Fine Structure of the Musculature of the Silkworm Larval Midgut

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The musculature of the larval midgut of the silkworm, Bombyx mori, is composed of outer set of longitudinal muscle fibers and inner set of circular muscle fibers. The circular muscle fibers are closely approximated and their plasma membranes are occasionally seen to be in close apposition. The muscle fibers are elongate uninucleate cells with a single myofibril in their sarcomplasm. The sarcomeres are divisible into A-, I-, H- and Z-bands. In the A-band region 12 thin myofilaments surround each thick myofilament. In fully contracted muscle fibers, the Z-band material is completely broken up into separate masses with spaces between them and there is an interdigitation of thick myofilaments from adjacent sarcomeres across these spaces. The sarcoplasmic reticulum frequently constitutes surface dyads with the plasma membrane but rarely associates with the transverse tubular system in the dyad configuration. The tracheal tissue appears to be the only cellular element that occurs in the interior of the musculature. Basal-granulated cells occur in the midgut epithelial layer.

INTRODUCTION

HUKUHARA et al. (1981) have observed active movements in the midgut of intact silkworm larva and in that of larva paralyzed with tetrodotoxin. Rings of constrictions, which are generated rhythmically at several regions of the midgut, move along the length of the midgut in both oral and aboral directions. The movements depend on the presence of contractile elements in its musculature. We have, therefore, attempted to correlate the structure and the type of motor activities exhibited by the midgut of the silkworm larva.

There are only a few reports on the fine structure of insect midgut musculature. SMITH et al. (1966), who studied the visceral muscles of several insects including the larval midgut muscles of Ephesia kühniella, concluded that the muscles contained contractile material which was constructed on essentially the same plan as that of vertebrate striated muscles. Similar observations were reported for the midgut muscles of Anopheles quadrimaculatus (SCHAEFFER et al., 1967), Periplaneta americana (SMITH, 1968) and Phormia regina (BEINBRECH, 1968).

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MATERIALS AND METHODS

Larval midguts were fixed by in situ perfusion of 2.5% buffered glutaraldehyde (pH 7.4) or by immersion of the dissected tissue in the fixative. The midgut muscles contracted considerably on contact with the fixative. Preparations of stretched midgut muscles were obtained as follows. A larva was first anesthetized with ether and a glass capillary tube was inserted through the anus into the hindgut. The larva was submerged in the buffer solution and the integument around the midgut was removed by dissection. The midgut content was increased by introducing the buffer solution through the capillary tube. At the same time, the larva was gently extended by pulling the anterior portion of the midgut. Thus, the midgut was expanded both in its diameter (30% increase) and in its length (50% increase). The buffer solution, in which the larva was immersed, was replaced with buffered glutaraldehyde solution.

After fixation in glutaraldehyde for 2–12 hours, the midgut was cut transversely into thin slices. Samples were postfixied for 1 hour in 1% buffered osmium tetroxide (pH 7.4), stained en bloc with 1% uranyl acetate for 3 hours, dehydrated in ascending grades of ethanol and acetone, and embedded in Epon 812. Transverse or longitudinal sections of muscle fibers were mounted without a supporting film on specimen grids. They were stained with uranyl acetate and lead salts (Sato, 1970), and viewed in a JEM-100C electron microscope.

RESULTS

General organization of the midgut wall

The midgut wall is composed of a single layer of epithelial cells lying on a basal lamina, which is covered with two sets of muscle fibers (Figs. 1 and 2). The superficial set consists of longitudinal muscle fibers, which are separated at well-marked intervals. There are about 110 longitudinal fibers around the circumference of the midgut of a fifth-instar larva. Individual fibers are enveloped by sarcolemma1, or basal lamina, 0.2–0.5 μm in thickness. They are connected with neighboring fibers by means of lateral communication of connective tissue elements. The communication appears to arise as two laminae passing from the sheath, which unite to form a membrane lying between the muscle coat and the hemocoele, 0.1 μm in thickness.

The deep set consists of closely approximated circular fibers. Some of the fibers twist above and below the others with varying degrees of overlapping, so that in a transverse section, most areas show more than two layers of circular fibers. The plasma membranes of two contiguous fibers are occasionally seen to be in close apposition (Figs. 5 and 7). Such cell interconnections run for considerable distance (up to 7 μm) along the overlapping tapering ends of the fibers. In many of the junctions, the plasma membrane of each fiber can be resolved and the separations are 10–20 nm. At some junctions, the outer membranes are fused to form a nexus. There are no desmosome nor other membrane specializations that have been demonstrated to hold cells together. Individual fibers are surrounded by a foliated connective tissue sheath consisting of several laminae, 30–40 nm in thickness (Fig. 4). The fibers not showing cell interconnections are connected with others by the fusion

1 The thin connective tissue sheath enveloping a muscle fiber.
Key to abbreviations—A, A band; B, basal lamina; C, circular muscle fiber; D, dyad; E, epithelium; H, H band; HD, hemidesmosome; HE, hemocoe; I, I band; L, longitudinal muscle fiber; M, mitochondrion; ME, membrane connecting longitudinal fibers; MT, microtubule; N, nucleus; NE, nexus; SD, surface dyad; SR, sarcoplasmic reticulum; T, tracheole; TU, T-tubule; Z, Z band.

Fig. 1. Transverse section of midgut. Magnification, 3,400×.
Fig. 2. Longitudinal section of midgut. Magnification, 2,400×.

of the outer sheath laminae (Fig. 2). The epithelium and the longitudinal fibers are loosely attached to the circular muscle layer by occasional connective tissue strands emerging from the muscle layer.

No enteric nervous plexus can be detected in the interior of the musculature, although there are rare nerve fibers on the upper surface of the muscle coat of the posterior midgut. The nerves are unmyelinated, enclosed within glial cells and run along the longitudinal muscle fibers. The connective tissue sheath enveloping the
nerve processes is continuous with that of the longitudinal muscle fibers.

The tracheal tissue appears to be the only cellular element that penetrates into the muscle coat. The tracheae pass down between muscle fibers, on which they rarely end, and divide into tracheoles on the basal lamina. The ultimate tracheoles are 0.3 μm in diameter.

Basal-granulated cells occur infrequently in the epithelial layer. They are readily distinguishable by their location and content of granules (up to 135 nm diameter)
which often occupy much of the cytoplasm (Fig. 3). The cells are lodged between the basal parts of columnar, goblet or regenerative cells and always have a broad base resting on the basal lamina. The granules are spherical or ovoid in shape, show various electron density, and have no apparent internal structure. Each granule is en-

Fig. 6. Circular muscle fiber showing striations. Magnification, 23,800×.
Fig. 7. Two circular muscle fibers which are in close apposition. Magnification, 22,700×. Insect shows a nexus-type junction. Magnification, 85,400×.
Fig. 8. Circular muscle fiber showing the arrangement of thick and thin myofilaments. Magnification, 110,000×.
Fig. 9. Longitudinal muscle fiber showing myofilaments and hemidesmosomes. Magnification, 22,600×.
closed by a membrane, which is either closely adhering or loose fitting. Some of the granules are almost in contact with the plasma membrane.

**Structure of muscle fibers**

The muscle fibers are elongate spindle-shaped cells, varying between 4 and 13 µm in width at the center. The muscle cells have a maximum length of about 60 µm based on measurements of longitudinal sections but they may be longer because of their possible oblique orientation. At the midpoint of each cell, there is a single oblong nucleus (2–3 × 6–11 µm) which occupies the greater part of the cell diameter. The nucleus is situated centrally in the longitudinal fiber and laterally in the circular fiber. The major fraction of the sarcoplasm is occupied by a contractile apparatus which corresponds anatomically to a single myofibril. The myofibril consists of myofilaments, which are contiguous with the plasma membrane at hemidesmosomes and lie juxtaposed to the membrane in other regions (Figs. 5 and 9).

![Diagram](image)

**Fig. 10.** Longitudinal muscle fiber showing thick filaments (arrow) penetrating the spaces between the Z-band masses. Magnification, 62,900×.

**Fig. 11.** Longitudinal muscle fiber showing T-tubule invaginations. Magnification, 42,400×.
The myofilaments present a regular transverse alignment and show the striation patterns of A-, I-, H- and Z-bands (Figs. 6 and 7). The total sarcomere length defined by adjacent Z-bands is about 15 μm in stretched muscle. The Z-band consists of several spindle-shaped masses that form, in longitudinal section, a broad zigzag line traversing the myofibril. In transverse section, the Z-band has a reticular structure consisting of closely-packed filaments, 5–6 nm in diameter (Fig. 5). The same filaments occur well-spaced in the I-band. In the A-band region, these filaments and thicker filaments (14–15 nm diameter) are arranged in a definite pattern: 12 thin filaments surround each thick filament (Fig. 8). The center-to-center spacing of the thick filaments is about 40 nm. The thick filaments are probably composed of myosin and the thin filaments of actin. The H-band is not readily apparent in longitudinal sections but is best observed in transverse profiles of myofibrils that show the presence of only thick filaments. No M-band occurs in the H-band region.

In contracted fibers, the I-bands are short and the thick filaments of the A-bands almost reach the level of the Z-bands. In fully contracted fibers, the Z-band material is completely broken up into separate masses with spaces between them and there is an interdigitation of thick filaments from adjacent sarcomeres across these spaces (Fig. 10).

Each muscle cell is limited by a typical plasma membrane, which invaginates into the cell to form a system of transverse tubules (T-tubules). In circular muscle the T-tubules are sparse and irregularly disposed (Fig. 4). In longitudinal muscle, they are more common and are arranged, in transverse sections, in a more precise radial pattern (Fig. 11). The sarcoplasmic reticulum, consisting of smooth membranes, is found predominantly around the myofibril and in the peripheral sarcoplasm (Fig. 7). Close juxtaposition of the sarcoplasmic reticulum and the T-tubule in the dyad configuration occurs very rarely (Fig. 11). More frequently, the sarcoplasmic reticulum constitutes surface dyads with the plasma membrane (Fig. 4).

Remaining organelles, such as mitochondria, ribosomes and rough-surfaced endoplasmic reticula, are located predominantly in the perinuclear position or scattered very sparingly within the contractile apparatus. The mitochondria are small (up to 1 μm in diameter) and limited in number. Microtubules are often located in the peripheral sarcoplasm running parallel with the myofilaments.

**DISCUSSION**

The muscle constituting the musculature of the silkworm midgut satisfies all the classical requirements of the definition as a striated muscle. The arrangement of myofibrillar elements which determine typical striations in the midgut muscle is similar to that in the other visceral and skeletal muscles of insects except for the myofilament array. Insect flight muscle exhibits a pattern of thick filaments each surrounded by six thin filaments, while both ordinary skeletal muscle and visceral muscle in insects have higher ratio of thin-to-thick filaments (Elder, 1975). Although the silkworm midgut muscle is striated, it behaves similar to the smooth muscle counterpart in the vertebrate alimentary canal; i.e., it contracts slowly and rhythmically and shows immense contractibility (Hukuhara et al., 1981).

The fast-acting muscles of insects and vertebrates are provided with a system of T-tubules, which act as the pathway for internal conduction of surface excitation.
The T-tubular system is poorly developed in the silkworm midgut muscle and is non-existent in the vertebrate smooth muscle. This ultrastructural feature is correlated with the slowness of the contractile response. The sarcoplasmic reticulum associated with the surface plasma membrane in these slow-acting muscles appears comparable to the dyad and triad found in fast-acting muscles and it may serve as the site of calcium storage (Somlyo, 1972).

Midgut muscle appears to be capable of a type of shortening designated by McAlear and Hoyle (1963) as supercontraction. They have demonstrated in barnacle striated muscle that the Z discs become perforated and expand during contraction and the thick filaments from neighboring sarcomeres pass through the spaces formed in the Z discs. Hoyle et al. (1965) have suggested that the supercontracting muscle bridges the gap between the ordinary striated muscle and the non-striated muscle by having highly mobile components in the Z disc, yet retaining them in some semblance of a plane. The smooth muscles of vertebrates are very extensible and capable of long, asymmetric contraction owing to the ‘disaggregation’ of their myosin and Z-material. Rice (1970), who has shown that the midgut muscle and other intrinsic visceral muscles of the adult tsetse fly are supercontractile, has considered that these muscles are well adapted to accommodate asymmetric contractions and to allow the visceral tubes to become greatly distended.

Kuwana (1935) has reported that no nervous elements occur in the middle one-third of the silkworm larval midgut but a few nerve fibers are present along the longitudinal muscles in the anterior and posterior one-thirds. Although the presence of nerve fibers is confirmed in the present study, their role in the midgut motor activity is not known. The midgut musculature does not appear to contain enteric nervous plexuses, which control the direction and strength of contraction waves in vertebrate alimentary canals (Hukuhara, 1973). Since the silkworm midgut exhibits normal contractile movements in the presence of tetrodotoxin, its motor activity is considered to be a myogenic, rather than a neurogenic, phenomenon (Hukuhara et al., 1981). There is no appreciable phase lag between the occurrence of contractions around the circumference of the midgut. If the propagation of contraction is electrical, this would imply that the electrical coupling is much better in this direction than along the length of the midgut. The nesal structure between the circular muscle fibers may represent a region of low electrical resistance.

There have been a large number of reports on hormonal peptides and biogenic amines, which are secreted from basal-granulated cells in mammals (Bloom, 1978; Essman, 1978). The motor activity of mammalian gastrointestinal is increased by the exogenous administration of some of these substances, although the physiological significance of the observations is not yet clarified (Cohen, 1975). Kobayashi (1971) has found a new type of secretory cells in the silkworm midgut epithelium. He has concluded on the basis of ultrastructural study that these cells are homologous to the mammalian basal-granulated cells. It should be a productive area of investigation to evaluate the role of these cells in the regulation of the midgut motor activity.

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