Invited Review

Ultrastructure of invertebrate muscle cell types

R. Paniagua, M. Royuela, R.M. García-Anchuelo and B. Fraile

Department of Cell Biology and Genetics, University of Alcalá de Henares, Madrid, Spain

Summary. The muscular cells of invertebrates can be divided into three major classes on the basis of their striation pattern: transversely striated, obliquely striated, or smooth muscle. Transversely striated muscles have either continuous or discontinuous Z lines and, thus, can be subdivided into two types respectively. Of all invertebrate muscles, the transversely striated muscle with continuos Z lines is the most similar to the vertebrate skeletal muscle and is present in arthropods, whose musculature (including the visceral muscles) only consists of this cell type. These muscles are multinucleate cells that contain myofibrils showing welldefined sarcomeres. Transversely striated muscles with discontinuous Z lines, consisting of multiple small electrondense patches, are found in the translucent portions of adductor muscles of some bivalves and in the heart muscle of the gastropods. This muscle is formed by mononucleated cells with centrally-located nuclei and a single myofibril. The obliquely striated muscle appears in nematodes, annelids, molluscs, brachiopods and chaetognathes and consists of mononucleated cells with both thick and thin myofilaments which form sarcomeres delimited by Z lines. Myofilaments are not perpendicular but oblique to the Z lines, so that both A and I bands may be seen together in each of the three spatial planes of view. Smooth muscle has been reported in coelenterates, annelids, molluscs, brachiopods and echinoderms, but is lacking in arthropods. These muscle cells have a centrally-located nucleus and abundant thin and thick myofilaments without apparent sarcomeres. The most relevant characteristics of invertebrate muscle cells are the following. The thick (myosin) myofilaments show a variable length (from 2.2 µm up to 6 µm) and width (from 14 nm up to 231 nm) and contain a central core of paramyosin, which is absent in vertebrate muscles. Thick filaments are homogenous in transversely striated muscles and either homogeneous or fusiform in the obliquely striated and smooth muscles. Thin filaments measure 6 nm in diameter. They contain tropomyosin and, only in striated muscles, also troponin.

Offprint requests to: Dr. R. Paniagua, Department of Cell Biology and Genetics, University of Alcalá de Henares, E-28871 Alcalá de Henares, Madrid, Spain

The thin/thick filament ratio varies from 3/1 to 6/1, even in smooth muscles. The plaques for filament anchorage (Z lines in striated muscles or electrondense bodies in smooth muscles) contain α -actinin. The striated (transversely or obliquely) muscles show long sarcomeres (up to 9 µm) and the number of thin filaments around each thick filament varies from 3 to 12, so that each thin filament is shared by two thick filaments. Z lines in the striated muscles show a variety of structures that differ from one species to another (filament bundles in nematodes, bars in annelids, small patches in molluscs, etc). Many striated muscles contain titin (connectin) and intermediate filaments and display a sarcotubular system consisting of T tubules and sarcoplasmic reticulum tubules. Both structures form dyads and, more rarely, triads. The location of T tubules as well as the configuration and distribution of sarcoplasmic reticulum vary among muscles and species. Invertebrate smooth muscle differs from that of vertebrates principally in the higher proportion and larger diameter of thick myofilaments. These may be fusiform and their size and number may vary widely among cells. These muscle cells may be classified by the characteristics of both the thick filaments and the electrondense bodies for filament anchorage.

Key words: Invertebrate muscle cells, Striated muscle, Transversely striated muscle, Obliquely striated muscle, Smooth muscle

1. Introduction

Vertebrate muscular tissue comprises two major muscular cell classes: smooth and striated. The latter is subdivided into two types: skeletal and cardiac. This classification is usually extended to the invertebrate muscle cells, although since the early electron microscopic studies important differences between vertebrates and invertebrates in both the striated and smooth muscle are evident. Invertebrate striated muscle cannot be subdivided into skeletal and cardiac, but should be classified according to the type of striation: transverse (like that of the vertebrate striated muscle), and oblique. However, within each of these two types, new subdivisions may be made responding to the distribution, length and width of myofilaments and the Z line components. Some of these muscle cell types are associated with a well-defined zoological group. There are also many types of invertebrate smooth muscles ranging from those that are ultrastructurally like the vertebrate smooth muscle to those that are more similar to invertebrate obliquely striated muscle. In addition, there are muscle cells of doubtful classification between transversely striated and obliquely striated muscle, or between the latter and smooth muscle.

The aim of this paper is to describe the most characteristic muscle cell types in invertebrates and to classify them according to their relevant ultrastructural features on the basis of previous literature and our own electron microscopic studies.

Transversely striated muscle

Arthropods (transversely striated muscle with continuous Z lines)

Of all invertebrate muscles, this is the most similar to the skeletal muscle of vertebrates and can be found in most arthropods whose musculature is exclusively formed by this cell type. The transversely striated muscle has been mainly studied in insect flight muscles from odonate, dipteran, hymenopteran, coleopteran and hemipteran insects (Smith, 1961, 1966; Auber and Couteaux, 1963; Shafiq, 1963, 1964; Ashhurst, 1967), jump and intersegmental muscles from dipterous larvae (Schouest et al., 1986; O'Donnell and Berstein, 1988; Kronert et al., 1991), and several skeletal muscles from crustaceans (Bouligand, 1962, 1964; Fahrenbach, 1963; Endo, 1964; Brehme, 1967; Rosenbluth, 1969; Jahromi and Charlton, 1979; Bonilla et al., 1992; Mieguel et al., 1992).

Although all the arthropod muscles present a common ultrastructural pattern, which may be described as transversely striated muscle with continuous Z lines, there are dissimilarities among zoological groups, and even within the same species, in relation to the anatomical type of muscle. Two anatomical types of muscles, specialized for different functions, may be distinguished in arthropods: skeletal (somatic) muscles, and visceral muscles (digestive tract and hemolymphatic vessels). Another type of muscle should be added in insects: the flight muscles, which may either be of the direct type (odonates, lepidopteran and orthopteran) or the indirect type (dipteran, hymenopteran, coleopteran and hemipteran).

The analogies between the ultrastructure and contracting mechanism of these muscles and the striated muscle of vertebrates are considerable. All of these muscles are multinucleated cells that contain several cylindrical myofibrils, separated from each other by rows of mitochondria. The myofilaments form welldefined sarcomeres, delimited by continuous Z lines, and present well-defined A and I bands showing transverse striation (Fig. 1a). The nuclei are usually longitudinally aligned in the centre of the myofibril, although peripherally placed nuclei have also been reported in indirect flight muscles (Ashhurst, 1967). Arthropod muscles differ essentially from vertebrate striated muscle in the size and arrangement of their thick and thin myofilaments. The arthropod muscle sarcomeres measure from 4 µm to 9 µm in length (Bonilla et al., 1992), while the length of the vertebrate sarcomere is 2.4 µm (Hanson and Huxley, 1953; Huxley and Niedergerke, 1954; Huxley, 1957a,b, 1960). The thin/thick filament ratio, which is 2/1 in vertebrate striated muscle, reaches higher values in arthropods: from 3/1 in most insect flight muscles (Fig. 1b) to 6/1 in the ventroabdominal flexor muscle of the crustacean Atya lanipes. Since the number of thin filaments around each thick filament varies from 6 to 12, each thin filament is shared by two thick filaments (Fig. 1c) (Shafiq, 1963, 1964; Ashhurst, 1967; Hagopian, 1966; Smith, 1966; Spiro and Hagopian, 1967; Hagopian and Spiro, 1968; O'Donnell and Bernstein, 1988). The intracellular structures known as leptomeres, which are joining Z lines of adjacent myofibrils in the skeletal (Ruska and Edwards, 1957) and cardiac (Fujita et al., 1979; Hosokawa et al., 1994) muscle of mammals, have not been observed in invertebrate striated muscle.

The arthropod thick myofilament also consists of myosin, as do vertebrate striated muscles, but the thick filaments are longer (2.2-6 µm) and thicker (up to 50 nm) in the arthropod muscle than in the vertebrate striated muscle (1.5-1.6 µm in length and 14 nm in diameter). The greater thickness of the arthropod thick myofilament is probably related to the higher thin/thick filament ratio. Since thick filaments are built up by helically-arranged myosin molecules whose heads form cross-bridges with the actin filaments, the larger the proportion of thin filaments, the higher the number of myosin molecules (heads) required in the thick filament (Wray, 1979; Crowther et al., 1985; Stewart et al., 1985). Ultrastructural studies in insect muscle cells reveal that many cross-sectioned thick filaments display a ring configuration rather than a compact structure (Fig. 1c). Since this microtubule-like pattern does not appear in all transverse sections within the same muscle cells, the two following explanations are possible: (1) part of the thick filament is cylindrical and part is compact; and (2) there are two filament types: cylindrical and compact. Ultrastructural studies of flight muscles in three insect species (Phornia terraenovae, Lethocerus uhleri and Apis mellifera) by Beinbrech et al. (1992), using a computerized tridimensional reconstruction of myofibrils, showed that compact thick filaments were present in the three species, whereas cylindrical thick filaments were only found in Phornia terraenovae and only in the periphery of the myofibrils. These results suggest that compact and cylindrical filaments are two different thick filament types and that the latter type is restricted to an undetermined number of species.

In addition to myosin, the arthropod thick myofilament contains another protein, paramyosin, which weighs 220 kDa and is absent in vertebrate muscles. The exact location of this protein is controversial, but it is assumed to form a core in the thick filament. Immunohistochemical methods have located paramyosin along the whole length of the thick filament in insect flight muscles (Fig. 1d). However, biochemical studies suggest that paramyosin is only found in the centre, rather than in the tips of the filaments, in *Drosophila melanogaster* (Levine, 1972), *Lethocerus* (Levine et al., 1974; Bullard et al., 1977) and *Apis mellifera* (Aust et al., 1980). The amount of this protein varies with the type of muscle and thick filament pattern. In *Drosophila*, paramyosin is more abundant in skeletal and visceral muscles than in flight muscles (Alamo et al., 1992; Vinós et al., 1992). In the muscles with two types of thick filaments, paramyosin is more abundant in the compact filaments (9%) than in the cylindrical filaments (2%). The function of paramyosin



Fig. 1. Transversely striated muscle from insects (*Drosophila melanogaster*). **a.** Longitudinally sectioned intestinal muscle showing an ultrastructural pattern similar to that of vertebrate striated muscle. Z: Z disc. **b.** Cross-sectioned flight muscle. **c.** Higher magnification of an area in Figure b. Each thick filament shows a ring configuration and is surrounded by six thin filaments which are aligned with the former, so that each thin filament is shared by two thick filaments. At the periphery of the myofibrils a tubule of the sarcoplasmic reticulum is seen (t). **d.** Longitudinally-sectioned flight muscle immunolabelled with paramyosin. Immunogold particles are observed along the thick filament. I: I band. **e.** Troponin immunolabelling. Immunoreaction is along the thin filaments. Z: Z disc; M: M line. a and b, x 8,000; c, x 400,000; d, x 43,000; e, x 22,000

is still unclear. However, two possibilities have been suggested: (1) they mechanically provide structural stability during tension development (Winkelman, 1976); and (2) they may influence the ATPase activity of the contractile proteins (Szent-Györgyi et al., 1971; Beinbrech et al., 1992).

A paramyosin-like 55 kDa protein, called miniparamyosin, has been reported in *Drosophila* and is probably another component of thick filaments (Becker et al., 1992; Maroto et al., 1995).

Thin filaments in arthropod muscles are essentially like those of vertebrate striated muscle and are also formed by actin molecules. In both arthropod and vertebrate striated muscle, thin filaments measure 6 nm in diameter, although the length of actin filaments is longer in arthropod muscle; this agrees with the higher length of the sarcomeres in these muscles (Mogami et al., 1982).

Western blot studies have revealed that arthropods possess several actin isoforms, according to their isoelectric point which is more basic than the vertebrate actin isoforms (Pascolini et al., 1992). Beifuss and Durica (1992) isolated the actin gene in *Drosophila melanogaster* and found that there was a small family of six genes codifying for actin. Two of these genes, called Act 5 C and Act 42 A, account for cytoskeletal actin. The other four genes account for muscular actin: Act 57 B and Act 87 E account for actin in integumental muscle in both larva and adult flies; Act 79 B accounts for thoracic muscles; and Act 88 F accounts for actin in indirect flight muscles.

As in vertebrate striated muscle, tropomyosin and troponin are found associated to actin in invertebrate striated muscle (Fig. 1e). Electrophoretic studies of these muscular proteins in jump muscles of *Drosophila* (trochanter, depressor and extracoxal) revealed that the relative proportions of actin/tropomyosin/troponin are 7/1/1 (Mogami et al., 1982). Similar proportions have been reported in the tail muscle of the American lobster *Homarus americanus* (Mieguel et al., 1992). Tropomyosin in the flight muscle of *Drosophila* consists of two chains (α and β) (Tansey et al., 1991), whereas in the cardiac muscle of this species only the tropomyosin α chain is present (Mieguel et al., 1992).

The Z line contains the protein α -actinin, which has a molecular weight of 78 kDa in *Drosophila* (Mogami et al., 1982; Vigoreaux et al., 1991). Experimental studies in this species suggest that this protein connects actin filaments to strengthen and stabilize the structure of the sarcomere, but that it does not influence the organization and distribution of myofilaments during morphogenesis and development.

Arthropod muscle also contains the filamentous

protein called titin or connectin, which is analogous to that of vertebrates. This protein has a weight of 3000 kDa and binds the thick filaments to the Z line (Maruyama, 1986). Wang et al. (1992) found that light meromyosin and the S1 myosin subfragment are the major domains of myosin interaction with titin.

Intermediate filaments of the desmin type have been found with electrophoresis in the flight muscle of *Drosophila*. This filament contains a 49 kDa protein which is localized in the Z line (Mogami et al., 1982).

The sarcotubular system of arthropod muscle consists of smooth endoplasmic reticulum (sarcoplasmic reticulum) and T tubules. The sarcoplasmic reticulum of the insect direct flight muscles forms a network of anastomosed cisternae and tubules which surrounds each myofibril. T tubules are plasma membrane invaginations which display a flattened profile that resembles cisternae rather than true tubules, and course perpendicular to the myofibrils. There are two T tubules per sarcomere, one tubule located on either side of the A band (at the level of the transitions from A band to I band). These tubules contact the sarcoplasmic reticulum tubules or cisternae that are at the level of the A-I band transitions and that separate the T tubules from the myofilaments. The pair of closely apposed double profiles (one T tubule and one sarcoplasmic reticulum cisterna) are known as dyads in comparison to the triad (one T tubule between two sarcoplasmic reticulum cisternae) found in the vertebrate skeletal muscle. The ultrastructural resemblance between this dyad and the triad includes the presence of 10 nmwide gap-like junctions between the T tubule and the adjacent sarcoplasmic reticulum tubule forming the dyad (Endo, 1964; Franzini-Armstrong and Porter, 1964; Page, 1964; Brehme, 1967). In the indirect flight muscles and in other somatic or visceral arthropod muscles the sarcotubular system is less developed and only a single T tubule per sarcomere may be observed in some muscles. In this case this T tubule is in the Z line or in the centre of the sarcomere.

Molluscs (transversely striated muscle with discontinuous Z lines)

Some molluscan species present transversely striated muscles in addition to obliquely striated and smooth muscles. The transversely striated muscle in these molluscs differs from the striated muscle of vertebrates and arthropods in its poorly-defined Z lines, which have a discontinuous appearance and consist of multiple small electrondense patches (Figs. 2a-2c). The poor definition of the sarcomeres in these muscles resulted in erroneous interpretations as either obliquely striated or smooth. This type of transversely striated muscle has been

Fig. 2. Transversely striated muscle from gastropod mollusc (heart from *Helix aspersa*). **a.** Light microscopy of a 2 µm thick section from the ventricle. Toluidine blue. **b and c.** Longitudinal sections of the same muscle showing the transversely striated ultrastructural pattern with Z discs that consists of discontinuous electrondense bodies (arrows) and T tubules (arrowheads). A: band; M: mitochondria; I: band. **d and e.** Cross sections of the same muscle showing the discontinuous pattern of electrondense bodies (arrows) and T tubules (arrows) and T tubules (arrows). M: mitochondria: Each thick filament is surrounded by 12 thin filaments. a, x 850; b, 2,600; c, x 40,000; d, x 49,500; e, x 80,000



reported in the translucent portions of adductor muscles of some bivalves such as *Placopecten magellanicus* and *Astarte undata* (Morrison and Odense, 1974), and in the heart muscle of the gastropods *Lymnaea stagnalis* (Plesch, 1977) and *Helix aspersa* (North, 1963).

These muscles are mononucleated cells with centrally located nuclei. In the heart muscles, mitochondria are more abundant and more developed than in the adductor muscles (Fig. 2c). The sarcomere length is around 1.5 μ m. The number of thin filaments that surrounds each thick filament is 10-12 with a thin/thick filament ratio of 5-6/1 (Figs. 2d-2e) (North, 1963). These small differences in the proportion of thin and thick filaments between muscles might be real or attributable to the margin of error in the calculations.

Thin filaments are like those of both vertebrate and arthropod striated muscle. Thick filaments are shorter



Fig. 3. Schematic representation of the obliquely striated muscle from nematodes (Ascaris lumbricoides) (modified from J. Rosenbluth, 1965).

than in arthropod muscles (0.8 μ m in the heart of *Lymnaea stagnalis*) (Plesch, 1977). Thick filament width varies from 17-20 nm in the adductor muscles of bivalves to 24-29 nm in the heart muscle. Personal observations indicate that the thick filament width also varies within the same species since the diameter of these filaments in the heart wall of the *Helix aspersa* are 24 nm in the auricle and 29 nm in the ventricle. Bridges between thick and thin filaments with a 32 nm interval have been reported in the heart of *Helix aspersa*.

Paramyosin is also a normal component of the thick filaments in the transversely striated muscles of molluscs, although this protein is less abundant than in the smooth muscle of molluscs (Squire, 1971; Szent-Györgyi et al., 1971). It has been suggested that paramyosin plays an important role in the performance of a strong isometric contraction, which may be maintained for long time periods at a low energy cost. Such contraction is required for the catch mechanism of adductor muscles (Cohen et al., 1971; Szent-Györgyi et al., 1971) and probably, for hemolymph pumping by the heart muscle.

The Z band is characteristic in these muscles and consists of multiple electrondense bodies, which are the plaques of thin filament attachments, intermingled with sarcoplasmic reticulum tubules and vesicles (Fig. 2d,e). The size of these plaques varies widely; their average dimensions in the heart of *Helix aspersa* are 150 x 140 nm.

The sarcotubular system is less developed than in vertebrate striated muscles, although, like these, there is a transverse component and a longitudinal component. This system was described by North (1963) in the pseudohearts of *Helix aspersa*. Some of the tubules that are present in the Z lines are T tubules which originate from invaginations of the plasma membrane. By joining sarcoplasmic reticulum tubules and vesicles in the Z lines, these tubules make the dyads which form the transverse component. The longitudinal component consists of vesicle chains (probably tubules with a sinuous course) which run parallel to myofilaments and connect the sarcoplasmic reticulum tubules of the adjacent transverse components.

2. Obliquely striated muscle

Like other striated muscles, the obliquely striated muscle also presents both thick and thin myofilaments, which are arranged forming sarcomeres delimited by Z lines. The most important characteristic that distinguishes this muscle cell type from other striated

Fig. 4. Obliquely striated muscle of the body wall from *Ascaris lumbricoides*. **a.** Cross-sectioned view of the nematode showing the muscular cell layer in the body wall. Each muscle cell comprises three portions: (1) a peripheral portion with myofilaments; (2) a cytoplasmic portion that contains the usual organelles; and (3) a cytoplasmic process which is devoid of myofilaments. Toluidine blue. C: cuticle. **b.** Longitudinal section in plane ZX of Figure 3. Myofilaments are not perpendicular to Z discs. A: A band; I: I band; Star: cytoplasmic portion labelled with 3 in Figure a. **c.** Detail of Figure B showing a sarcomere. A: A band; I: I band; Z: Z bars. **d:** Cross-sectioned cell in plane XY of Figure 3. Although myofilaments are transversely sectioned, A bands (A) and I bands (I) are seen in the same plane of section. Star: cytoplasmic portion labelled with 3 in Figure a. **a**, x 500; b, x 21,000; c, x 36,000; d, x 18,200



muscles is that myofilaments are not perpendicular but oblique to the Z lines, so that, in each of the three spatial planes of view, which are perpendicular to one another (planes XY, YZ and ZX), both the A and the I bands appear together (Fig. 3). Since myofilaments are also oblique to the cell longitudinal axis and the aligned sarcomeres spiral through the cell, this muscle type has also been called spiral striated muscle.

The ultrastructure of the obliquely striated muscle has been studied in nematodes, annelids, molluscs, brachiopods and chaetognathes. Since the ultrastructural differences among these groups are relevant, each zoological group is described separately.

Nematodes

The first light microscopy description of these





muscles was made by Plenk (1924) in the muscular wall of *Ascaris megalocephala*. The first ultrastructural studies were reported by Rosenbluth in the muscular body wall of *Ascaris lumbricoides* (1965) (Fig. 3). The muscular wall of *Ascaris* consists of a single layer of muscular cells, each of them displaying three welldefined portions: central portion, which contains the nucleus, some cytoplasmic organelles and abundant glycogen particles; U-shaped cell processes (with the U vertex anchored in the epidermis) which contain the myofilaments; and a clear cytoplasmic portion, which is devoid of myofilaments and direct towards either the dorsal or the ventral nerve cord (Fig. 4a).

The myofilaments are exclusively contained in the periphery of the cell processes. The centre of these processes is occupied by cytoplasmic organelles, mainly mitochondria (Fig. 4a,b). Myofilament arrangement presents the ultrastructural pattern of obliquely striated muscles. The sarcomere length is 10-12 μ m. The thin filament/thick filament ratio is 10-12/1 (Rosenbluth, 1965).

Thick myofilaments measure 6 μ m in length and 23 nm in width. Paramyosin also seems to be a normal component of thick filaments in nematodes. This protein has been studied in *Caenorhabditis elegans* by Epstein et al. (1985) who found that paramyosin is from 2% to 18% of thick filament weight and forms a central core in these filaments. Whether this protein extends along the whole thick filament length or is limited to a definite segment is still unknown (Waterston et al., 1974, 1977). In addition to paramyosin, another protein component, called miniparamyosin, has been found associated to paramyosin in the thick filaments of *C. elegans* (Epstein et al., 1985, 1988).

Thin myofilaments are 6 nm in diameter and consist of actin and associated proteins, as in all the other striated muscles. However, the analysis of actin codifying genes in *C. elegans* suggests that these actin molecules are more similar to the non-muscular actin than to the muscular actin of vertebrates (Mounier and Prudhomme, 1986; Mounier et al., 1987, 1992; Krause et al., 1989). An actin-associated protein present in nematodes is titin which, as in the transversely striated muscle of vertebrates and invertebrates, joins actin filaments to the Z lines (Maruyama, 1986; Fürst et al., 1988, 1989; Whiting et al., 1989; Labeit et al., 1990, 1992). The titin of *C. elegans* weights 700 kDa; this is

Fig. 6. Obliquely striated muscle of the body wall from *Eisenia foetida*. **a and b.** Light microscopy of body wall showing the circular (C) and longitudinal (L) muscular layers in longitudinal (a) and transverse (b) sections. The muscle cells in the longitudinal layer are smaller than those of the circular layer and are grouped in nests anchored in the connective-tissue septa (arrow). **c.** Cross-sectioned view of a muscle cell in the circular layer showing the characteristic ultrastructural features of obliquely striated muscle. The helicoidally arranged myofilaments have been sectioned in three planes (XY, YZ and ZX) (see drawing in figure 5). A bands (A) and I bands (I) are visible. Z bars appear longitudinally (ZB)- or obliquely (zb)-sectioned. Subsarcolemmal electrondense plaques (arrows) are observed. **d.** Cross-section view of muscle cells in the longitudinal layer. Myofilaments are sectioned in the XY plane or in planes oblique to it. Subsarcolemmal electrondense plaques (arrows) are observed. **d.** Cross-sectioned myofilaments in the circular layer (f) and longitudinal layer (g). Thick myofilaments are wider in the circular layer. The differences in thick myofilament diameter within the same section are due to the spindle shape of these filaments. In both sections, the thin/thick filament ratio is 1/10. **h.** Higher magnification of an area similar to that labelled as Zx in Figure c, showing A bands (A), z bars (zb) and sarcoplasmic reticulum tubules (arrows). **i.** Subsarcolemmal electrondense plaques (arrow) in the muscle cells of the circular layer (g). Thick myofilaments are wider in the circular layer. The differences in thick myofilament diameter within the same section are due to the spindle shape of these filaments. In both sections, the thin/thick filament ratio is 1/10. **h.** Higher magnification of an area similar to that labeled as ZX in Figure c, showing A bands (A), z bars (zb) are seen. a and b, x 360; c, x 9,000; d, x 9,500; e, x 42,000; f and g, x 48,000; h, x 36,000; i, x 28,000



lower than vertebrate titin (3000 kDa). Titin seems to be codified by the same genetic complex (unc-22) that controls myosin synthesis (Moerman et al., 1988; Nave et al., 1991).

Z lines are less electrondense and wider than in vertebrate striated muscles and contain fibril bundles (termed Z bundles), electrondense bodies, T tubules and sarcoplasmic reticulum tubules (Rosenbluth, 1965, 1967) (Fig. 4b-d). There are also electrondense plaques for myofilament anchorage beneath the plasma membrane. These plaques contain α -actinin and vinculin, whereas Z lines only contain α -actinin.

The sarcotubular system is more simple than that of vertebrate striated muscle. Some of the tubules in the Z lines are in continuity with the plasma membrane and may be considered T tubules. Other tubules are sarcoplasmic reticulum tubules that discontinuously attach to the T tubules to form dyads and triads (Rosenbluth, 1965) (Fig. 3).

Biochemical studies suggest the presence of desmintype intermediate filaments, although their location is still unknown (Bartnik et al., 1986; Bartnik and Weber, 1987).

Annelids

Many ultrastructural studies have been made of the obliquely striated muscle in annelids. These studies concern the muscular body wall of the oligochaetan species Eisenia foetida (Kawaguti and Ikemoto, 1957a; Ikemoto, 1963; Morita and Van Breemen, 1963; Chapron and Valembois, 1967; Royuela et al., 1995) (Figs. 5, 6), Lumbricus terrestris (Hanson, 1957; Kawaguti, 1962; Staubesand and Kersting, 1964; Heuman and Zebe, 1967; Lanzavecchia, 1968; Mill and Knapp, 1970), Allolobhora chlotica (Aguirre et al., 1981), Rinchelmis limosella (Lanzavechia et al., 1987), Pelodrilus leruthi (De Eguileor et al., 1990); the polychaetan species Haplosyllis depresa (Bouligand, 1966), Glycera (Rosenbluth, 1968), Nereis irrorata (Defretin and Wissocq, 1969), Syllis amica (Wissocq, 1970); and the hirudinean species Hirudo niponia (Kawaguti and Ikemoto, 1958b), Hirudo medicinalis (Röhlich, 1962; Lanzavecchia et al., 1985), and Glossiphonia complanata (De Eguileor et al., 1993). Recently, this muscle type has also been described in the intestinal wall (Fig. 7a,b) and pseudohearts (Fig. 7c,d) of the oligochaete E. foetida (Royuela et al., 1995).

The muscle cells of the body wall are usually elongated mononucleated cells, which measure from 200

to 600 µm in length, and occupy several metameres (Wissocq, 1970) (Fig. 6a,b). The distribution of the nucleus and organelles varies with the annelid species (Valvassori and De Equileor, 1991) and muscle type studied (Royuela et al., 1995). Usually the nucleus and most of the cytoplasmic organelles lie on the cell periphery in the muscular body wall cells (Valvassori and De Eguileor, 1991), whereas they are centrally located in the intestinal wall and outer muscular layer of the pseudoheart (Royuela et al., 1995). Most of the cytoplasm is occupied by thick and thin myofilaments which are grouped forming spirally-arranged sarcomeres measuring 6-8 µm in length (Wissocq, 1970) (Figs. 6c,d, 7b). The thin filament/thick filament ratio also varies among muscle types. Although the most common value is 6/1 (Ikemoto, 1963; Bouligand, 1966; Wissocq, 1967, 1970), ratios of 10/1 have also been reported in the muscular body wall of E. foetida by Chapron and Valembois (1967) and Royuela et al. (1995) (Fig. 6e-6i).

Thick filaments are usually fusiform and their length and diameters vary among species and among muscles within the same species. The most common length is about 4 µm (Wissocq, 1967, 1970). The diameters of thick filaments, measured in their central portion, in the muscular body wall of oligochaetes are 20 nm in *Haplosyllis depresa* (Bouligand, 1966) and 30-36 nm in *Syllys amica* (Wissocq, 1970). In both species, thick filament diameters measured only 7-10 nm at their ends. In *Eisenia foetida* the central diameter of the tick filaments varies according to the muscle: 32 nm in the longitudinal layer of the body wall musculature; 42 nm in the circular layer of this musculature; and 50 nm in both intestinal wall muscle and pseudoheart (Royuela et al., 1995)

The thin filaments are like those in other striated muscles and also contain troponin (Fig. 7e). They show a uniform width of 6 nm and consist of actin and associated proteins including troponin and tropomyosin.

Thick filaments consist of myosin and paramyosin (Fig. 7f). Isolation and purification of myosin from the muscular body wall of *Lumbricus terrestris* revealed two isoforms which differed in the regulation of one of the two myosin light chains (Serwe et al., 1993). These myosin isoforms have been designated as RLC25 and RLC28, according to the molecular weight of their variable light chain (25 kDa and 28 kDa respectively). The essential light chain in both isoforms weights 18 kDa (Serwe et al., 1993). As in other invertebrate muscles, paramyosin does not seem to occupy the entire filament length.

Fig. 7. a.b Obliquely striated muscle of the intestinal wall from *Eisenia foetida*. **a.** Cross section of the intestinal wall observed by light microscopy. The muscle cells form a thin layer (arrows). H-E. **b.** Electron microscopy of a muscle cell shown in Figure a. A bands (A) and I bands (I) are seen. M: Mitochondria. **c and d.** Obliquely striated muscle of the pseudoheart from *Eisenia foetida*. **c.** Cross section of the pseudoheart observed by light microscopy. The muscle cells form an inner (arrowhead) and an outer (arrows) layer. L: pseudoheart lumen. **d.** Electron microscopy of a muscle cell from the inner muscular layer showing the characteristic pattern of the obliquely striated muscle. A bands (A) and I bands (I) are seen. **e.** Troponin immunoreaction in a muscle cell of the body wall from *Eisenia foetida*. Immunogold particles are observed in the A bands (A) and I bands (I), which are sectioned in the plane YZ. Z: Z bar. **f.** Paramyosin immunolabelling in a similar cell sectioned on the plane ZX Immunogold particles are observed in the J bands (I). a, x 450; b, x 30,000; c, x 360; d, x 17,000 e, x 32,000; f, x 43,200



Z lines are well defined and consist of electrondense cylindres, called Z bars, alternating with sarcoplasmic reticulum tubules. Both structures are parallel to each other and perpendicular to the myofilaments (Figs. 5, 6h,i). Subsarcolemmal electrondense plaques are also found (Fig. 6i).

De Eguileor et al. (1988), using electrophoresis and Western blot analyses, identified desmin in the muscular body wall of several annelids, including hirudinean (*Hirudo medicinalis*), oligochaetan (*Lumbricus terrestris, Enchytraeus albidus* and *Rinchelmis limosella*), and polychaetan (*Prinospio caspersi*) species. In *H. medicinalis*, intermediate filaments are observed under the plasma membrane running parallel to the main axis of the muscular cells. In *Lumbricus terrestris* the intermediate filaments are also located under the sarcolemma and lie in two main directions: perpendicular to myofilaments, and parallel to the main axis of the fibre (De Eguileor et al., 1988).

The sarcoplasmic reticulum is usually abundant in the obliquely striated muscle of annelids and consists of tubules that are mainly perpendicular to myofilaments at the level of the Z lines (Fig. 6h). Although some authors have failed to demonstrate continuity between these tubules and the plasma membrane (true T tubules) in some species (Rosenbluth, 1972), ultrastructural studies by De Eguileor and Ferraguti (1980) in Branchiobdella pentodonta revealed a number of tubular sarcolemmal invaginations, which remained in contact with expansions of sarcoplasmic reticulum tubules oriented in parallel and formed structures morphologically similar to dyads. In Branchiobdella pentodonta (De Eguileor and Ferraguti, 1980) and in some hirudinean species (Lanzavecchia et al., 1985) longitudinally arranged cisternae of sarcoplasmic reticulum that form the longitudinal component of the sarcotubular system have also been observed.

Molluscs

The existence of these obliquely striated muscles was first reported in certain cephalopods by Plenk (1933) who, with only light microscopy, observed oblique or helical striation. Afterwards, the ultrastructure of these muscle cells was described by Kawaguti and Ikemoto (1957a,b,c) in the mantle muscles of the sepia, and by Bowden (1958) in some lamellibranchs. Other ultrastructural studies refer to the tentacular muscles of several cephalopods such as Sepia, Octopus and Eledone (Plenk, 1933; Kawaguti and Ikemoto, 1957b; González-Santander and Socastro García-Blanco, 1972); the translucent portion of the adductor muscles in bivalves such as Pecten albicans (Hanson and Lowy, 1959; Nunzi and Franzini-Armstrong, 1981), Crassostrea virginica and Arctica islandica (Morrison and Odense, 1974), Tridacna crocea (Matsuno and Kuga, 1989), Chlamys nobilis (Matsuno et al., 1993), Mitilus crassitesa (Kawaguti and Ikemoto, 1975c), Spondilus cruentus (Kawaguti and Ikemoto, 1959), Meretrix lusoria

(Kawaguti and Ikemoto, 1960), *Crassostrea angulata* (Hanson and Lowy, 1961); and buccal mass, gizzard and deferent ducts of the gastropod *Lymnaea stagnalis* (Plesch, 1977).

Molluscan obliquely striated muscle cells are fusiform and have mononucleate fibres with a centrally located nucleus and a few large mitochondria at the ends of the muscle cells (González-Santander and Socastro García-Blanco, 1972; Kuga and Matsuno, 1988). Most of the remaining cytoplasm is occupied by myofilaments which form helically arranged sarcomeres (Fig. 8). In many molluscan species each thick filament is surrounded by 12 thin filaments, giving a thin/thick filament ratio of 6/1 (González-Santander and Socastro García-Blanco, 1972; Kuga and Matsuno, 1988), although nine thin filament arrays around each thick filament have been observed in *Lymnaea stagnalis* (Plesch, 1977).

Thick myofilaments are fusiform and their length and width vary from one muscle to another. In some



Fig. 8. Representation of the myofilament arrangement in the obliquely striated muscle of molluscs (modified from R. González-Santander and E. Socastro Garcí-Blanco, 1972).

cephalopods thick myofilaments measure approximately 5 μ m in length and their diameter is 30 nm at the centre of the filament and 5 nm at the tips (González-Santander and Socastro García-Blanco, 1972). In *Lymnaea stagnalis* thick filaments measure 2-4 μ m in length and have a width of 20-30 nm at their centre (Plesch, 1977). In the bivalves *Crassostrea virginica* and *Arctica islandica*, the diameters at the centre of thick filaments are 20 nm (Morrison and Odense, 1974). In *Chlamys nobilis*, Matsuno et al. (1993) found two types of obliquely striated cells whose thick filament diameters, measured at their central portion, were 30-35 nm and 50-60 nm, respectively.

Thick filaments are made by both myosin and paramyosin (Hanson and Lowy, 1961; Szent-Györgyi et al., 1971). In the horseshoe crab (*Limulus*) and in some molluscan species, the myosin molecule has three light chains with molecular weights of 19, 21 and 31 kDa, respectively (Sellers et al., 1981). Myosin covers paramyosin molecules, which form a central core (Elliot, 1979). There is no data on the extension of paramyosin in the thick filament. In the translucent portion of the adductor muscles of some bivalves (*Mytilus*, and *Mercenaria*) the paramyosin/myosin ratio is 2/1 (Squire, 1971; Levine et al., 1976).

Thin filaments are similar to those reported in other muscles. Actin molecules in the obliquely striated muscles of *Pecten* and *Loligo* are similar to nonmuscular actin of vertebrates (Vanderkerckhove and Weber, 1984; Mounier et al., 1992). Several actin isoforms, which differ in their isoelectric point, have been found in *Aplysia californica* and *Pecten maxims* (Hue et al., 1988; Hue and Benyamin, 1989). Other actin-associated proteins present in the obliquely striated muscles of molluscs are troponin and tropomyosin (Millman, 1967; Lehman and Szent-Györgyi, 1975; Sellers et al., 1981).

Inconspicuous in the obliquely striated muscle of molluscs, Z lines show a discontinuous pattern (Matsuno and Kuga, 1989) which has led to misinterpretations of these muscles as smooth muscles. The Z line pattern has been described as a row of either granular accumulations (González-Santander and Socastro García-Blanco, 1972) or small electrondense bodies (Kawaguti and Ikemoto, 1959), located in the centre of the I bands. As in other muscles, these electrondense bodies are plaques for thin filament attachment. It has been proposed that, in contrast with what occurs in other muscles, when the thin filaments approach the Z line closely, they do not maintain their parallel arrangement but converge and insert themselves into the nearest electrondense body of those forming the Z line (González-Santander and Socastro García-Blanco, 1972). According to this hypothesis, the discontinuous pattern of Z lines is due to the convergence of thin filament groups at this level.

Using an antibody that cross-reacts with all mammalian intermediate filament proteins, intermediate filaments have been identified in obliquely striated muscles of gastropods, although the exact type and location of these filaments is not yet known (Bartnik et al., 1986; Bartnik and Weber, 1987).

The sarcotubular system has been described in bivalves (Morrison and Odense, 1974) and cephalopods (González-Santander and Socastro García-Blanco, 1972) and is very similar to that reported in the transversely striated muscle of molluscs. There is an oblique component or system (equivalent to the transverse component of the transversely striated muscle) which extends through the Z lines, and a longitudinal component, which extends along the myofilaments. The oblique system consists of tubules invaginated by the plasma membrane (T tubules) and of sarcoplasmic reticulum tubules both of which bind and form dyads. The longitudinal system consists of rows of interconnected tubules and vesicles that extend between adjacent Z lines.

Brachiopods

The adductor muscles of brachiopods are histologically similar to the adductor muscles of bivalves (Kawaguti and Ikemoto, 1958b; Lowy and Hanson, 1962; Nunzi and Franzini-Armstrong, 1981). The translucent portion in the adductor muscles of *Lingula unguis* (Kuga and Matsuno, 1988) and *Terebratalia transversa* (William et al., 1982) consists of an obliquely striated muscle and is very similar to the translucent muscle portion of many bivalves. In the adductor muscle cells of these brachiopods thick filaments are 22.5 nm wide and most of the cytoplasmic organelles are located at the cell periphery (Kuga and Matsuno, 1988).

Chaetognathes

Duvert (1969a,b) and Duvert and Savineau (1986) have reported the ultrastructure of the muscular body wall of the chaetognathe *Sagitta setosa*. This wall consists of obliquely striated muscle with very short sarcomeres measuring $1.55 \,\mu\text{m}$ in length (1.2 μm for the A band and only 0.33 μm for the I band). Z lines are not very electrondense and are formed by the interconnection of adjacent thin filaments through a net of filamentous bridges designated C filaments. The transition from I band to Z line is delimited by a single row of bridges (Z-I bridges), and the transition from I band to A band is also delimited by another single row of bridges (A-I bridges). The arrangement of thin filament in the A band is 6 thin filaments around 1 thick filament, with a thin/thick filament ratio of 3/1.

Smooth muscle

Smooth muscle is widely extended in invertebrates. It has been reported in coelenterates, annelids (Fig. 9), molluscs (Fig. 10), brachiopods and echinoderms (Matsuno, 1987) but is absent in arthropods. Like vertebrate smooth muscle, invertebrate smooth muscle cells are fusiform cells with a centrally-located nucleus, peripherally-placed mitochondria and sarcoplasmic reticulum, and abundant thin and thick myofilaments without an apparent sarcomeric organization, but the invertebrate smooth muscle differs from the vertebrate muscle in the proportion and size of its myofilaments (Figs. 9, 10).

The thin/thick filament ratio in invertebrate smooth muscle varies among species and muscles but is usually higher than in vertebrate smooth muscle and similar to the values found for obliquely striated muscles (from 6 to 12) (Figs. 9e, 10c).

The thick myofilaments of invertebrate smooth muscle, like those of obliquely striated muscle, vary widely in thickness and may be homogeneous or fusiform. The reported diameters, measured at the centre of the thick filament of the retractor muscle of several bivalve species are: 25 nm in *Helix pharynx* (Hanson and Lowy, 1957); 36.4 nm in *Helix aspersa* (personal observation); and 125 nm in *Mytilus edulis* (Selbi and Bear, 1956; Hanson and Lowy, 1959). The maximum reported thick myofilament diameter is 231 nm in the opaque portion of the adductor muscle of *Astarte undata* (Morrison and Odense, 1974).

The thick myofilaments of invertebrate smooth muscle also contain a central core of paramyosin (Fig. 9f), like invertebrate striated (obliquely or transversely) muscles (Elliot, 1974). This protein is not found in vertebrate smooth muscle. Therefore, paramyosin is exclusive to invertebrates, and independent of the muscle class (smooth or striated).

Most of smooth muscle paramyosin studies have used molluscs (Kawaguti and Ikemoto, 1960; Sobieszek, 1973; Morrison and Odense, 1974; Plesch, 1977; Matsuno, 1987; Maroto et al., 1995). X-ray studies by Elliot (1974, 1979) of the *Oyster* smooth muscle suggest that paramyosin molecules are regularly distributed along the thick filaments at an interval of 14.4 nm. A similar periodicity has been reported in the retractor muscle of Mytilus edulis (Castellani et al., 1983; Ishii and Takahashi, 1983) and *Meretrix* (Nonomura, 1974). A slightly higher paramyosin period (18 nm) has been found in the adductor muscle (opaque portion) of Chalamys nobilis (Matsuno et al., 1993). Studies in Mytilus edulis and Mercenaria (Castellani and Cohen, 1987a,b; Watabe et al., 1990) have led to the suggestion that paramyosin is involved in myosin light chain phosphorylation, which is required for muscular contraction.

In all smooth muscle cell types, the thin myofilaments are 6 nm-wide actin filaments and are associated to tropomyosin. The principal difference with the thin filaments of all the striated muscle types is the absence of troponin in the smooth muscle. This absence is also a differential character between striated and smooth muscle in vertebrates.

Rather than Z lines, invertebrate smooth muscle shows electrondense bodies, similar to those found in vertebrate smooth muscle (Figs. 9a,b, 10a,b). These bodies, which are plaques for thin filament attachment are not laterally aligned. However, they appear to be somewhat regularly arranged in some molluscan smooth muscles, such as the retractor muscle of *Mytilus edulis* (Lowy and Hanson, 1962).

The morphology of the invertebrate smooth muscle cells varies widely among species and even within the same species (Fig. 10e). An ultrastructural study of several muscles in the gastropod, Lymnaea stagnalis (head retractor, circular, diagonal, tentacle retractor, columellar, feet muscle, digestive tract and associated gland muscle, retractor and protractor muscles of the penis, and retractor muscle of the mouth) revealed important differences between them regarding cell size, myofilament size and number, mitochondrial number, and sarcoplasmic reticulum development (Plesch, 1977). In addition, examining the same anatomical muscle (the opaque portion of the adductor muscle) in several bivalve species, Morrison and Odense (1974) found that the diameter of the smooth muscular cells varied from one species to another, and that the cell size was associated with the thick filament diameter: the larger the cell the larger the thick filament diameter. The values found by these authors were (cell diameter and thick filament diameter): 4 µm and 60 nm in Placopecten magellanicus; 7 µm and 85 nm in Arctica islandica; 8 µm and 110 nm in Crassostrea virginica; and 12 µm and 231 nm in Astarte undata.

A single muscle may be formed by several ultrastructurally different muscle cell types. Matsuno and Kuga (1989) described two different portions (translucent and opaque) in the perfusor adductor muscle of the bivalve Tridacna crocea. The translucent portion consists of obliquely striated muscle and the opaque portion was made up of two types of smooth muscle cells: (1) cells with 50-65 nm wide thick filaments; and (2) cells that, in addition to this type of thick myofilament, showed another type measuring 85-100 nm in diameter. The former smooth muscle cells are also present in the muscular body wall of many bivalves and seem to account for body movements (Plesch, 1977; Matsuno, 1987). The second cell type is also observed in bivalve muscles, such as the shell adductor muscle of Pecten albicans (Kawaguti and Ikemoto, 1958a), in the adductor muscle of Oyster (Elliot, 1979) and retractor muscle of Mytilus (Sobieszek, 1973), and seems to be

Fig. 9. Smooth muscle of the inner muscular layer of the pseudoheart from *Eisenia foetida*. **a and b**. Low power micrograph of smooth muscle cells. No Z bars or associated sarcoplasmic reticulum system are seen, but electrondense plaques for filament anchorage (arrows) and grouped mitochondria (M) can be observed. **c.** Nucleus (N) and numerous mitochondria (M) in the centre of a smooth muscle cell. **d**. No immunoreaction to troponin is seen in longitudinally (L)- or transversely (T)-sectioned myofilaments. **e.** Cross-sectioned myofilaments seen at high magnification. Each thick filament is surrounded by approximately 12 thin filaments (the thin/thick filament ratio is 1/6). **f.** Positive paramyosin immunoreaction in the thick filaments. a, x 8,000; b, x 10,000; c, x 17,500; d, x 40,000; e, x 47,000; f, x 60,000





Fig. 10. Smooth muscle cells from the gastropod *Helix aspersa*. **a.** Longitudinal section of a smooth muscle cell from the retractor muscle showing abundant thick myofilaments, which are not apparently arranged into sarcomeres. Electrondense plaques are seen beneath the plasma membrane (asterisk) and are irregularly distributed throughout the cytoplasm (arrows). Numerous caveoles (arrowhead) are seen in the cell periphery. M: mitochondria. **b.** Transverse section of a smooth muscle cell from the retractor muscle. Together with transversely-sectioned thick myofilaments (T), a bundle of longitudinally-sectioned thick myofilaments (L) can be seen. Electrondense plaques (asterisk) and caveoles (arrowhead) are seen. **c.** Higher magnification of cross-sectioned myofilaments seen in Figure b. Each thick filament is surrounded by approximately 12 thin filaments (the thin/thick filament ratio is 1/6). **d.** Two small muscle cells in the intestinal wall with developed subsarcolemmal electrondense plaques (arrows). **e.** Smooth muscle cells in the intestinal wall showing transverse (T)- and longitudinal (L)-sectioned myofilaments. Notice the large diameter of the cross-sectioned thick filaments. M: mitochondria; Star: large electrondense plaque. a, x 28,200; b, x 41,500; c, x 70,400; d, x 9,500; e, x 68,750

involved in catch contractions (Lowy and Millman, 1963; Matsuno and Kuga, 1989). A similar dual composition of adductor muscles consisting of a translucent portion made up of obliquely striated muscle, and an opaque portion composed of two smooth muscle cell types (one with only one type of thick myofilament, and another with two classes of these myofilaments) has also been reported in the brachiopod Lingula unguis (Kuga and Matsuno, 1988). However, this organization of adductor muscles does not seem to be common to all brachiopod and bivalve species since the opaque portion of the adductor muscle in Chlamys nobilis only contains the second type of smooth muscle cell with two types of thick filaments: one measuring 7.3µm x 26.2 nm, and the other measuring 13.4 µm x 41.8 nm (Matsuno et al., 1993).

Due to the wide variety of smooth muscle cell types present in invertebrates, Matsuno (1987) proposed a classification of smooth muscle cells into four types. This classification is principally based on the diameter of thick filament, and the size and organization of the electrondense bodies for filament anchorage.

Type A cells. Small fusiform cells with a centrallylocated nucleus and peripherally-placed cytoplasmic organelles. Thick myofilaments measure 14 nm in width. The electrondense bodies are ovoid, small, scanty and apparently disordered. The sarcoplasmic reticulum is scanty. This cell type is characteristic of vertebrates and is also found in some invertebrates, such as adductor muscles of the bivalves *Spondylus cruentus* (Kawaguti and Ikemoto, 1959) and *Tamanovalva limax* (Kawaguti and Ikemoto, 1960), spines and ovarian wall of several echinoderms (Kawaguti and Ikemoto, 1965), muscular body wall of sea cucumbers (Kawaguti and Ikemoto, 1965) and tentacles of the celenterates *Stephanoscyphus* and *Atorella* (Matsuno, 1987).

Type B cells. Elongated cells have a centrallylocated nucleus and peripherally-placed cytoplasmic cell organelles. They differ from type A muscle in their thick filaments which measure approximately 40 nm in diameter (Fig. 10). The electrondense bodies are disordered but they are larger than in Type A cells. The sarcoplasmic reticulum is scanty. This cell type is mainly present in molluscs and echinoderms, and has also been reported in the anterior retractor muscle of *Mytilus* (Kawaguti and Ikemoto, 1957a; Sobieszek, 1973), muscular body wall of the gastropod *Lymnaea stagnalis* (Plesch, 1977), oral disc muscle of several celenterates (Matsuno, 1987), and outer muscular layer in pseudohearts of the oligochaete *Eisenia foetida* (Royuela et al., 1995).

Type C cells. These large and elongated cells have a centrally-located nucleus. The thick filament diameter varies from 60 to 120 nm. The sarcoplasmic reticulum is scanty. The electrondense bodies are large, scanty and without order. Type C cells have been reported in the opaque portion of adductor muscles of bivalves such as *Meretrix* (Kawaguti and Ikemoto, 1960), *Astarte* (Morrison and Odense, 1974), *Atrina* (Kawaguti and Ikemoto, 1957a,b,c) and *Tellin* (Kawaguti and Ikemoto, 1961).

Type D cells. These cells present a greater development of cell organelles than the preceding smooth muscle cell types. The electrondense bodies are small but they are more numerous and more regularly arranged through the cytoplasm than in the other smooth muscle cell types. The sarcoplasmic reticulum is developed. The diameter of thick myofilaments varies from 14 nm to 40 nm. The ultrastructural appearance of these cells is similar to that of obliquely striated muscle. This cell type has only been reported in the intestinal wall musculature of the echinoderm sea cucumber and the oligochaete *Branchiura sowerbyi*, large hemolymphatic vessels of *Phoronis* and the ampullae of several echinoid species (Naitoh and Matsuno, 1985; Matsuno, 1987).

In conclusion, the invertebrate smooth muscle varies widely among zoological groups although the proportion, width and organization of thick myofilaments is higher than in the vertebrate smooth muscle, and some invertebrate smooth muscle cells (the Type D cells in Matsuno's classification) are intermediate between smooth and obliquely striated muscle cells.

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