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Ultrastructure and morphology of midgut visceral muscle in early pupal *Aedes aegypti* mosquitoes

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Abstract

These studies focus on the pupal *Aedes aegypti* midgut muscularis for the first 26 h following larval–pupal transition. The midgut muscularis of *Ae. aegypti* pupae during this first half of the pupal stadium is a grid of both circularly and longitudinally oriented muscle bands, arranged in a manner resembling that of the larvae. While many muscle bands exhibit signs of degeneration during the time period studied, not all bands degrade, nor is this degradation simultaneous. Band deterioration involves destruction of internal elements while the muscle fiber plasma membrane remains intact. Deterioration of contractile elements may involve proteosome-like structures and associated enzymes. Many features of the larval muscularis including cruciform cells, bifurcating circular bands, and bifurcating longitudinal bands of muscle are retained during the time period investigated. Neuromuscular junctions along some muscle bands are retained through at least 16 h into the pupal stadium. The selective nature of muscle fiber degradation, coupled with the retention of larval features and neural input, may allow for limited functionality of the muscularis during metamorphosis. Evidence of sexual dimorphism in the midgut muscularis of male and female *Ae. aegypti* pupae was not observed during the time period studied.

Keywords

Aedes aegypti; Midgut; Visceral muscle; Pupae; Development; Ultrastructure

1. Introduction

The mosquito *Aedes aegypti* is a vector for several blood borne diseases including dengue, yellow fever, and equine encephalitis. The larval midgut of this mosquito participates in digestion, nutrient absorption, ion transport, and osmoregulation. The larval midgut (Fig. 1A) is a tubular structure composed of eight gastric caeca as well as distinct anterior, intermediate, and posterior regions (Clark et al., 2005). The larval caeca deteriorate during the pupal stadium and do not contribute to the formation of the adult midgut (Clements, 1992). The larval posterior midgut provides the anatomical basis for the formation of the adult stomach, responsible for blood digestion in the female, while the larval anterior midgut is transformed into a long tubular conduit responsible for conducting ingested food from the esophagus to the stomach (Fig. 1C and D). The pupal midgut (Fig. 1B), during the first half of the pupal stadium, resembles the larval midgut more than that of the adult (Fig. 1C, female; Fig. 1D, male); the structure does, however, become much longer and thinner than that seen in the 4th instar.

The muscularis associated with the mosquito midgut is responsible for generating peristaltic activity along the underlying midgut epithelium, facilitating the performance of this organ in its vital functions. In *Ae. aegypti*, the larval midgut muscularis arises by the second larval instar and develops extensively throughout the larval stadia (Bernick et al., 2007). Both the larval and adult midgut muscularis take the form of a grid of circularly and longitudinally oriented bands of muscle (Bernick et al., 2007; Park and Shahabuddin, 2000). In larvae, grid intersections include regions of band overlap and cruciform fibers with both circularly and longitudinally oriented filaments. Extensive bifurcation of individual muscle bands are present (Bernick et al., 2007). Sharing of filaments between otherwise discrete bands occurs in both larvae and adults (Bernick et al., 2007; Park and Shahabuddin, 2000).

Many studies have been conducted on blood-feeding dipterans, but the information relating to larval and pupal mosquitoes remains limited. The muscularis of mosquito larvae and pupae has received very little attention, despite its integral role in the facilitation of digestion and absorption. The metamorphosis of the midgut muscularis during the pupal stadium needs to be characterized. In the present studies the structure, morphology, and development of the midgut muscularis in *Ae. aegypti* pupae during the first half of the pupal stadium are presented.

2. Methods

2.1. Mosquitoes

Ae. aegypti eggs (Vero Beach strain) were obtained from a colony continuously maintained by Dr. Marc Klowden, University of Idaho (Moscow, ID). Larvae were hatched into a 1:1 mixture of local tap water and deionized water containing a small amount of yeast. The water mixture was replaced daily. The larvae were exposed to a regular photoperiod of 17L:7D and maintained at 26 °C. Feeding involved ground Tetramin flakes (Tetrawerke, Melle, Germany) and occurred daily. Specimens used for these studies were separated as they pupated. Pupae were kept in a 1:1 mixture of local tap water and deionized water at room temperature. Animals were dissected, fixed and prepared for microscopic examination (both TEM and SEM). As the pupal stadium is a non-feeding stage, no food was provided. Pupae were sexed according to Christophers (1960) immediately prior to dissection. Only definitively sexed pupae were used for these studies.

2.2. Transmission electron microscopy

Preparation for transmission electron microscopy occurred in a manner quite similar to that occurring in our previous studies on the larval midgut muscularis (Bernick et al., 2007). The major difference involved the infiltration of the liquid SPURRS resin for pupal tissues, which included an overnight incubation of two parts acetone:one part SPURRS not used during previous larval experiments.

Ultrathin sections (~50–75 nm; silver to gold color) were made using either glass or diamond knives on a Reichert Ultracut R microtome (Cambridge Instruments, W. Germany) and placed on formvar coated grids. Staining of larval preparations included exposure to 2% uranyl acetate followed by Reynolds' Lead. Specimens were then viewed on a JOEL JEM1200EX TEM (JOEL USA, Inc., Peabody, MA, acceleration beam approx. 70 uA). Images were captured via digital camera and processed with analySIS software version 3.2 (Soft Imaging System).

2.3. Scanning electron microscopy

For *scanning electron microscopy*, preparation of pupal tissues occurred in a manner identical to that used in previous experiments on larval *Ae. aegypti* midguts (Bernick et al., 2007). Following preparation, specimens were gold coated on a Technics Hummer V sputter coater (Technics, San Jose, CA). Viewing of samples occurred on a Hitachi S 570 SEM (Hitachi LTD,

Tokyo, Japan, acceleration beam 20–25 kV). Images were captured and stored digitally with Quartz PCI v. 4.2- Scientific Image Management System (Quartz Imaging Corporation).

3. Results

3.1. Gross morphology and development of the pupal midgut muscularis

During the first 26 h of the pupal stadium, the pupal *Ae. aegypti* midgut muscularis consists of a network of both circular and longitudinal muscle bands (Fig. 2– Fig 5). Fig. 2a–d illustrates changes in the gross appearance of the pupal midgut during the time period investigated. Bifurcating circular muscle bands are present in both the anterior and posterior midgut (anterior seen in Fig. 3a), a feature identified in the larval muscularis (Bernick et al., 2007). Bifurcating longitudinal muscle bands, yet another larval feature, were identified in the pupal posterior midgut, approaching the junction with the hindgut (Fig. 3b). Some muscle bands appear quite robust (Fig. 4a and b), and others show signs of atrophy and degeneration as early as 8 h into the pupal stadium (Fig. 4c–e). This atrophy becomes obvious earlier in the posterior midgut than in the anterior. The borders of the degenerating muscle bands become very difficult to discern as a result of their deterioration (Fig. 4d and e and Fig 5a and b). Incomplete circular muscle bands and cruciform cells are present in the anterior midgut (Fig. 4d). Some circular muscle bands become flattened and amorphous (Fig. 5c and d). Incomplete circular bands remain more robust than complete circular bands for a longer period (Fig. 4a). Selective complete band degeneration continues as the organism progresses through the first half of the pupal stadium (Fig. 5b–d).

Despite the differences in midgut morphology between adult male and female mosquitoes, no differences in midgut shape and structure of the muscularis were observed between male and female pupae during the first 26 h of the pupal stadium.

3.2. Fine structure of the pupal midgut muscularis

All muscle fibers examined, in both anterior and posterior regions of the midgut, retain an apparently intact contingent of contractile elements during the first 2–3 h of the pupal stadium (Fig. 6a–d). These early circular and longitudinal muscle fibers contain well-staining actin and myosin filaments. Cruciform fibers containing circular and longitudinally oriented filaments, a feature present in the larval muscularis (Bernick et al., 2007), are observed in the pupal anterior midgut (Fig. 6a–b).

By the time animals are 8 h into the pupal stadium, small rod-shaped, dark staining structures ranging in size from 20 to 40 nm begin to appear in some, but not all, muscle fibers (Fig. 7a–d). The small structures are in the same size range as proteosomes (Holzl et al., 2000; Sharon et al., 2006). The animals exhibiting this feature range from 8 to 25 h following larval–pupal transition. While many fibers exhibit these structures, other muscle fibers at the same stage do not (Fig. 8a and Fig 9a). In some fibers containing the proteosome sized structures, the contractile elements within appear robust (Fig. 7a–c). Others appear to exhibit some degree of degeneration. Some muscle fibers contain vacant areas in which contractile filaments are either less dense or completely absent (Fig. 7d and Fig 8b). The cytoplasm of many of these muscle fibers has acquired a grainy appearance (Fig. 7c and d). Neither the presence of the proteosome-sized structures nor the loss of contractile elements was observed in all muscle fibers. Both the proteosome-sized structures and grainy, filament vacant regions, were observed in both male and female specimens.

Some longitudinal muscle fibers, despite showing signs of degeneration, contain several mitochondria of normal appearance (Fig. 9b–d). Such mitochondria-rich muscle fibers occur predominantly, but not exclusively, in the anterior midgut. Fine strands can be observed in

some fibers located in the anterior midgut (Fig. 9d). Some fibers have short, tangentially oriented filaments similar in diameter to thick observed in other specimens in which the contractile machinery is intact (Fig. 9e).

Membrane invaginations, reminiscent of transverse tubules, are present in some fibers (Fig. 10a and b). No sarcoplasmic reticulum was observed in association with these invaginations, as is true with those associated with the larval muscularis (Bernick et al., 2007). Mitochondria were observed in many muscle bands of pupae of both genders, across several time points surveyed (Fig. 6a and b, Fig 7b and Fig 9a–e). These mitochondria were present in both filament rich muscle fibers and degenerate fibers containing only sparse filaments.

Neuromuscular junctions were observed in pupae as late as 15.5–16 h following larval–pupal transition (Fig. 10a–b), a time at which some muscle bands show strong signs of degeneration. The muscle associated with the neuromuscular junction seen in Fig. 10 appears quite robust, lacking both proteosome-sized structures and obvious signs of degeneration. Synaptic vesicles can be observed in the axon (Fig. 10b).

Z lines were observed in many circular muscle bands (Fig. 11a and b and Fig 12a and b). In some bands, the Z lines are quite robust and dark staining (Fig. 12a). The pupal Z lines, as those seen in the larvae, are often perforated. Some fibers, including those indicated in Fig. 11a and b, appear to have myosin filaments extending from one sarcomere into the next through these perforations. Other muscle bands, as seen in Fig. 12b, contain Z lines less distinct than those observed in robust fibers.

4. Discussion

The muscularis associated with the adult stomach has been described in insects including the mosquitoes *Ae. aegypti* and *An. gambiae* (Park and Shahabuddin, 2000) and fruit fly *Drosophila melanogaster* (Goldstein and Burdette, 1971), as well as the larval *M. sexta* (Copenhaver et al., 1996) and *Ae. aegypti* (Bernick et al., 2007). Park and Shahabuddin's study of the midgut muscularis in adult *Ae. aegypti* and *An. gambiae* is limited to the stomach, a region derived from the larval posterior midgut and responsible for processing of the blood meal. The muscularis associated with the tube-like anterior midgut has been ignored in adult *Ae. aegypti*.

The main goals of this project have been to study the processes of development and metamorphosis in the *Ae. aegypti* midgut muscularis during the first half of the pupal stadium. A major result of these studies is that the muscularis is not abolished and regenerated at once. Instead, individual muscle fibers undergo atrophy at different times without apparent cell death. To this extent, our data are consistent with O'Brien's (1965) finding that the *Ae. aegypti* midgut muscularis persists through metamorphosis. In his study, O'Brien briefly discussed the muscularis associated with the pupal midgut, stating only that the larval muscularis is retained through metamorphosis. However, we observed that during this period, significant changes occur in both gross and fine structure of the midgut muscularis. Some muscle fibers appear to undergo degradation, beginning as early as 6–8 h into the pupal stadium. Externally, degenerating muscle bands acquire an amorphous appearance. Internal changes include the loss of contractile elements as the cytoplasm becomes increasingly grainy. These changes occur selectively in the pupal *Ae. aegypti* midgut muscularis, with some fibers showing signs of atrophy as early as 8 h into the pupal stadium while others appear robust at 25 h. The changes can be characterized as atrophic because they appear mainly to reflect a loss of muscle mass without elimination of muscle fibers during the time frame of our studies. Selective loss of muscle fibers is known to occur in the midgut muscularis of *Drosophila melanogaster* (Klapper, 2000).

Two forms of cellular apoptosis have been described. Type I apoptosis is characterized by early nuclear collapse accompanied by DNA fragmentation. Type II apoptosis is a more gradual process in which the primary event is loss of cytoplasm accompanied by pycnosis of chromatin. Type II apoptosis is the form characteristic of elimination of inter-segmental muscle in pupal *M. sexta*. In these fibers, atrophy is initiated by a decrease in ecdysteroid titers late in the pupal stadium. A further decline in ecdysteroids is required to commit the fibers to die. Loss of actin and myosin is characteristic of early apoptotic changes in this model, and both 20S proteasomes and lysosomes are participants in the destruction of the cytoplasmic elements (Schwartz, 1992; Beaulaton and Lockshin, 1976; Haas et al., 1995).

It is presently unclear whether all muscle fibers associated with the *Ae. aegypti* midgut undergo atrophy, or only a sub-set of both circular and longitudinal muscle bands experience this phenomenon. One tenable hypothesis is that muscle bands undergo atrophy and subsequent rebuilding in an asynchronous manner, with the result that all larval muscle fibers are overhauled during metamorphosis. Alternatively, some muscle bands may undergo atrophy while others do not. Those which do not could either be retained, in larval form, into adulthood, undergo gradual transition to the adult form, or become lost later during the pupal stadium.

4.1. Causal factors of muscle degeneration in pupal *Ae. aegypti*

Some bands of muscle, in both the anterior and posterior regions of the midgut, exhibit several features indicative of degeneration early into the pupal stadium. At the SEM level, this degeneration can be seen through the flattening of some muscle bands and the amorphous quality of the bands in some specimens. Internally, some muscle fibers exhibit large, vacant areas of cytoplasm, unoccupied by contractile elements. Additionally, the cytoplasm acquires a grainy appearance. In those fibers in which degradation of contractile elements occurs, the cell membrane remains intact.

The cytoplasm of muscle fibers undergoing atrophy develops small, dark staining organelles similar in size to proteasomes. Proteasomes and proteasome-like organelles play a pivotal role in the cellular degradation of proteins (Holzl et al., 2000; Sharon et al., 2006). Their dumbbell shape includes a hollow core allowing for the ATP-ase driven degradation of ubiquitin-labeled proteins.

The most commonly recognized proteasome species, the 20S proteasome, has been implicated in apoptosis of larval muscles in *M. sexta* pupae (Löw et al., 2000). The 20S proteasome is highly conserved evolutionarily, and the arthropod form is grossly similar to forms in other metazoans (Mykles, 1997). While comparable in size to proteasomes, the small structures we observed in some pupal *Ae. aegypti* muscle bands do not exhibit the dumbbell shape characteristic of 20S proteasomes. Most strikingly, they appear to lack the larger 19S caps and, alternatively, retain a constant diameter across their entire length.

It is plausible that these structures are not true proteasomes, but related structures serving in a similar capacity. Alternatively, *Ae. aegypti* midgut visceral muscle may develop unique proteasome isoforms with caps of differing mass than the traditional 19S subunits. At least one other giant protease complex, TPP II, is reported from insects. This complex appears as a rod-like particle when fixed under non-reducing conditions (Rockel et al., 2002) and is within the size range of the particles observed in *Ae. aegypti*. However, without more definitive studies, our current findings are only suggestive; we cannot identify the structures as belonging to the reported proteasome-like families.

There is a significant peak in 20-HE levels approximately 6 h into the pupal stadium (Margam et al., 2006). This pulse triggers apolysis of the pupal cuticle and transforms the pupa into a pharate adult, with profound effects on the overall bauplan. However, the response of 20-HE

activated genes to this peak was found to occur in a highly staggered manner; not all genes were expressed at the same time, or to the same degree, following exposure.

Upon entry into the target cell, 20-HE forms a complex with two receptor types, EcR and USP. Hegstrom et al. (1998), in their study of the first dorsal external oblique muscle in *M. sexta*, found that cellular expression of the EcR-A receptor subtype was correlated with the degeneration of larval muscle fibers. Cellular expression of the EcR-B1 receptor subtype was localized in the only larval muscle fiber contributing to the adult muscle counterpart in this species and was presumed to contribute towards muscle regeneration.

The *Ae. aegypti* midgut muscle appears to differ from the *M. sexta* intersegmental muscle model in that in the later, the onset of atrophy reflects a process that is tied to a developmental program that eliminates specific muscle fibers and ultimately results in cell death. The factors that control the timing of atrophy in individual midgut muscle fibers in *Ae. aegypti*, as well as the ultimate fate of these fibers in the adult, remain uninvestigated.

4.2. Significance of selective muscle band degeneration in pupal *Ae. aegypti*

Key functional questions are raised by the observations relating to the nature of degradation of muscle bands in the pupal *Ae. aegypti* midgut muscularis. What is the purpose of such selective degeneration of muscle bands? What is the purpose of retaining the cell membrane of the muscle fiber during its internal destruction?

The degradation of content within the muscle fibers, while keeping the outer cell membrane intact, may play a significant role in the renovation of the midgut muscularis. Such a developmental strategy would conserve the basic structure of the muscularis and maintain some degree of functionality during a period in which the larval midgut epithelium is completely abolished and subsequently regenerated from stem cells. In a previous study (Moffett and Moffett, 2005), we found that the pupal midgut maintains a remarkable level of apparently functional serotonergic and peptidergic innervation, as well as a large population of enteroendocrine cells.

There are both morphogenic and functional rationales for conserving the structure and function of the midgut and associated muscularis. The muscularis may maintain the structural relationship of the gut innervation and serve as a scaffold for subsequent morphogenic changes. Both Truman and Reiss (1995) and Hegstrom and Truman (1996) discuss the phenomenon of neuromuscular metamorphosis in *M. sexta*. The authors indicate that the loss of axonal branches at neuromuscular junctions is dependent upon cues emitted by the muscle fiber. Regrowth of the muscle triggers development of axonal sprouts at motor end plates. It is possible that metamorphosis of midgut visceral muscle in *Ae. aegypti* plays a similar role in sustaining the innervations of the gut in the pupa and stimulating the development of the adult stomatogastric nervous system.

It is also arguable that a functional pupal gut could participate in nutrient cycling. During pupal life, meconium, which includes the products of the shed larval gut epithelium as well as a fraction of the secretion from the Malpighian tubules, accumulates in the midgut lumen. Digestion and recovery of valuable components of the meconium would be of advantage to the developing mosquito. Maintenance of some degree of midgut motility might be important for this process.

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Fig. 1.

Midguts from *Ae. aegypti* 4th instar larvae (A), pupae (B), and adults (C and D). The anterior midgut, labeled with a grey interior, has eight associated gastric cecae in the larvae. The cecae are lost early into the pupal stadium and are not regenerated. The posterior midgut, filled with cross-hatchings, develops into the adult stomach. The Malpighian tubules are located at the junction of the posterior midgut and the hindgut. Image C represents an adult female, and D represents an adult male.

Fig. 2.

(A) Midgut from a female *Ae. aegypti* pupa, approximately 6.75–7.25 h following larval–pupal transition. Anterior midgut is located in the upper right, and the posterior midgut in the lower left. Scale bar represents 600 μ m. (B) Midgut from a female *Ae. aegypti* pupa, approximately 9 h following larval–pupal transition. Anterior midgut is located in the upper right, and the posterior midgut in the lower left. Scale bar represents 600 μ m. (C) Midgut from a female *Ae. aegypti* pupa, approximately 16.5–17 h following larval–pupal transition. Anterior midgut is located in the upper right, and the posterior midgut in the lower left. Scale bar represents 600 μ m. (D) Midgut from a female *Ae. aegypti* pupa, approximately 21.5 h following larval–pupal

transition. Anterior midgut is located in the upper right, and the posterior midgut in the lower left. Scale bar represents 600 μm .

Fig. 3.
 (A) Bifurcating circular muscle bands (BC) in the anterior midgut of a female *Ae. aegypti* pupa, approximately 6.75–7.25 h following larval–pupal transition. Longitudinal bands of muscle (L) can also be observed. Scale bar represents 27 μm. (B) Bifurcating longitudinal muscle bands (BL) found in the posterior midgut of a female *Ae. aegypti* pupa, approximately 5.5–6 h following larval–pupal transition. Circular muscle bands (C) and Malpighian tubules (MT) are present. Scale bar represents 50 μm.

Fig. 4.

(A) Longitudinal (L) and circular (C) muscle bands in the posterior midgut of a female *Ae. aegypti* pupa, approximately 5.5–6 h following larval–pupal transition. Scale bar represents 50 μ m. (B) Muscularis associated with the posterior midgut of a female *Ae. aegypti* pupa, approximately 9 h following larval–pupal transition. Defined longitudinal (L) and circular (C) bands of muscle can be seen. Malpighian tubules (MT) are located at the junction between the posterior midgut and hindgut. Scale bar represents 50 μ m. (C) Muscularis associated with the posterior midgut of a male *Ae. aegypti* pupa, approximately 16.5–17 h following larval–pupal transition. Longitudinal (L) and circular (C) bands of muscle are present, but appear flattened and less robust than those observed in younger pupae. Scale bar represents 50 μ m. (D)

Cruciform cells (CC) and incomplete circular muscle bands (IC) found in the anterior midgut of a male *Ae. aegypti* pupa, approximately 19 h following larval–pupal transition. Longitudinal (L) and circular (C) bands of muscle are also present. Scale bar represents 75 μm . (E)

Muscularis associated with the posterior midgut of a female *Ae. aegypti* pupa, approximately 17 h following larval–pupal transition. Circular muscle bands (C) are severely atrophied, as evidenced by decreased diameter of some muscle bands, and longitudinal bands of muscle are difficult to discern. Filamentous branches (FB) are associated with the midgut. Scale bar represents 50 μm .

Fig. 5.

(A) Muscularis associated with the anterior midgut of a female *Ae. aegypti* pupa, approximately 6.75–7.25 h following larval–pupal transition. Longitudinal (L) and circular (C) bands of muscle are present. Scale bar represents 60 μm . (B) Muscularis associated with the anterior midgut of a male *Ae. aegypti* pupa, approximately 19 h following larval–pupal transition. Incomplete bands of circular muscle (IC) are present, but circular and longitudinal muscle bands are difficult to discern. Scale bar represents 43 μm . (C) Muscularis associated with the anterior midgut of a female *Ae. aegypti* pupa, approximately 21.5 h following larval–pupal transition. Longitudinal (L) and circular (C) muscle bands are present. Scale bar represents 50 μm . (D) Muscularis associated with the anterior midgut of a female *Ae. aegypti* pupa,

approximately 21.5 h following larval–pupal transition. Higher magnification of boxed area in (C). Scale bar represents 25 μm .

Fig. 6.

(A) Cruciform muscle fiber containing both circularly (CF) and longitudinally (LF) oriented contractile elements from the anterior midgut of a male *Ae. aegypti* pupa, approximately 2.5–3 h following larval–pupal transition. A membrane invagination (MI) resembling a transverse tubule is present. Mitochondria (M) are present in the cytoplasm unoccupied by contractile elements. Scale bar represents 1 μ m. (B) Cruciform muscle fiber containing both circularly (CF) and longitudinally (LF) oriented contractile filaments from the anterior midgut of a male *Ae. aegypti* pupa, approximately 2.5–3 h following larval–pupal transition. Mitochondria (M) are present in the cytoplasm unoccupied by contractile elements. Scale bar represents 0.5 μ m. (C) Circular (C) and longitudinal (L) muscle associated with the posterior midgut of a male *Ae. aegypti* pupa, approximately 2.25–2.75 h following larval–pupal transition. A nucleus (N) and mitochondria (M) are present in the cytoplasm of the circular muscle. The circular muscle lies close to the basement membrane (BM). Scale bar represents 1 μ m. (D) Longitudinal muscle, rich with actin and myosin filaments (AMF) from the posterior midgut of a male *Ae. aegypti* pupa approximately 2.25–2.75 h following larval–pupal transition. Scale bar represents 1 μ m.

Fig. 7.

(A) Circular muscle (C) from the posterior midgut of a male *Ae. aegypti* pupa, approximately 8 h following larval–pupal transition. Small proteosome sized flecks are present in the muscle fiber. The basement membrane (BM) lies between the muscle and midgut epithelium (ME). Scale bar represents 1 μm . (B) Circular (C) and longitudinal (L) muscle from the posterior midgut of a male *Ae. aegypti* pupa, approximately 15.5–16 h following larval–pupal transition. Mitochondria (M) are present in the circular muscle. Both longitudinal and circular muscle bands contain small, dark staining structures. Scale bar represents 0.5 μm . (C) Longitudinal muscle (L) lying close to the midgut basement membrane (BM) from the anterior midgut of a female *Ae. aegypti* pupa, approximately 16.5–17 h following larval–pupal transition. A membrane invagination (MI) resembling a transverse tubule is present. Scale bar represents 0.5 μm . (D) Longitudinal muscle (L) lying close to the basement membrane (BM) from the anterior midgut of a female *Ae. aegypti* pupa, approximately 14–14.5 h following larval–pupal transition. Regions of the cytoplasm are vacant (V), unoccupied by contractile elements. Scale bar represents 0.5 μm .

Fig. 8. (A) Circular (C) and longitudinal (L) muscle fibers from the posterior midgut of a female *Ae. aegypti* pupa, approximately 23.5–24 h following larval–pupal transition. Scale bar represents 1 μm. (B) Circular muscle (C) from the anterior midgut of a female *Ae. aegypti* pupa, approximately 23–23.5 h following larval–pupal transition. Cytoplasm unoccupied by contractile elements appears grainy. The midgut epithelium (ME) and basement membrane (BM) can be observed. Scale bar represents 1 μm.

Fig. 9.

(A) Longitudinal muscle from the posterior midgut of a male *Ae. aegypti* pupa, approximately 2.25–2.75 h following larval–pupal transition. The fiber contains intracellular spaces (IS) and abundant actin and myosin filaments (AMF). Scale bar represents 0.5 μm . (B) Longitudinal muscle (L) from (A) at a higher magnification. Mitochondria (M) are present. Midgut epithelium (ME) and associated basement membrane (BM) can be observed. Scale bar represents 0.5 μm . (C) Longitudinal muscle from the anterior midgut of a female *Ae. aegypti* pupa, approximately 23–23.5 h following larval–pupal transition. Numerous mitochondria (M) are found in the fiber, largely vacuolated of contractile elements. Scale bar represents 1 μm . (D) Longitudinal muscle from the anterior midgut of a female *Ae. aegypti* pupa approximately 23–

23.5 h following larval–pupal transition. Fine, dark staining strands (FS) are present in the cytoplasm. Scale bar represents 1 μm . (E) Longitudinal muscle from the anterior midgut of a female *Ae. aegypti* pupa, approximately 21–21.5 h following larval–pupal transition. Mitochondria (M) and tangentially oriented filaments (TF) are present. Scale bar represents 0.5 μm .

Fig. 10.

(A) Longitudinal muscle, rich with actin and myosin filaments (AMF) from the posterior midgut of a male *Ae. aegypti* pupa, approximately 15.5–16 h following larval–pupal transition. Membrane invaginations (MI) resembling transverse tubules are present. A neuron (N) is closely associated with this muscle fiber. Scale bar represents 0.5 μm. (B) Neuromuscular junction (NMJ) seen in (A) at a higher magnification. Neurosecretory vesicles (NSV) are present in the neuron. Actin and myosin filaments (AMF) can be observed in the longitudinal muscle fiber. Scale bar represents 0.2 μm.

Fig. 11.

(A) Circular muscle (C) from the posterior midgut of a male *Ae. aegypti* pupa, approximately 2.25–2.75 h following larval–pupal transition. The muscle fiber lies closely to the midgut epithelium (ME) and basement membrane (BM). Z lines (Z) are easily observable. Mitochondria (M) are present. Scale bar represents 1 μm . (B) Circular muscle (C) from the posterior midgut of a female *Ae. aegypti* pupa, approximately 17–18 h following larval–pupal transition. The muscle fiber lies closely to the midgut epithelium (ME) and associated basement membrane (BM). A faint Z line (Z) is present in the upper left portion of the muscle fiber. Scale bar represents 2 μm .

Fig. 12.

(A) Circular muscle (C) from the posterior midgut of a male *Ae. aegypti* pupa, approximately 15.5–16 h following larval–pupal transition. The muscle lies close to the midgut epithelium (ME). Perforated Z lines (Z) are present. Scale bar represents 1 μ m. (B) A perforated Z line from (A) at a higher magnification. Some of the myosin filaments associated with the circular muscle (C) to the Z line, as opposed to penetrating the perforated regions. Scale bar represents 0.5 μ m.