Mem. Fac. Sci., Shimane Univ.,
16, pp. 61–67 Dec. 20, 1982

Electron Microscopy on Striated Muscles in the Ephyra of *Aurelia aurita*

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(Received September 4, 1982)

Introduction

Coelenterates generally have smooth muscles in bodies (Hyman, 1940; Kawaguti, 1966, Kawaguti and Yoshimoto, 1973, Matsuno, 1981b), excepting some peculiar occasions (Chapman, 1978). But in the free swimming medusae and ephyrae, cross striated muscles are observed, although they are simple and undeveloped in their structures as compared with those of mammals and arthropods. That is, they are characterized to have short sarcomeres, short A and I bands (Kawaguti and Hamakoshi, 1963; Keough and Summers, 1976) and thin myofibrils. And sometimes they lack the sarcoplasmic reticular systems (S. R) (Matsuno, 1981a).

Free swimming medusae develop through the developmental course of ephyrae which were released from the strobila. So it must be reasonable to assume that cross striated muscles in medusae develop through the developmental course of the ephyra. *Aurelia aurita* is a convenient material to examine such development, that is, the polyps are easily transformed into strobilae under a temperature treatment in the laboratory (Kakinuma, 1962).

Cross striated muscles in an *Aurelia* ephyra were observed in a circular muscle around the mouth and radial muscles running a longitudinal direction in the lappets. These two muscles contain a similar type of cross striated muscle cells which resemble those of the medusae, but those two muscle cells were different from the fine structures.

This paper deals with the fine structures of both cross striated muscle cells in comparison with those of other medusae and ephyrae, and discusses the relation of the circular and radial muscular systems.

Materials and Methods

The specimens used in this study were ephyrae of *Aurelia aurita* derived from polyps which had been kept in laboratory. The polyps were kept at 8°C in several weeks to transform into strobilae. Ephyrae of 7–9 days after the release show brownish color and swim actively. An external profile is shown in Fig. 1. These ephyrae were fixed

for 1.5 hrs in 1.5% paraformaldehyde and 1.5% glutaraldehyde with 0.1 M cacodylate buffer (pH 7.3) at room temperature. In some cases, they were anaesthetized in the sea water containing a few grains of menthol until their movements ceased. The pre-fixed specimens were washed for 30 min on ice in the same buffer solution except both aldehydes, and post-fixed for 2 hrs on ice in a solustion of 1% osmium tetroxide in 0.1 M phasphate buffer pH adjusting to 7.3. The fixed specimens were dehydrated in a graded series of ethanol, and embedded in Epoxy resin after passing through propyleneoxide. Sections were cut with glass knives and mounted on formbar-coated grid meshes. They were stained with saturated aqueous uranyl acetate and lead cirtrate. Then, they were observed under a JEM 100 C type electron microscope and photographed at 2,000– 20,000 magnifications.

Results and Discussion

An ephyra of *Aurelia aurita* which was kept at 8°C for 7–9 days after the release shows a typical ephyra-form as shown in Fig. 1. The ephyra has 8 lappets that bear sense organs at the top regions, and 4 gastral filaments in the gastric cavity. The animal swims actively by pulsations of the circular muscle which is constructed with cross stiated muscle cells. The circular muscle is distributed around the mouth in the subumbrella, binding the roots of lappets. On the other hand, radial muscles run through the lappets longitudinally (Fig. 1). The exact possition of a pair of radial muscle in a lappet is in the epitherial layer of both sides of the radial canal in the lappet. These two muscular systems observed electron microscopically will be described separately in the following sections.

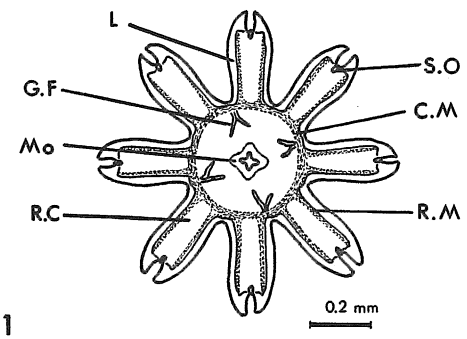

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Fig. 1. An aboral view of an ephyra of *Aurelia aurita* at 7–9 days after the release. C.M: circular muscle, G.F: gastral filament, L: lappet, Mo: mouth, R.C: radial canal, R.M: radial muscle, S.O: sense organ.

Circular Muscle

The circular muscle measuring about 0.14 mm in its cross section contains about

160 muscle cells. 40–50 cells gather in a unit and arrange in a wavy shape (Fig. 2). Each cell consists of a cell body and a slender muscle fiber or fibers. The cell body measured about 12 μm × 20 μm has a nucleus, endoplasmic reticular systems (E. R), glycogen granules etc (Fig. 2). The muscle fiber measures 0.9 μm in width and over 50 μm in length, and contains a thin myofibril, mitochondria and a few cytoplasm. A successful, longitudinal section of a part of myofibril bears 46 sarcomeres in about 40 μm length (Fig. 3, arrows).

In a cross section of a muscle fiber, many mitochondria are distributed near a myofibril (Fig. 4). The muscle cell sometimes includes 2 or 3 myofibrils. Two kinds of myofilaments are recognized, that is, thick and thin about 13 nm and 6 nm in diameter respectively. These sizes resemble those of the cross striated muscle of mammals and insects. Thick ones arrange in a regular lattice of about 30 nm intervals, and thin ones encircle a thick one hexagonally (Fig. 5). Thick ones sometimes appear as a tubules as seen in insect flight muscles (Ashhurst, 1967; Fig. 5).

In a longitudinal section, the circular muscle shows a similar structure as in the typical cross striated muscle including short A and I bands, M-line and Z-disk. The sarcomere measures about 0.8 μm, an A-band measures 0.58 μm and a I-band shows 0.18 μm in length. The short sarcomere (0.8 μm) is shorter than that of *Spirocodon* (Kawaguti and Hamakoshi, 1963), *Pennaria* (Keough and Summers, 1976) and *Atorella* (Matsuno, 1981a). In addition to those short sarcomeres, the characteristics of the muscle cells are recognized in the structures of a Z-disk. That is, Z-disks in the muscle cells do not appear as a complete Z-disk but as an accumulation of J-granules (Fig. 6). These structures of Z-disk are also recognized in a circular muscle cells of an ephyra of *Atorella* (Matsuno, 1981a), but not observed in medusae of *Spirocodon* (Kawaguti and Hamakoshi, 1963) and *Pennaria* (Keough and Summers, 1976).

Myofibrils bear no S. R systems, nor E. R systems around them. Many mitochondria are recognized around the myofibrils (Fig. 4). These features are different from those of *Spirocodon* (Kawaguti and Hamakoshi, 1963) and *Pennaria* (Keough and Summers, 1976), but resemble those of an *Atorella* circular muscle in an ephyra stage (Matsuno, 1981a). Lacking the S. R systems which must work for ionic regulations for contractions is a prominent characteristic in the cross striated muscle cells of this ephyra.

Radial Muscle

A pair of radial muscles measuring about 0.06 mm in thickness run longitudinally in the lappet from the top region of the radial canal to the circular muscle (Fig. 1). A cross section of the muscle contains about 55 muscle cells (Fig. 7). The muscle cell shows a similar external feature to that of the circular one, that is, it has a large cell body in the center and a slender muscle fiber or fibers from the base. The muscle cell sometimes contains 2 or 3 muscle fibers as same as the circular muscle (Fig. 8, arrows). The muscle fiber contains a myofibril, mitochondria and other cell organells,

but not any S. R or E. R systems around the myofibril (Fig. 8). The myofibril contains thick and thin myofilaments. Thick one measures about 13 nm and thin one about 6 nm in diameter respectively (Fig. 9). The thick filaments array in a regular lattice of about 30 nm intervals, and thin ones distribute around a thick filament hexagonally (Fig. 9).

In a longitudinal section, the radial muscle shows similar appearances to those of a circular one. That is, a sarcomere, A-bands and I-bands are measured as 0.8 μm, 0.59 μm and 0.18 μm respectively (Fig. 10). S. R and E. R systems are not recognized around the myofibril (Fig. 10). A Z-disk appears also in the similar structure to that of a circular one (Fig. 11).

The observations descrived above suggest that the cirular and radial muscles have the same type cross striated muscle cells. The following observations afford the basis for the suggestion; 1) Both muscle cells in two muscular systems have similar sizes of a sarcomere, an A-band and a I-band. 2) Thick and thin myofilaments are common in sizes and show similar arrangements. 3) S. R or E. R systems are not seen around the myofibrils. A slight difference between the two muscle cells is recgnized in the size of myofibrils, that is, the circular myofibril shows a rectangule of about 0.7 μm $\times$ 1.5 μm in cross section, but the radial one shows a polygon of about 1 μm in diameter. In addition to those structural similarity, a close relation of the two muscles is shown in Fig. 12. An arrow in Fig. 12 indicats the point at which the radial muscle branches off from the circular one.

The reason why an ephyra of *Aurelia* has cross striated muscle cells in the lappets, though the lappets and the muscles disappear during the development of ephyrae, may be explanable; that is, the lappets work as a locomotive organ when the ephyra swim.

From another standpoint, these cross striated muscle cells in circular or radial muscle systems, appear somewhat more primitive in structures than those of medusae. It may be explanable by a surmise that the muscle cells in an ephyra transform well-developed ones during the developmental course to a medusa. We must observe the

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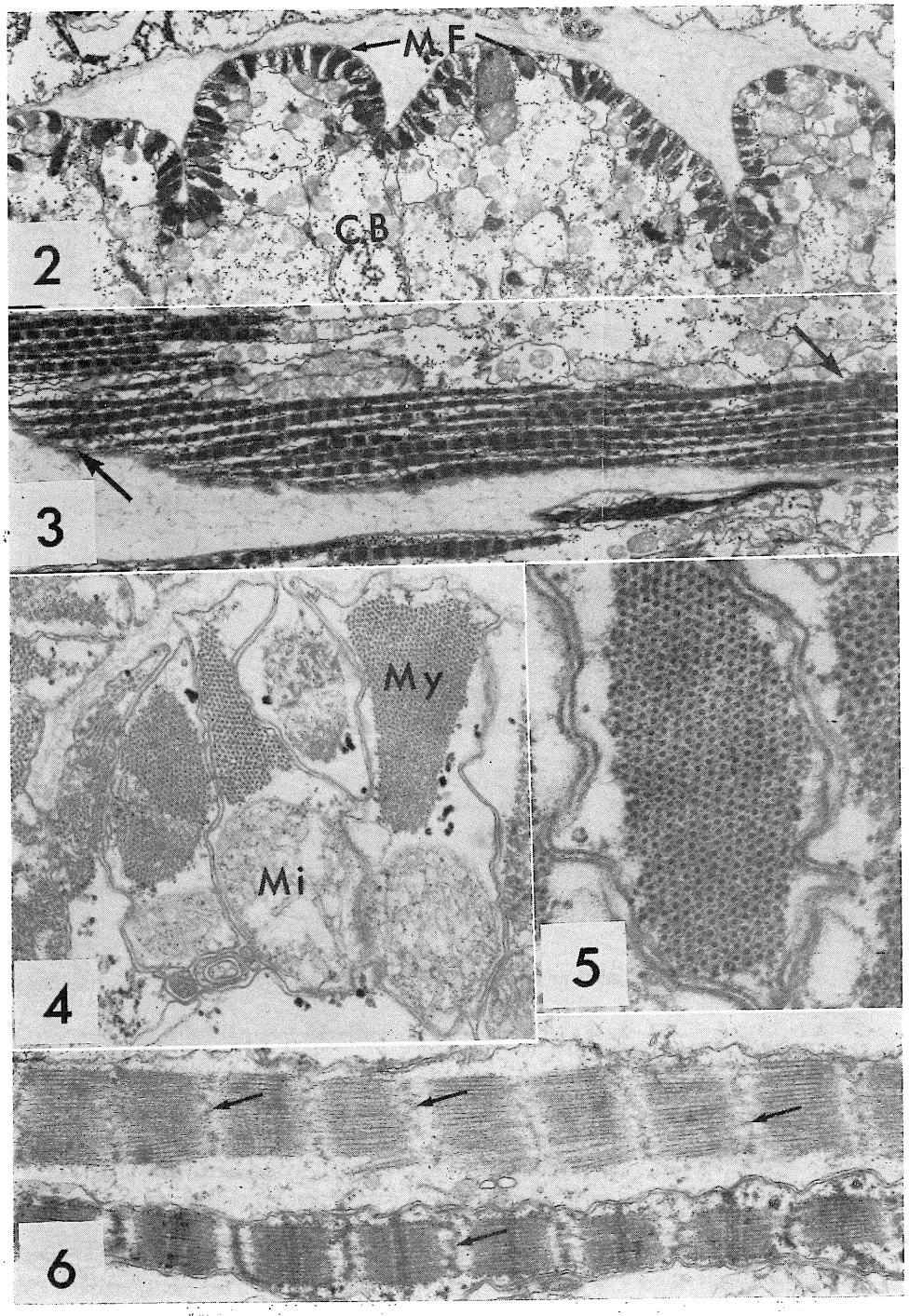
Fig. 2. A cross section of a part of a circular muscle. 40–50 muscle cells gather in a unit, and array in a wavy shape. Muscle cells have large cell bodies (C. B) and slender muscle fibers (M.F). $\times$2,500.

Fig. 3. A longitudinal section of a part of a circular muscle. A myofibril indicated by two arrows bears 46 sarcomeres. $\times$2,800.

Fig. 4. A cross section of muscle fibers. Mitochondria (Mi) arrange near the myofibril (My). $\times$22,000.

Fig. 5. An enlarged view of a myofibril. Thick myofilaments array in a regular lattice of about 30 nm intervals. They show tubular structures. Thin ones arrange hexagonally around a thick one. Sizes of the two kinds of myofilaments are about 13 nm and 6 nm in diameter respectively. $\times$50,000.

Fig. 6. A longitudinal section of myofibrils. Myofibrils bear A and I bands, M-lines and Z-disks. Z-disks appear as accumulations of J-granules (arrows). $\times$18,000.


M.F
C.B
2
3
My
Mi
4
5
6

muscle cells in an *Aurelia* medusa to compare with those of an ephyra in an early stage.

Acknowledgments

The authors wish to express their sincere thanks to Prof. S. Kawaguti of Kawasaki Paramedical College for his critical and kind advices. We are indebted to Prof. M. Yoshida of Ushimado Marine Laboratory of Okayama University for his kindness offering materials of this work. We also thank to Prof. T. Iga of Shimane University for his critical reading of this manuscript.

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Fig. 7. A cross section of a radial muscle. About 55 muscle cells are recognized in the muscle. ×2,000.

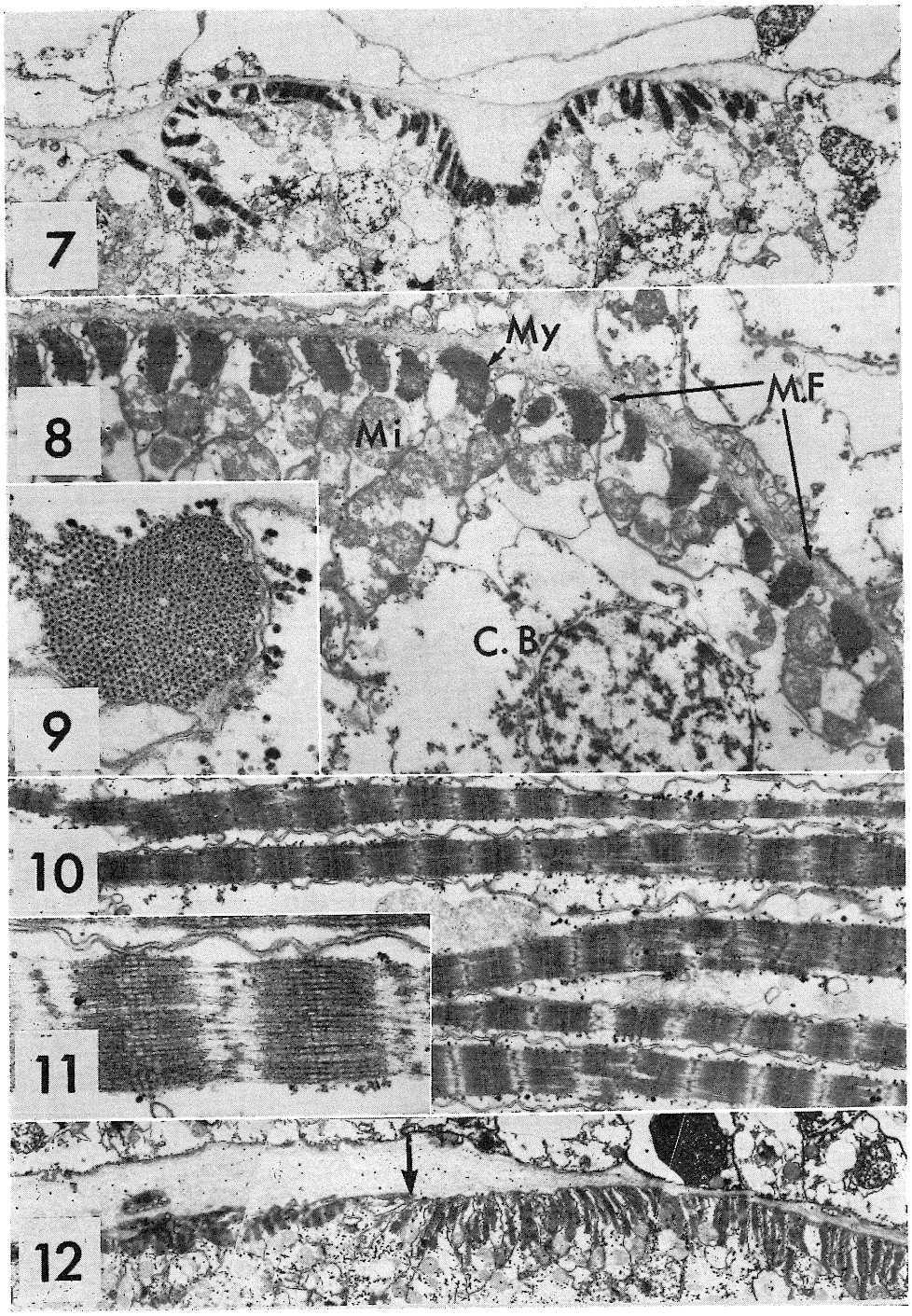
Fig. 8. A cross section of a part of a radial muscle. A muscle cell consists of a large cell body (C.B) and a slender muscle fiber (M.F) or fibers. Arrows indicate these two muscle fibers. Mitochondria (Mi) are recognized near a myofibril (My). ×6,600.

Fig. 9. An enlarged view of a myofibril of a radial muscle cell. Thick and thin myofilaments arrange in a similar pattern as shown in Fig. 5. ×33,000.

Fig. 10. A longitudinal section of myofibrils. They show regular cross striations. ×10,000.

Fig. 11. An enlarged view of a part of a myofibril. Z-disks appear as accumulations of J-granules. ×28,000.

Fig. 12. A cross section of a circular muscle. A right area is a cross section of a circular muscle, and a left one shows a longitudinal section of a radial muscle. An arrow indicateds a point where the radial muscle branches off from the circular muscle. ×2,200.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8
My
M.F
Mi
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