histologic preparations, as Roskin noted (40), but in sections less than 0.1 μ thick, such as those used for electron microscopy, a coil would be expected to pass in and out of the plane of section and look like a series of interrupted bundles, as is apparently the case here.

Goldschmidt (12) considered these bundles of fibrils to be part of a semirigid intracellular skeleton, or supporting network, with enough resiliency to restore the original length and shape of the fiber after contraction. According to his view, the fibrillar bundles are extensively interconnected longitudinally, transversely, and radially within the fibers and are continuous also with a comparable intracellular skeleton in the muscle bellies and arms.

DISCUSSION

Inferred Three-Dimensional Structure of the Contractile Apparatus

From the regularly repeating patterns in transverse sections, a three-dimensional model has been inferred which corresponds in essence to the sliding filament model of Huxley and Hanson (19) but which has other structural characteristics, much like those of the oyster adductor (14) and a number of other invertebrate muscles (1, 21, 34, 43), that account for the complex patterns seen in longitudinally sectioned specimens.

In describing the model, three planes will be referred to: the transverse, or XY plane, and two longitudinal planes, XZ and YZ, which are perpendicular to each other and to the XY plane (see Fig. 15). The contractile part of the fiber consists of two sets of myofilaments, thick and thin, which interdigitate with each other and which appear to be interconnected by cross-links. The filaments are roughly perpendicular to the XY plane. Thin filaments are discontinuous across the H bands, and thick filaments are discontinuous across the I bands and the dense bands.

The principal difference from the Huxley-Hanson model appears in the XZ plane. Adjacent parallel filaments are not lined up with one another, but are staggered in a regular fashion. Consequently, a line drawn through the ends of either the thick or thin filaments is oblique rather than transverse to the filament axis in this plane. Each thick filament is set ahead of the neighboring thick filament by about 10 per cent of its length as

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1 The YZ plane is equivalent to the "sagittal" plane as defined by Kawaguti (21) and the XZ plane to his "tangential" plane.
FIGURE 15  Diagram of a muscle fiber showing the pattern of striation in three planes. In the XZ plane the myofilaments are staggered with the result that the striations are oblique rather than transverse. A second consequence of the stagger is that the adjacent rows of myofilaments do not reach the XY plane in phase resulting in the appearance of striation in this plane also. The YZ plane shows cross-striation.

indicated by the fact that only about ten rows of thick filaments appear in each “myofibril” in the XY plane. In the transverse plane the filaments in each row are cut across at a different distance from their ends: the thick filaments in the middle of the H band are cut midway between their ends; those at one A-I junction are cut close to one end, and those at the other A-I junction are cut close to the opposite end. As a result of the stagger, sections in the XZ plane always appear obliquely striated (Fig. 16), and sections in the XY plane always pass across I, A, H, A, and I bands in that order between successive dense bands (Fig. 15). Although the model does not specify the exact angle of the striations in the XZ plane it is possible to make an approximate calculation of this angle from the estimated length of the thick filaments (~6 μ) together with the distance from one A-I junction to the next as seen in transverse sections (~0.6 μ). This angle turns out to be very small (~6°). In the diagrams shown in Figs. 15-17 the filaments are drawn parallel to the fiber axis and the striations oblique. Actually, both filaments and striations are probably slightly oblique to the fiber axis as well as to each other. In addition, for the sake of clarity, the angle of striations to filament axis in the XZ plane has been greatly exaggerated in the diagrams (cf. Fig. 8).

510  THE JOURNAL OF CELL BIOLOGY • VOLUME 25, 1965
FIGURE 16  Schematic drawing of the XY, XZ, and YZ planes, showing the relationship between the arrangement of the myofilaments and the pattern of striation characteristic of each plane. Inset (upper right). Diagram of the XZ plane showing thick filaments only. The dashed line represents a transverse section passing through the caudal end of a thick filament at A and through the rostral end of a thick filament at C. The distance AC corresponds to the distance from one A-I junction to the next as seen in transverse section. This distance amounts to approximately 0.6 \( \mu \) (cf. Fig. 5). The distance AB is equal to the length of a thick filament, approximately 6 \( \mu \) as estimated from longitudinal sections (cf. Fig. 8). The angle ABC is the angle of striation with respect to the filament axis. The tangent of angle ABC is AC/AB, or approximately 0.1. The angle itself is therefore approximately 6°.

Fig. 16 inset for a diagrammatic explanation of this point.

In the YZ plane adjacent myofilaments are not staggered and therefore sections cut in this plane appear cross-striated with dense bands located in just the position where Z lines would be found in classical striated muscles (Fig. 16). (The last observation would also be true of the XZ plane if the myofilaments were aligned rather than staggered.) In both XZ and YZ planes the myofilaments are not subdivided into bundles, but extend uniformly across the thickness of the cortex. Hence, strictly speaking, the cortex contains only a single myofibril occupying the full width of the cortex from the surface of the fiber to its core.

This proposed model accounts for the occurrence of banding in the transverse sections and also accounts for at least two different patterns of striation in longitudinal sections, oblique and transverse, both of which have been seen, as described above (cf. reference 21). As Fig. 17 indicates, sections in planes other than XY, XZ, and YZ can produce striations with all degrees of obliquity between horizontal and transverse.

The complex patterns of striation illustrated in Fig. 2a can be ascribed simply to undulation of the individual muscle fibers such that even though the plane of section is parallel to the long axis of the whole worm, it passes through the fibers themselves with varying degrees of obliquity. In consequence the apparent direction of the striations in an individual fiber changes continuously as a function of the changing orientation of the fiber, even though the pattern may be entirely regular with respect to the axis of the fiber itself. Similarly the apparent direction of the myofilaments is a function of the plane of section, so that even though they are probably only slightly oblique to the fiber axis, they may appear to run very obliquely, or longitudinally, or radially with respect to the cut surface of the fiber.

FIGURE 17  Diagram showing the effect of the plane of section on the apparent direction of the striations. In the upper figure, plane A is perpendicular to the YZ plane and cut at angle a to the XY plane; b is the angle of the striations in the XZ plane, and c is the angle of the striations in plane A. c can be calculated from the equation: \( \tan c = \tan b / \sin a \). The angle c varies between the value of b (when \( a = 90^\circ \)) and 90° (when \( a = 0^\circ \)). In the lower figure, plane B is parallel to the striations in the XZ plane but cut at 45° to the XZ plane itself. The striations in plane B are longitudinal with respect to the cut edge of the plane. If plane B were perpendicular to the XZ plane, it would have no striations.
Another difference from the Huxley-Hanson model is that Z lines as such are absent. Small bundles of thin filaments apparently become bound together forming miniature counterparts to the Z lines (fragmented Z lines, as it were) which are dispersed through the dense bands. This mode of insertion of thin filaments also occurs in other invertebrate muscles (14, 21) and is believed to occur in vertebrate smooth muscles as well (3, 5). The bound sheaves of thin filaments, referred to here as Z bundles, presumably serve to link small groups of thin filaments from the same sarcomere together in parallel and to link small groups of thin filaments from adjacent sarcomeres together in series. They may also attach thin filaments to the plasma membrane (37). The Z bundles are closely associated with the dense bodies and may be attached to them. The latter are in turn anchored to the plasma membrane and to its invaginations all along the length of the fiber and presumably transmit the force of contraction of the myofibrils to the sarcolemma.

The original controversy about whether this muscle is smooth or striated can now be considered again. Obviously the contractile part of the muscle exhibits a periodic structural organization. It contains two kinds of myofilaments grouped together in a regular array in which I, A, and H zones are distinguishable. In one plane (YZ), at least, the bands are in register. In these respects the muscle resembles a classical striated muscle. On the other hand typical Z lines are missing and are replaced by much less highly ordered counterpart structures. Furthermore the myofilaments are markedly staggered in one plane. These features are not characteristic of classical striated muscles. Thus although the *Ascaris* muscle is not smooth it does not quite fit into the striated category either. It appears to be an intermediate form possessing characteristics of both smooth and striated muscle.

Although more closely related to striated muscle it has not quite attained the high degree of order usually associated with classical striated muscles. The question of how to classify this muscle becomes a semantic one (see reference 14 for discussion) and is perhaps most easily resolved by referring to it as “obliquely striated.”

Some of the earlier confusion about this muscle can be attributed to the fact that the dense bands, which are rather highly ordered, contain, intimately entwined, both myofibrillar components, as Plenk contended, and sarcoplasmic and supporting components, as Roskin believed. Probably an even greater source of confusion is the paradoxical course of the striations and their occurrence in transverse sections. As has been explained above, these puzzling effects can be ascribed in part to the pronounced stagger of the myofilaments resulting in obliquity of the striations, and in part to the undulation of fibers within the worm. Moreover, the angle of the striations with respect to the fiber axis may vary with the state of contraction in muscles of this general type (13).

**Plasma Membrane Invaginations and Sarcoplasmic Reticulum**

The contractile apparatus of the muscle fibers is closely associated with a membranous system composed of two discrete constituents, finger-like infoldings of the sarcolemma, which extend deep into the fiber, and shallow intracellular membranous sacs apposed to the cytoplasmic surface of the infoldings. These elements correspond to the central and lateral components respectively of the dyads and triads which are so widely distributed in striated muscles (28), particularly rapid phasic muscles (10).

There is growing reason to believe that in vertebrate skeletal muscles, as in the *Ascaris* muscle described here, the central component of the triad (the T system) is also nothing but a direct continuation of the plasma membrane (11, 18). Its status as such has been uncertain because, like certain other plasma membrane invaginations, it has a tendency to disintegrate into chains of apparently intracytoplasmic vesicles after osmium tetroxide fixation (10). After permanganate (36) or glutaraldehyde (11) fixation, however, such plasma membrane invaginations are manifestly continuous with the cell surface and the space they surround is clearly extracellular. In the *Ascaris* muscle, and in certain other muscles (22, 42), these invaginations do not fragment; hence there is no mistaking their identity as part of the plasma membrane. Presumably the sarcolemmal invaginations described here and the T system of vertebrate muscles both serve to conduct electrical potential changes rapidly from the cell surface to its interior (24).

Although the functional significance of the central components of the triads seems clear, that of the lateral components remains a mystery. The lateral components do not penetrate any deeper into the fiber than the plasma membrane invaginations and would therefore seem to be in no
position to extend the distribution of surface potential changes beyond the depth of the plasma membrane invaginations themselves. Some discussions of the lateral components have stressed their cisternal character and implied that they are important because of what they contain or what they convey. Fawcett and Revel (10) have suggested that they serve to convey metabolites from their site of production to their site of use. Their potential role in the uptake of calcium has been discussed by Porter (27). Essner and Quintana (8) have reported the presence of an ATPase within them. However, in the {Ascaris} muscle described here, the cisternal character of the lateral components is unimpressive. They are remarkably flat, and their interconnections are sparse.

Perhaps the most striking feature of the lateral components is the closeness of their association with the invaginated plasma membrane—a relationship which is emphasized by the presence of an intermediate line between the respective membranes. This configuration suggests another function, which has been discussed at length in connection with neuronal "subsurface cisterns," structures which are morphologically identical to the lateral components of the triads (35). It was suggested, in short, that intracellular membranes closely applied to the plasmalemma may interact with it in such a way as to alter its properties, thus producing a patchy cell surface (cf. reference 25).

Recently Falk and Fatt (9) have reported that in frog sartorius fibers, there are indeed two parallel pathways for current flow between the inside and outside of the cells. One of these has the properties usually associated with the plasma membrane, but the other has a resistance only one tenth as great. One wonders whether the low-resistance pathway corresponds to the patches at which the membranes of the lateral components of the triads are applied to the membrane of the T system. If indeed the lateral components have the effect of lowering the impedance of the plasma membrane, then their concentration along the invaginated plasma membrane in {Ascaris} muscle fibers, or along the limiting membrane of the T system in vertebrate striated muscle fibers, would lead to a kind of salutory conduction with ionic currents channeled selectively through this region of the cell surface.

Such an effect would also bear on the observations of Huxley and Taylor (16) that there are sensitive spots along the surface of muscle fibers at which subthreshold stimuli are able to elicit local contractions. It is currently assumed that the sensitive spots correspond merely to the openings of the T system to the surface of the fiber. But if the T system consists of nothing more than an extension of the plasma membrane why should it be more sensitive to subthreshold stimuli than the plasma membrane at the surface of the cell? Again the "sensitivity" of this membrane may derive from the association of the lateral components with it. Thus the significance of the lateral components of the triads may prove to lie not so much in what they contain as in their interaction with the plasma membrane and their effect on its properties.

In this paper little attention has been given to the sarcoplasmic core of the muscle fiber, which contains not only bundles of supporting fibrils but also innumerable mitochondria and an abundance of glycogen. Since this portion of the muscle fiber is related functionally to the muscle belly, it will be considered separately with that portion of the muscle (39).

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