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ULTRA-HIGH RESOLUTION SCANNING ELECTRON MICROSCOPIC STUDIES ON THE SARCOPLASMIC RETICULUM AND MITOCHONDRIA IN VARIOUS MUSCLES: A REVIEW

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Abstract

The three-dimensional structure of the sarcoplasmic reticulum (SR), transverse (T)-axial tubular system and mitochondria in various muscles was examined by means of ultra-high resolution scanning electron microscopy (SEM) after removal of the cytoplasmic matrices and myofilaments by the aldehyde-osmium-DMSO-osmium procedure. The striated muscles reviewed and presented are twitch and slow fibers of the frog, twitch and slow fibers of the chicken, twitch extrafusal fibers and intrafusal fibers of the rat, and cardiac muscle fibers of the rat and dog. In all of these striated muscle fibers, T-tubules run transversely and are coupled with terminal cisternae forming triads or dyads. Sarcotubules arising from the terminal cisterna form meshes around the myofibrils. Considerable variations are seen in the location of the T-tubules, the structure of the terminal cisternae, the SR and the mitochondria among these muscles. The changes of these organelles in the experimental pathological conditions, (i.e., experimental mitochondrial myopathy and hypertrophic myocardium of spontaneously hypertensive rats) are also presented. In addition the SR and mitochondria in the smooth muscles of the rodents are described and discussed.

Key Words: T-tubule, sarcoplasmic reticulum, mitochondria, muscle, muscle fiber, scanning electron microscopy

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Introduction

The three-dimensional models of the membrane systems in muscle fibers, specifically the sarcoplasmic reticulum (SR) and transverse (T)-axial tubular system, and mitochondria have been proposed by some investigators based on transmission electron microscope (TEM) observations of thin, sometimes serial, sections (Porter and Palade, 1957; Peachey, 1965). The three-dimensional organization of the membrane systems in muscle fibers has been examined under a high voltage electron microscope after impregnation with metal (Segretain et al., 1981; Forbes and Van Niel, 1988). However, the three-dimensional architecture of these systems is not readily appreciated in two-dimensional sections. Recent developments in specimen preparation techniques for scanning electron microscopy (SEM), in particular, the osmium-dimethyl sulfoxide-osmium (ODO) method (Tanaka and Naguro, 1981) and an improved variation, the aldehyde-osmium-dimethyl sulfoxide-osmium (A-ODO) method (Tanaka and Mitsushima, 1984) have proved to be effective for observing the intracellular structures of muscle fibers at high magnification. Application of this method to the various muscle tissues has provided the direct visualization of the membrane systems. In addition, the recent introduction of an ultra-high resolution SEM with a resolving power of 0.7 nm enabled us to visualize the minute structure of these membrane systems. The purpose of this review is to present the three-dimensional ultrastructural features of the membrane systems in various normal muscle tissues exposed by the A-ODO method and observed under an ultra-high resolution SEM. In addition, the SEM findings on a few experimental pathological conditions are briefly reviewed.

Methods

For observation of the membrane systems in muscle fibers, we used a slightly modified version of the original A-ODO method (Tanaka and Mitsushima, 1984). In short, the muscles were fixed by perfusion with 0.5% paraformaldehyde + 1.0% glutaraldehyde solution in 0.067 M cacodylate buffer, pH 7.4, for 15 min. The muscles are then cut into small pieces and fixed further for

15-45 min in the same fixative (Ogata and Yamasaki, 1987). After rinsing with a buffer solution, the specimens are successively immersed in 15, 30, and 50% dimethyl sulfoxide (DMSO) for 30 min each. The specimens are frozen on a metal plate chilled with liquid nitrogen. They are cracked with a single-edged razor blade by striking them with a hammer on a freeze-cracking apparatus, Eiko TF-1. The cracked pieces are immediately placed in 50% DMSO at 20°C. After thawing, the specimens are rinsed in the buffered solution until the DMSO has been completely removed. Then they are postfixed with 1% OsO_4 in 0.067 M cacodylate buffer (pH 7.4) for 1 hour, and left standing in 0.1% OsO_4 in the same buffer at 20°C for 72-96 hours to remove the cytoplasmic matrix (Tanaka and Mitsushima, 1984). Tissue specimens prepared in this way are impregnated with osmium by a conductive staining method (Murakami, 1973): they are immersed for 1 hour in 1% OsO_4 in 0.067 M cacodylate buffer (pH 7.4), for 4 hours in 2% tannic acid in distilled water, for 1 hour in 1% OsO_4 in the same buffer, and washed well between each step with the same buffer. Afterwards they are dehydrated in ethanol and dried in a Hitachi HCP-1 critical-point dryer. The dried specimens are again impregnated with osmium by mounting them on a specimen stub and placing them in an Erlenmeyer flask together with a pitted ampule containing OsO_4 crystals, and exposing them to the vapor for 10-20 minutes (Kubotsu and Ueda, 1980). They are left in air for 10-80 minutes to remove excess osmium vapor, then placed in a vial in a dish containing hydrazine hydrate for 5-10 minutes, and again left in air to remove excess hydrazine vapor. This cycle is repeated until sufficient conductivity is obtained. The specimens are observed with an ultra-high resolution, field-emission type SEM, the Hitachi S-900.

Results

The A-ODO method used in the present study proved very useful for disclosing the architecture of the membrane systems in the muscle fiber. The results indicate that myofilaments and cytoplasmic matrices are effectively removed by maceration in the dilute OsO_4 solution, and that the membrane structures can be exposed without introducing significant artifacts.

Skeletal Muscle

Most skeletal muscles are composed of a mixture of different muscle fiber types. Numerous nomenclatures are used to classify the skeletal muscle fiber type. In order to avoid confusion, the nomenclature of skeletal muscle fiber types is briefly described.

Nomenclature of skeletal muscle fiber type

Vertebrate skeletal muscle fibers are classified into two major groups: twitch (fast) and slow (tonic) muscle fibers. Morphologically, the former has straight Z-lines and well-developed T-tubular and SR systems and is singly innervated. The latter has zigzag Z-lines, a less-well developed T-SR system and is multiply innervated.

Numerous nomenclatures have been used for the different twitch fiber types and there is no universal nomenclature. In this review the terms red (mitochondria-rich), intermediate (mitochondria-moderate), and white (mitochondria-poor) fibers are used, because these terms are widely used and are more suitable for SEM studies, since the discrimination of fiber types under SEM is mainly based on the differences in the number and structure of mitochondria. In addition, nomenclatures based on physiological features the slow-twitch oxidative fiber (SO), fast-twitch oxidative glycolytic fiber (FOG), and fast-twitch glycolytic fiber (FG) or based on the histochemical staining pattern of ATPase - type I, IIA and IIB, are used for the classification of mammalian twitch fibers. However, these are not always applicable for the classification of the muscle fiber types in all vertebrate classes (Ogata, 1988).

Independent from the classification of the twitch and the slow fibers, the vertebrate skeletal muscle fibers are classified into two groups: the "Z-fiber" and "A-I fiber", according to the location of triads. In the Z-fiber one sarcomere has one triad, but in the A-I fiber one sarcomere has two triads. For a detailed discussion on the nomenclature of skeletal muscle fiber types see Ogata's review (1988).

Amphibian muscle

Amphibian muscle fibers are classified into two major groups; twitch and slow fibers (Ogata, 1988).

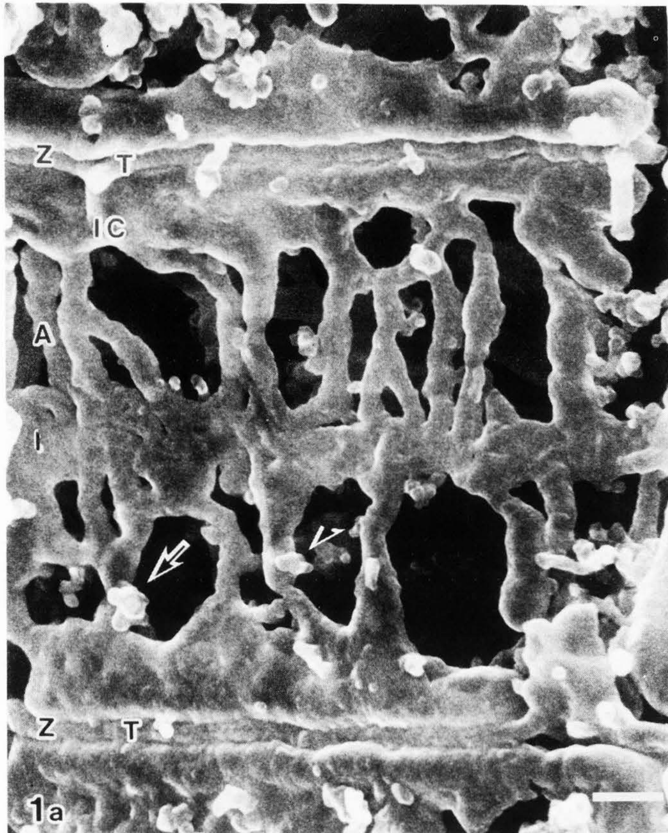
Figure legends

Abbreviations used in Figure 1-8

A: A-band level, AT: axial tubule, CS: corbular SR, H: H-band level, I: I-band level, IC: intermediate cisterna, M: mitochondria, T: T-tubule, Z: Z-line level, *: cisternal SR

Fig. 1. SEM pictures of the frog twitch white fiber. a. The T-tubule runs at the Z-line level and is sandwiched between thick terminal cisternae and forms a triad. Terminal cisternae transform into thin, flat intermediate cisternae, from which numerous sarcotubules extend to form meshes in front of the A-band and then continue to the H-band (or fenestrated) collar. Arrows show ribosomes. Bar=0.2 μm b. Higher magnification of a triad. Note a row of indentations (arrowheads) arranged along the border zone between terminal cisternae and intermediate cisternae. Bar=0.1μm

Fig. 2. The slow fiber of the frog. a. T-tubules run at each Z-line level. At intervals of 0.4 to 1 μm, small terminal cisternae (arrows) are coupled with the T-tubule and form the triads or the dyads. From the terminal cisternae, a few sarcotubules extend and form meshes in front of the A-band and then continue to the poorly developed H-band collar. Bar=0.2μm b. Higher magnification. Arrows show triads. Bar=0.1 μm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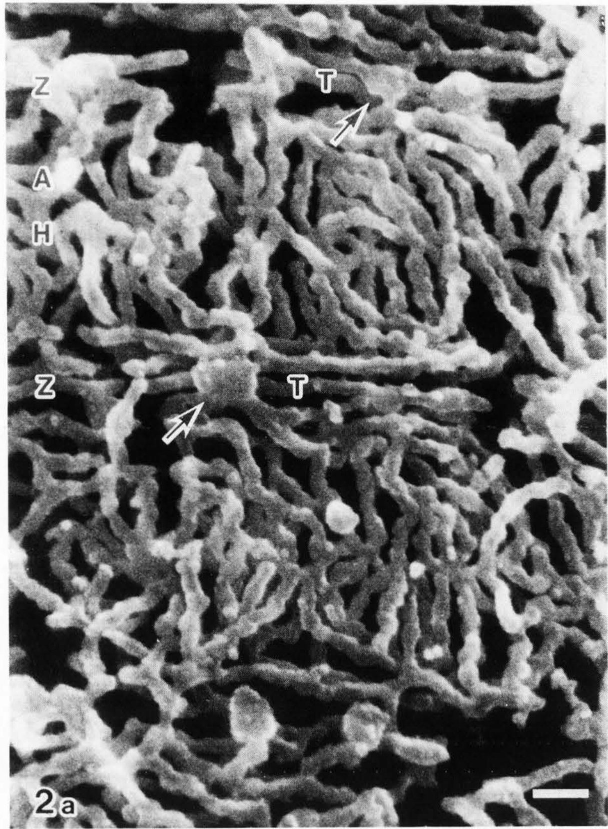
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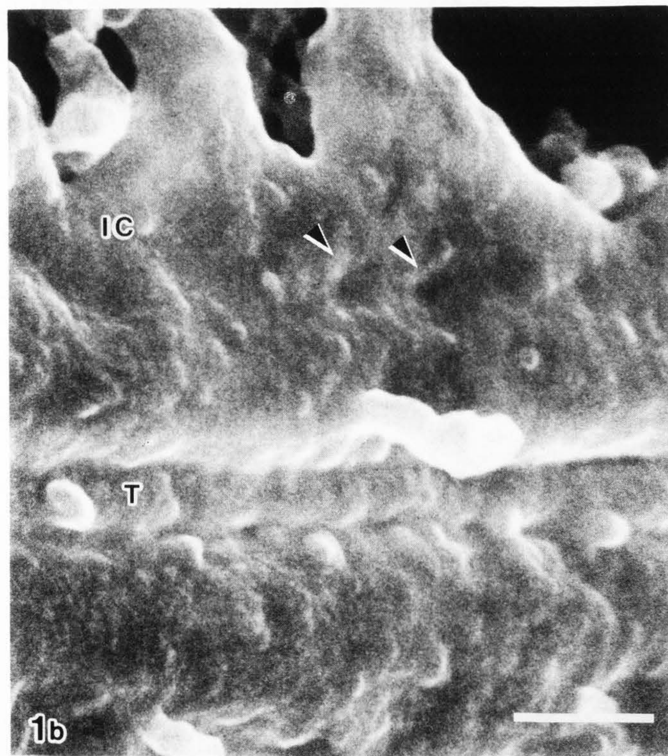
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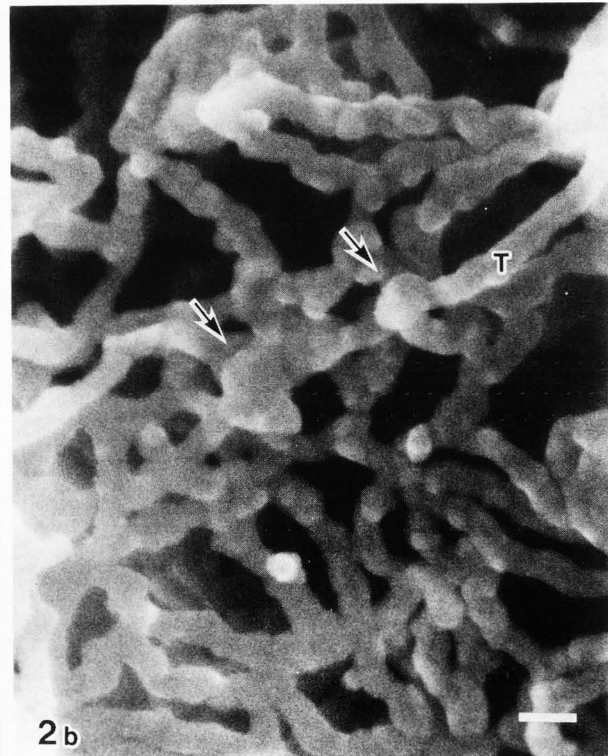
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Twitch fiber

The frog twitch muscle fibers are classified into three types, the red, white and intermediate fibers (Ogata, 1958; Ogata and Mori, 1964). In TEM micrographs, the red fiber has many large mitochondria and wide Z-lines. The white fiber has fewer and smaller mitochondria and thinner Z-lines, and the intermediate fiber has mitochondria and Z-lines intermediate between these two types (Ogata and Yamasaki, 1987; Ogata, 1988).

In the SEM micrographs, the small red fibers have numerous large diameter mitochondrial columns, while the large white fibers have fewer mitochondrial columns, smaller in diameter. In the medium-size intermediate fibers, the number and diameter of the mitochondrial columns are intermediate between those of the red and white fibers (Ogata and Yamasaki, 1987; Ogata, 1988).

In all three types of twitch fibers, slender T-tubules run at the level of each Z-line and are sandwiched between broad terminal cisternae forming triads (Fig. 1a). The terminal cisternae continue into thin, flat intermediate cisternae. Along the transitional area between them a row of tiny indentations is observed (Fig. 1b). The indentations (25-35 nm diameter) appear as cone-shape depressions of the membrane. Numerous slender sarcotubules extend from the intermediate cisternae and form oval networks in front of the A-band, continuing to the H-band (fenestrated) collar. Tiny fenestrations about 15-25nm in diameter and hollows about 10 nm in diameter are sporadically seen in the H-band collar. The large flat SR, known as the cisternal SR, is occasionally intercalated among the SR meshes. Ribosomes are occasionally attached on the surface of the SR. The three-dimensional structure of the SR is basically the same in all three fiber types. However, the SR is scarce on the surface of mitochondria, hence the red fiber appears to have a smaller total volume of SR than the white fiber. Moreover, the volume of SR of the intermediate fiber lies between the two.

Slow fiber

In the TEM micrographs, the Z-lines of slow fibers are much wider than those in twitch fibers and are usually jagged. The M-line is lacking or indistinct. There is one T-system per sarcomere at the Z-line level. Terminal cisternae form small triads or dyads. The fenestrated collar is less developed than that of the twitch fibers (Ogata, 1988).

In the SEM micrographs, slender T-tubules run at each Z-line level (Fig. 2a). At intervals of about 0.4 to 1.0 μm, small terminal cisternae couple with the T-tubule and form spherical or ovoid triads or dyads (Fig. 2b), on whose surface tiny indentations are occasionally seen. Each terminal cisterna gives rise to a few sarcotubules that run in various directions, divide frequently and form circular or oval meshes of diverse size in front of the A- and I-bands. The sarcotubules usually form small meshes in the middle of the A-band, but occasionally fuse and form a poorly developed H-band collar (Ogata and Yamasaki, 1989).

Avian muscle

Avian muscles are composed of two main types of muscle fibers: twitch fibers and slow fibers (Ogata, 1988).

Twitch fiber

Avian twitch fibers are classified into three types, the red, white and intermediate fiber (Ogata and Mori, 1964). In the TEM micrographs, the red fiber has thick Z-lines and numerous large mitochondria, the white fiber has thin Z-lines and few small mitochondria, and the intermediate fiber has intermediate Z-lines and mitochondria (Ogata, 1988).

In the SEM preparations of the chicken gastrocnemius muscle, the small red fibers have numerous large subsarcolemmal and column-forming mitochondria, while the large white fibers have fewer and smaller mitochondria. In the medium size intermediate fibers, the number and diameter of those mitochondria are intermediate between those of the red and white fibers. The I-band limited mitochondria and I-band branches of the column-forming mitochondria seen in mammalian muscle fibers are absent in the avian muscle.

T-tubules run at each A-I junction level and are coupled with small to medium size terminal cisternae forming triads at some intervals. Small cone-shaped indentations about 30 nm in diameter are sporadically seen on the surface of the terminal cisterna. The sarcotubules arising from the terminal cisterna form single-layered networks at the A-band level, and multi-layered networks at the I-band level.

Slow fiber

The chicken anterior latissimus dorsi muscle exclusively consists of two types of slow fibers. In TEM micrographs, slow fibers are classified into two types; "thin Z-line slow fiber" and "thick Z-line slow fiber" (Ogata, 1988). The thin Z-line slow fiber has more numerous and larger subsarcolemmal and column-forming mitochondria than the thick Z-line slow fiber. M-lines are distinct in the thin Z-line slow fiber, whereas they are ill-defined in the thick Z-line slow fiber. The basic structure of the SR is the same in both types of fibers, but the SR is more prominent in the thin Z-line slow fiber than in the thick Z-line slow fiber. Both of them have triads at the A-I junction.

In SEM micrographs, the column-forming mitochondria are longitudinally arranged in the intermyofibrillar space (Fig. 3a). The size of these mitochondria varies from place to place even within the same fiber. Therefore, it is still difficult to clearly distinguish the thick Z-line slow fiber from the thin Z-line slow fiber under SEM (Ogata and Yamasaki, 1990a).

The T-tubule runs transversely at the A-I junction level (Fig. 3b). It frequently divides, runs longitudinally and transforms into the axial tubules. Small spherical or ovoid terminal cisternae couple laterally with the T-tubule or the axial tubule at intervals of 0.4-1.0 μm forming a triad or a dyad. Sarcotubules arising from the terminal cisternae form circular meshes of about 50 nm in diameter at the A- and I-band level. The large flat cisternal SR occasionallyare intercalated among the SR meshes.

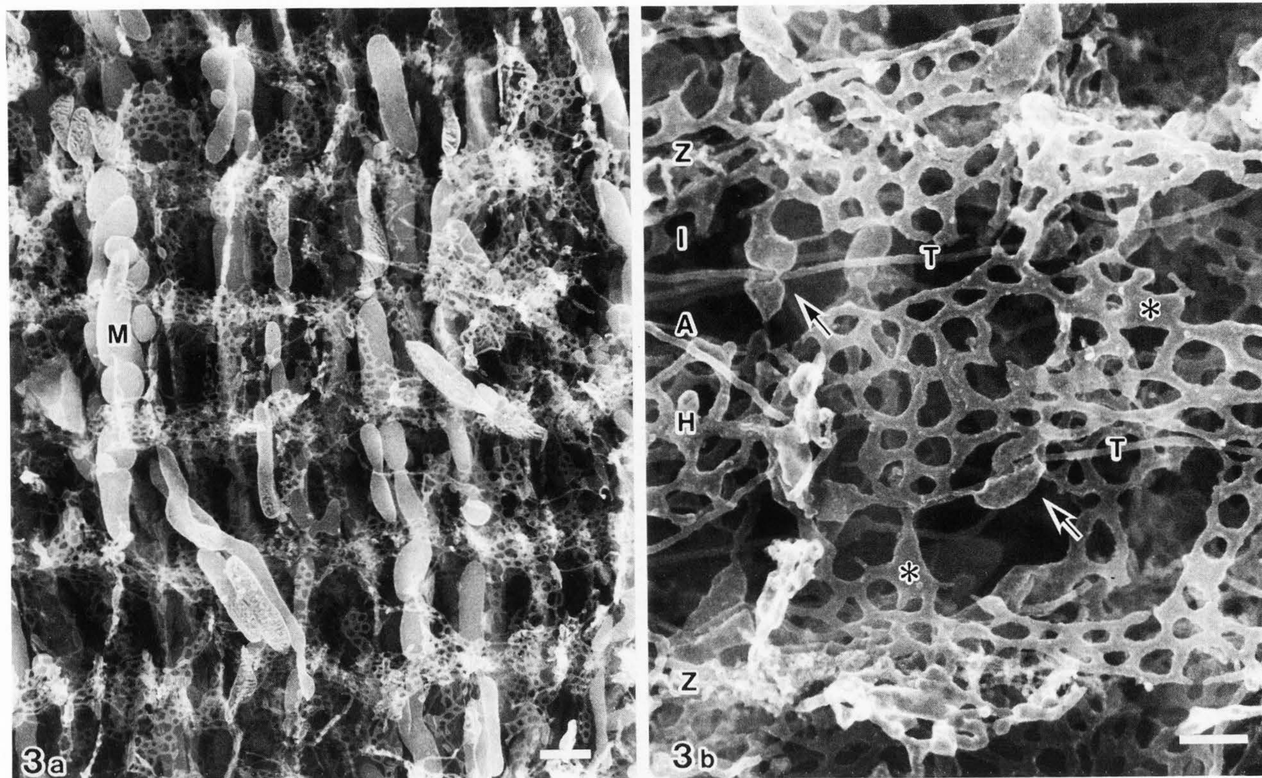

Fig. 3. SEM view of the chicken slow fiber. **a.** Column-forming mitochondria longitudinally arranged in the intermyofibrillar space. The SR appears as lace-like networks. Bar=1μm **b.** Higher magnification. T-tubules transversely run at the A-I junction level and are coupled with small terminal cisternae (arrows) at some intervals forming triads or dyads. The sarcotubules arising from the terminal cisterna form round meshes at the A-and I-band level. Bar=0.3μm

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Mammalian muscle

Extrafusal muscle fiber

Almost all mammalian muscles are composed of exclusively twitch fibers. The slow fibers are only see in the extraocular and middle ear muscles and a few other muscles in mammals. Twitch fibers are classified into three types; red, white and intermediate muscle fibers (Ogata, 1958; 1988; Padykula and Gauthier, 1967). In TEM micrographs, the red fibers have numerous large mitochondria and thick Z-lines while the white fibers have a few smaller mitochondria and thin Z-lines. In the intermediate fibers the number and size of mitochondria and the thickness of Z-lines lie between those of the red and white fibers (Padykula and Gauthier, 1967; Ogata, 1988).

Using the ODO method, the three-dimensional structure of the SR, triad and mitochondria in the rat skeletal muscle was first reported by Ohmori (1984). However, this study did not mention the structural differences of the membrane systems in each fiber type. The structural differences of mitochondria in each fiber type were clearly demonstrated by SEM (Fig. 4) (Ogata and Yamasaki, 1985a, b; Ogata, 1988). In the subsarcolemmal spaces, the ovoid or irregular-shaped subsarcolemmal mitochondria are accumulated. The subsarcolemmal mitochondria are numerous and large in the red fiber, intermediate in the intermediate fiber, and smaller and fewer in number in the white fiber. In the intermyofibrillar spaces, two types of mitochondria are seen (Fig. 4). One is the paired slender mitochondria which are located on both sides of the Z-line and partly embrace the myofibrils at the I-band level. They are located within the I-band level only and neither extend to the Z-line level nor to the A-band level. Hence, they were named "I-band limited mitochondria" (Ogata and Yamasaki, 1985a). The I-band limited mitochondria occur in all three types of fibers. The second type of mitochondria, column-forming mitochondria, arrange in a longitudinal direction and form mitochondrial columns. In the rat muscle, these columns can be classified into two types: thin and thick mitochondrial columns (Ogata and Yamasaki, 1985a). The thin mitochondrial column is relatively small in size and is formed by a succession of columnar-shaped mitochondria. Usually, a single mitochondrion occupies the intermyofibrillar space corresponding to one sarcomere in length, namely, between two Z-lines. However, occasionally a very long mitochondrion extends several sarcomeres in length. Slender

arm-like branches transversely extend from both extremities and partly embrace one or more myofibrils at the I-band level. This type of branch is named the "I-band branch of the column-forming mitochondria (Ogata and Yamasaki, 1985a). The thick mitochondrial column has a larger diameter and is formed by multiple mitochondria, each covering with an intermyofibrillar space corresponding to one sarcomere in length. Usually slender I-band branches extend transversely from these mitochondria, but they are lacking in some columns. In the red fiber, columns are abundant and the ratio of the thick and thin columns appears to be the same (Fig. 4a), while in the intermediate fiber most of the columns belong to the thin type (Fig. 4b). The white fiber displays rare, very thin columns (Ogata and Yamasaki, 1985a).

In all three types of fibers, the T-tubule runs at the A-I junction level and is sandwiched between large terminal cisternae along its entire length forming a triad (Figs. 4 and 8). On the surface of the terminal cisterna, indentations (25-35 nm diameter) occasionally appear as cone-shaped depressions of the membrane (Fig. 4d). The intermediate cisternae, which are seen between the terminal cisternae and sarcotubules in the frog are obscure in the mammals. Numerous slender sarcotubules, originating from the A-band side terminal cisternae, form oval- or irregular-shaped meshes of various sizes in front of the A-band. Occasionally, a large plate-like cisternal SR is intercalated among the SR networks. The A-band sarcotubules continue into tiny meshes of sarcotubules (H-band collar or fenestrated collar) in front of the H-band. The A-band SR appears as a single sheet of anastomotic tubules (Figs. 4 and 8). Numerous sarcotubules originating from the I-band side terminal cisternae extend in all directions and form multi-layered three-dimensional networks over the I-band and Z-line regions (Figs. 4 and 8). Near the I-band side terminal cisternae a relatively large-sized network is seen, whereas only a tiny mesh is located over the Z-line region. The size of the mesh is almost the same as that of the H-band mesh. At the I-band level paired and transversely oriented mitochondria partly embrace the myofibrils. The SR network is poorly developed on the surface of these mitochondria, though it is well developed in places devoid of these mitochondria. Yet the SR network is always present in the narrow space between paired I-band limited mitochondria, or between paired I-band branches of column-forming mitochondria. In SEM micrographs, the three-dimensional structure of the SR is basically the same in all three fiber types. However, the SR is sparse on the surface of mitochondria, so the mitochondria-rich red fiber appears to have a smaller total volume of SR than the mitochondria-poor white fiber. Moreover, the volume of the SR of the intermediate fiber appears intermediate between the two (Ogata and Yamasaki, 1985b). However, further stereological studies are needed to clarify the accurate difference of the SR volumes in the three fiber types.

Intrafusal muscle fiber

The intrafusal fibers of the mammalian muscle spindle are classified into three types; the nuclear chain fiber (chain fiber), the nuclear bag_1 fiber (bag_1 fiber) and the nuclear bag_2 fiber (bag_2 fiber). In TEM micrographs, the chain fiber has thin Z-lines and numerous mitochondria. The bag_1 fiber has thick Z-lines with a few smaller mitochondria and the bag_2 fiber has thin Z-lines and intermediate size mitochondria. From the SEM observations, in the intracapsular sleeve region of the rat muscle spindle, chain fibers are seen to have thick to moderate size column-forming mitochondria occasionally having I-band branches (Ogata and Yamasaki, 1991). The T-tubules run at the level of the A-band side of the A-I junction and are sandwiched between two large terminal cisternae, forming triads (Fig. 5a). The sarcotubules arising from the terminal cisternae form single-layer networks at the A-band level, and well developed, multi-layered, three-dimensional networks at the I-band level. Bag_2 fibers have thick to medium diameter column-forming mitochondria without I-band branches (Fig. 5b). The T-tubules run at the level of A-band adjacent to the A-I junction and are coupled with terminal cisternae of various lengths at some intervals. As a rule, the SR forms single-layered networks at the level the A-band and multi-layered networks at the I-band level. Swollen terminal cisternae and broad cisternal-like or thick cylinder-shape cisternal SR are frequently seen in bag_2 fiber in the extracapsular region (Ogata and Yamasaki, 1992).
However, these two structures are rare in bag_2 fibers in the sleeve region (Ogata and Yamasaki, 1991). Bag_1 fibers have slender column-forming mitochondria devoid of I-band branches. The T-tubules are located at the level of the A-I junction (Fig. 5c). Both the T- and the axial tubules are occasionally coupled with small terminal cisternae, forming dyads or triads. At the I-band level, the SR is well developed and

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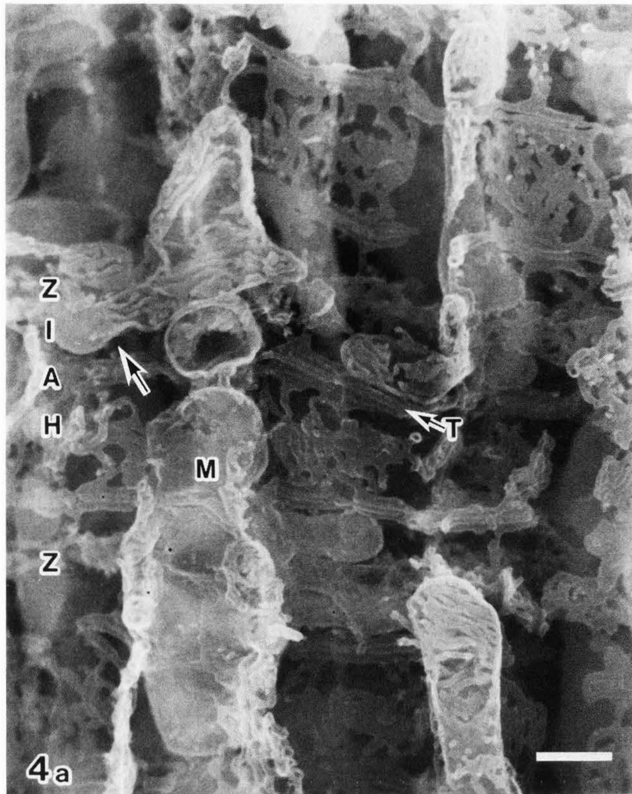
Fig. 4. Twitch fibers of the rat extensor digitorum longus muscle. a. Red fiber. Large column-forming mitochondria are arranged in the intermyofibrillar space. The slender I-band branch (arrow) transversely extends from the column-forming mitochondria and partly embraces the myofibrils at the I-band level. T-tubules run at the A-I junction level and are sandwiched between two large terminal cisterna. The sarcotubules form single sheet networks in front of the A-band, and three-dimensional multi-layered networks over the I-band region. Bar=0.5μm b. Intermediate fiber. A thin column-forming mitochondrion with I-band branches (arrow). Bar=0.5μm c. White fiber. Note only I-band limited mitochondria are seen. Bar=0.5μm d. Higher magnification of the white fiber. Cone shaped indentations (arrowheads) are seen on the surface of the terminal cisternae. Note that sarcotubules form single layer networks at the A-band level, while they form double layer three-dimensional networks at the I-band level. Bar=0.2μm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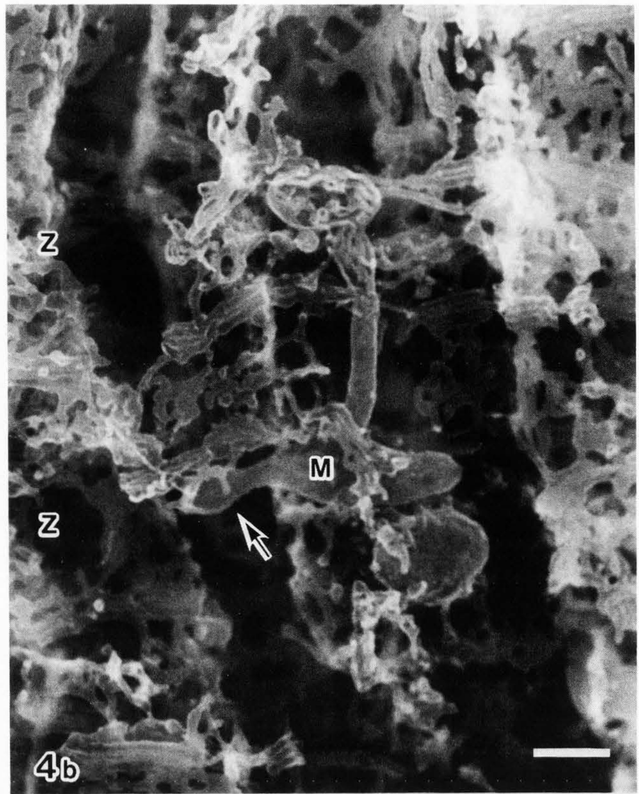
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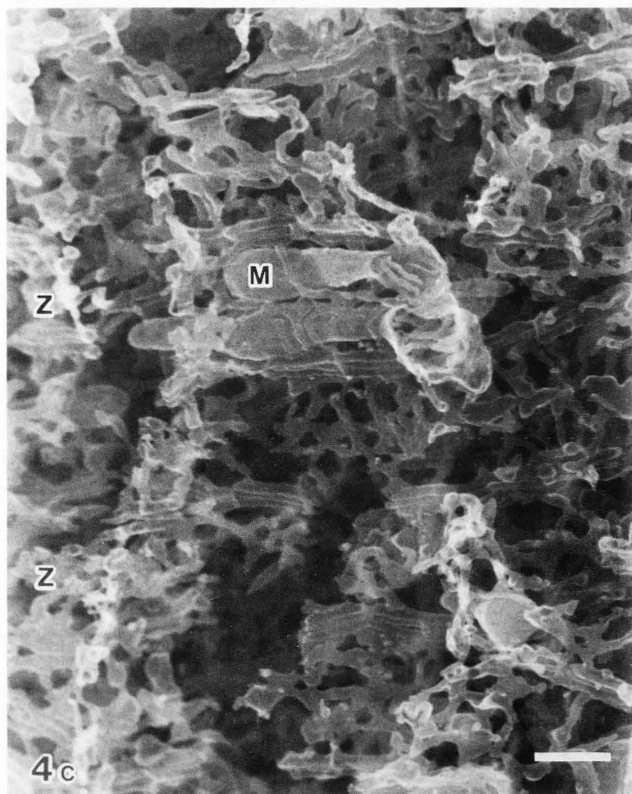
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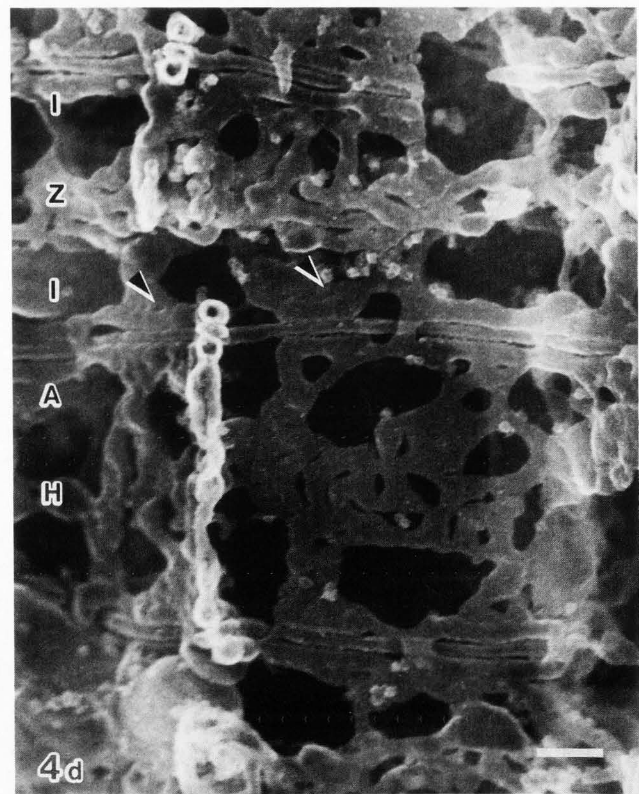
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single-layer networks are formed. However, at the A-band level only a few longitudinally arranged sarcotubules and axial tubules are observed (Ogata and Yamasaki, 1991).

Cardiac Muscle

The three-dimensional structure of mitochondria and the SR of the rat cardiac muscle treated by the ODO method was first reported by Ohmori (1984). He mentioned that the triads were difficult to identify in the cardiac muscle. In the dog, the three-dimensional structure of the T-tubule, triads, the SR, mitochondria and intercalated disc were observed by SEM (Yoshikane et al., 1986). However, in these studies, the observations were made on the metal coated specimens by conventional field emission type SEM, and the minute structures of membrane systems were overlooked. Precise description of the three-dimensional structure of the membrane system of the rat's left ventricular muscle only became possible when osmium impregnated specimens without metal coating were observed by ultra-high resolution SEM (Ogata and Yamasaki, 1990b). In the intermyofibrillar space, numerous spherical or ovoid mitochondria are accumulated (Fig. 6). The T-tubules are accompanied by longitudinally oriented axial tubules and together form a transverse-axial system. The terminal cisterna (or junctional SR) is usually small but occasionally medium or large in size and couples with the T- or axial tubules. On the surface of the terminal cisterna facing the T- or axial tubules, tiny junctional processes are seen. One or two sarcotubules, the so-called Z-tubules, frequently run parallel to the T-tubule (Ogata and Yamasaki, 1990b). The sarcotubules derived from the junctional SR or from the Z-tubule run longitudinally or obliquely and form polygonal meshes around the myofibrils. On the surface of the SR at the H-band level, small fenestrations of 12-40 nm in diameter, and tiny hollows 8-20 nm in diameter are seen. Bulbous swellings of the SR, the corbular SR, are preferentially seen near the Z-band. Large, flat cisternal SR is occasionally intercalated among the SR meshes. In the subsarcolemmal space, the sarcotubules form a multilayered network (peripheral SR). The cisternal SR is frequently intercalated in these meshes and closely associate with the inner surface of the sarcolemma (Ogata and Yamasaki, 1990b).

The intercalated disc appears as a prominently undulated membrane demarcating the border between two adjacent heart muscle cells, and occasionally small projections 60-90 nm in diameter and 200-600 nm in length are displayed on its surface (Ogata and Yamasaki, 1990b).

Smooth Muscle

A few articles have been published on the intracellular structures of smooth muscle cells by SEM. Under SEM, Sawada (1981) first observed the SR and surface caveolae in the guinea pig taenia coli by SEM. His results, however, were unsatisfactory because the SR was embedded within myofilaments and cytoplasmic matrix, and because he used specimens torn after critical point drying. Using the A-ODO method, the SR and mitochondria were clearly demonstrated in smooth muscle cells of the mouse small intestine (Inoué and Osatake, 1986; Inoué, 1990). Inoué (1990) classified the SR of smooth muscle cells into three types: peripheral, perinuclear and connecting. The peripheral SR is found underneath the plasma membrane, spreading in close relationship with the caveolae. From their morphology, the three types of the SR are subclassified into: the tubular, the fenestrated and the netty SR. The perinuclear SR is associated with the accumulation of mitochondria at both poles of the nucleus. It is tubular in shape and forms complex three-dimensional networks. The connecting SR links the peripheral SR with the perinuclear one, corresponding to the intermediate SR described by Popescu and de Bruijn (1982). Similar results are observed in the SR of the smooth muscle cells in the rat stomach (Fig. 7). Mitochondria of smooth muscle cells are mainly situated at the perinuclear region or beneath the plasma membrane. Inoué (1990) classified them into two types, peripheral and perinuclear. Peripheral mitochondria show elongated forms and arrange parallel to

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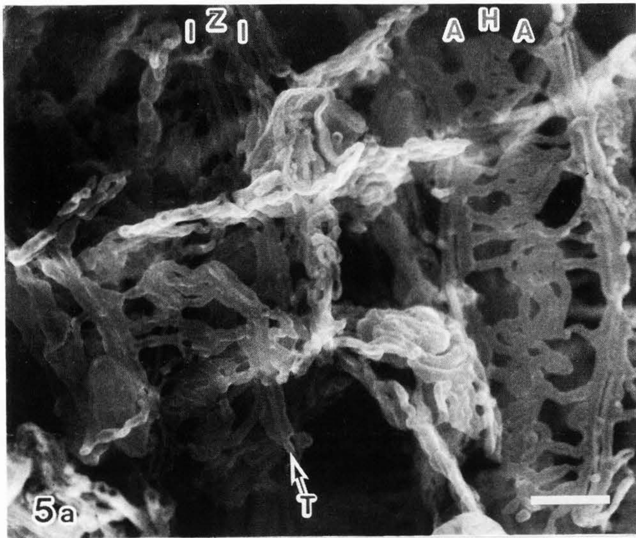
Fig. 5. Intrafusal fibers in the intracapsular sleeve region of the rat muscle spindle. **a.** The chain fiber. The SR forms one-layer networks at the A-band level (arrows) and two layered three-dimensional networks at the I-band level (arrowheads). Note that T-tubules are coupled with terminal cisternae along their entire length. Bar=0.5μm **b.** The bag$_2$ fiber. The T-tubule is coupled with short (arrowheads) to medium length terminal cisternae (arrow) at intervals. The sarcotubules on the A-band side form single sheet networks, while those on the I-band side are arranged three-dimensionally. Note that the sarcotubules arising from the short terminal cisternae form narrow sheets of meshes at intervals. Bar=0.5μm **c.** The bag$_1$ fiber. Single-layer SR networks at the I-band level form cylinder-like structures (arrow). At the A-band level, only a few sarcotubules and axial tubules run longitudinally (arrowheads). Small arrows show the triads (Ogata and Yamasaki, 1991, reproduced with permission from Archiv. Histol. Cytol.). Bar=0.5μm

Fig. 6. Rat left ventriculus. T-tubules run at the Z-band level. The axial tubule connects with a T-tubule at both ends. Note that the diameter of these tubules is larger than that of the sarcotubules. T-tubules are coupled with junctional SR forming a triad (arrows) or a dyad. Sarcotubules arising from the junctional SR form meshes around the myofibrils. Numerous large ovoid mitochondria arrange themselves in the intermyofibrillar spaces. (From Ogata and Yamasaki, 1989, reproduced with permission from Anatomical Record). Bar=0.5μm

Fig. 7. The peripheral SR of a rat stomach smooth muscle fiber with numerous caveolae (arrows). **a.** The tubular type SR. Bar=0.1μm **b.** The netty type SR. Bar=0.1μm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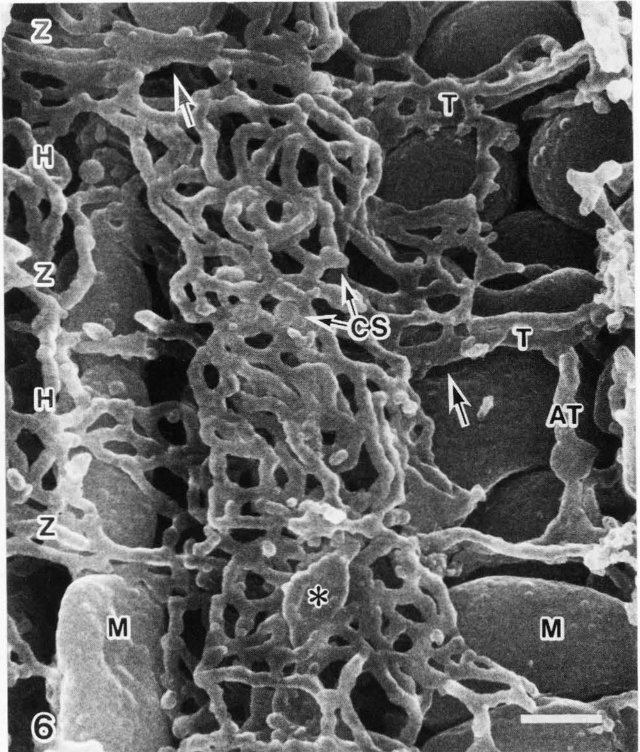
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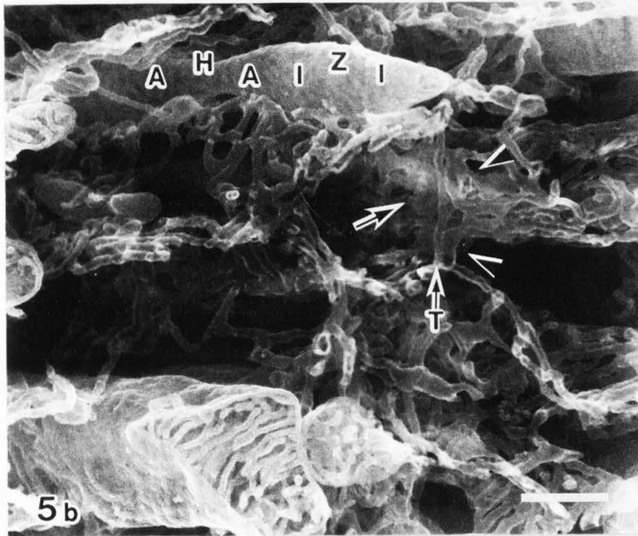
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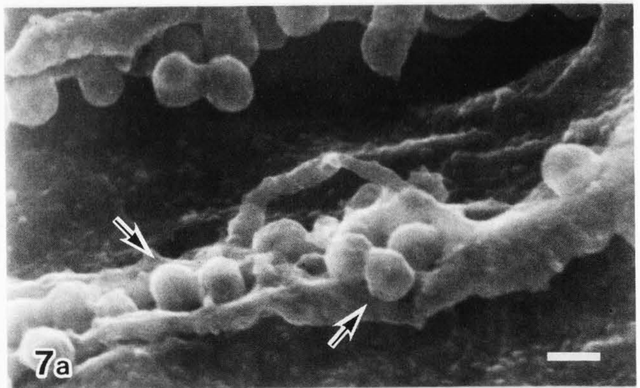
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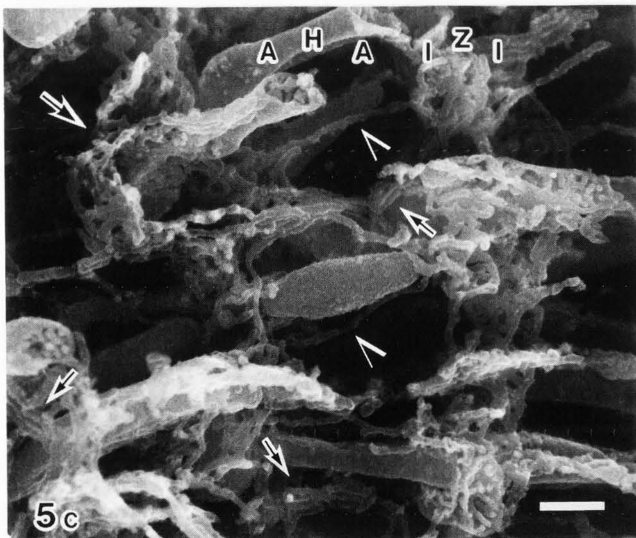
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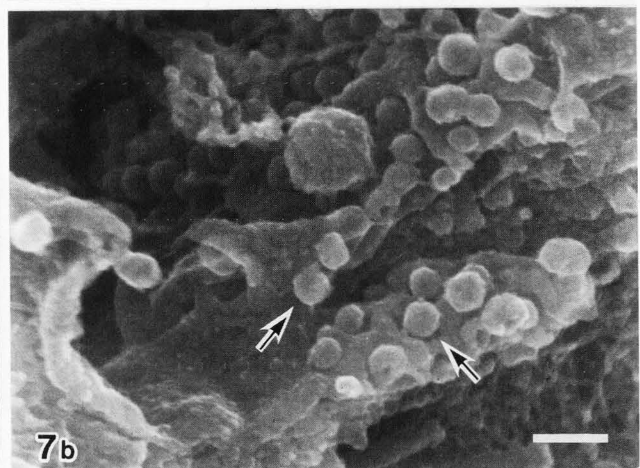
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the long axis of the cell. Most of mitochondria have a rod-like appearance without branching, but some are bifurcated or curved showing J- or H-shapes. Perinuclear mitochondria are accumulated in the juxtanuclear region. They are interwoven in a remarkably complex pattern.

Discussion

In all the striated muscles studied by SEM, the T-axial tubular system, the SR and mitochondria were clearly demonstrated. However, the structure of these membrane systems markedly differs with the muscles and within the different muscle fiber types. From the type of coupling of the terminal cisterna to the T-tubule, the striated muscle fibers are classified into two types, the continuously coupled type, in which the T-tubule is coupled with long terminal cisterna along its entire length, and the intermittently coupled type, in which the T-tubule is coupled with short to medium size terminal cisternae at some intervals. Twitch extrafusal fibers of the frog and the rat, and nuclear chain fibers of the rat belong to the former. Slow fiber of the frog, slow and twitch fibers of the chicken, cardiac muscle fibers of the rat, and the nuclear bag_1 and bag_2 fibers of the rat belong to the latter. From the physiological point of view, the former are rapidly contracting fibers, while the latter are slowly contracting fibers, except the chicken twitch fibers. However, the terminal cisternae of the chicken twitch fibers are larger and better developed than those of the chicken slow fibers. From the difference in the location of the T-tubule, the striated muscle fibers are classified into two types, Z-fibers and A-I fibers (Ogata, 1988). The frog twitch and slow fibers, and rat cardiac muscle fibers belong to the Z-fibers; while the chicken slow and twitch fibers, and rat extrafusal and intrafusal fibers belong to A-I fibers. The A-I fiber has two types of SR, the A-band side SR and the I-band side SR. The A-band side SR forms single layer networks, but the I-band side SR forms multi-layered three-dimensional networks. The functional differences between the Z-fiber and the A-I fiber are unknown.

The indentations are regarded as tiny concavities of the membrane of the terminal cisternae. A row of cone-shaped indentations have been revealed in the terminal cisternae of the guinea pig (Rayns et al., 1975), rat and frog (Dulhunty and Valois, 1983). These indentations were clearly demonstrated to be present on the surface of the terminal cisternae of all muscle fiber types examined in the present study.

The structure of mitochondria varies among the different muscle fiber types. Generally the muscle fibers which have a high metabolic activity, such as the red fibers and cardiac muscle fibers, have more mitochondria than the muscle fibers with low metabolic activity, such as the white fibers and the slow fibers. The column-forming mitochondria, which are longitudinally arranged in the intermyofibrillar space, are seen in all the muscle fibers examined. From the present SEM observation and the previous TEM study (Ogata 1988), the I-band limited mitochondria and I-band branches of the column-forming mitochondria are lacking in the avian, reptilian, amphibian and fish muscle fibers. They are only seen in the mammalian skeletal muscle.

The A-ODO method was applied for the studies on some pathological muscles. The morphological changes of the skeletal muscle cells of the rat experimental myopathy induced by 2, 4 dinitrophenol were examined by SEM after A-ODO treatment (Kawahara et al., 1991). In the experimental mitochondrial myopathy, a large accumulation of mitochondria are observed in the subsarcolemmal region. Mitochondria in the perinuclear and intermyofibrillar region show swelling and occasionally are accompanied by abnormal concentric cristae. A regular network of the SR disappears and the arrangement of T-tubules is disturbed.

The SR, caveolae and mitochondria in the myocardium of spontaneously hypertensive rats were observed by SEM after A-ODO treatment and compared with those of age- sex-matched normal rats (Goto et al., 1990). In ten week-old hypertensive rats, with mild cardiac hypertrophy, a significant increase in the number of caveolae and accumulation of mitochondria with dense cristae were seen. Moreover the density of the SR network also increased and some SR exhibited giant and squamous profiles. In 20 week hypertensive rats with established cardiac hypertrophy, the caveolae and SR observed in 10 week hypertensive rats are no longer present, but caveolae are distributed in bands with variable width. In addition, a wavy arrangement of mitochondria, fragmentation and stacks of mitochondrial cristae are observed. The structure of the SR in the smooth muscle differs from that of the striated muscle. The T-axial tubular system is lacking and the SR is much less developed than in the striated muscle. Probably, a poorly developed SR system is enough for slow contracting smooth muscle fibers.

References

Dulhunty AF, Valois VA (1983) Indentations in the terminal cisternae of amphibian and mammalian skeletal muscle fibers. J. Ultrastruct. Res. 84: 34-49.

Forbes MS, Van Niel EE (1988) Membrane systems of guinea pig myocardium: Ultrastructure and morphometric studies. Anat. Rec. 222: 362-379.

Goto Y, Yoshikane H, Honda M, Morioka S, Yamori Y, Moriyama K (1990) Three-dimensional observation on sarcoplasmic reticulum and caveolae in myocardium of spontaneously hypertensive rats. J. Submicrosc. Cytol. Pathol. 22: 535-542.

Inoué T, Osatake H (1986) Three-dimensional architecture of the sarcoplasmic reticulum in smooth muscle cells observed by scanning electron microscopy. Proc. XIth Int. Cong. Electron Microsc., Kyoto, 3115-3116.

Inoué T (1990) The three-dimensional ultrastructure of intracellular organization of smooth muscle cells by scanning electron microscopy. In: Ultrastructure of Smooth Muscle, Motta PM (ed),

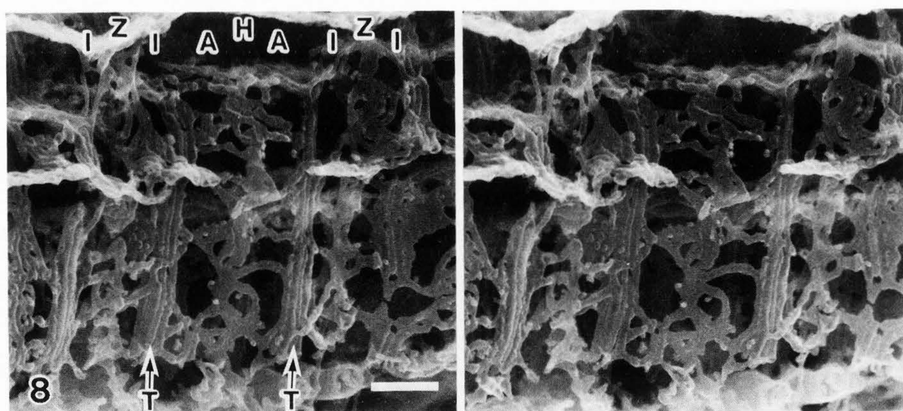

Fig. 8. Stereo pair of rat white fiber. Note the A-band side SR forms single layer networks, while the I-band SR forms multi-layered networks. Bar=0.5μm

Kluwer Academic Publishers, Norwell (USA), 63-77.

Kawahara H, Houdou S, Inoue T (1991) Scanning electron microscopic observations on muscle cells of experimental mitochondrial myopathy produced by 2, 4-dinitrophenol. J. Submicrosc. Cytol. Pathol. 23: 397-403.

Kubotsu A, Ueda M (1980) A new conductive treatment of the specimen for scanning electron microscopy. J. Electron Microsc. 29: 45-53.

Murakami T (1973) A metal impregnation method of biological specimens for scanning electron microscopy. Arch. Histol. Jpn. 35: 323-326.

Ogata T (1958) A histochemical study of the red and white muscle fibers. Part 1. Activity of the succinoxydase system in muscle fibers. Acta Med. Okayama 12: 216-227.

Ogata T (1988) Morphological and cytochemical features of fiber types in vertebrate skeletal muscle. CRC Critical Rev. Anat. Cell Biol. 1: 229-275.

Ogata T, Mori M (1964) Histochemical study of oxidative enzymes in vertebrate muscles. J. Histocem. Cytochem. 12: 171-182.

Ogata T, Yamasaki Y (1985a) Scanning electron-microscopic studies on the three-dimensional structure of mitochondria in the mammalian red, white and intermediate muscle fibers. Cell Tissue Res. 241: 251-256.

Ogata T, Yamasaki Y (1985b) Scanning electron-microscopic studies on the three-dimensional structure of sarcoplasmic reticulum in the mammalian red, white and intermediate muscle fibers. Cell Tissue Res. 242: 461-467.

Ogata T, Yamasaki Y (1987) High-resolution scanning electron-microscopic studies on the three-dimensional structure of mitochondria and sarcoplasmic reticulum in the different twitch muscle fibers of the frog. Cell Tissue Res. 250: 489-497.

Ogata T, Yamasaki Y (1989) High resolution scanning electron-microscopic study on the three-dimensional structure of the sarcoplasmic reticulum in the slow (tonic) muscle fibers of the frog, Rana nigromaculata. Cell Tissue Res. 255: 669-672.

Ogata T, Yamasaki Y (1990a) High resolution scanning electron microscopic study on the three-dimensional structure of the transverse-axial tubular system, sarcoplasmic reticulum and mitochondria of the slow muscle fibers of the chicken. J. Electron Microsc. 39: 342.

Ogata T, Yamasaki Y (1990b) High-resolution scanning electron microscopic studies on the three-dimensional structure of the transverse-axial tubular system, sarcoplasmic reticulum and intercalated disc of the rat myocardium. Anat. Rec. 228: 277-287.

Ogata T, Yamasaki Y (1991) Ultra-high-resolution scanning electron microscopic studies on the sarcoplasmic reticulum and mitochondria of the rat intrafusal muscle fibers. Part I. The capsular sleeve region. Arch. Histol. Cytol. 54: 471-490.

Ogata T, Yamasaki Y (1992) Ultra-high-resolution scanning electron microscopic studies on the sarcoplasmic reticulum and mitochondria of the rat intrafusal muscle fibers. Part II. The extracapsular region. Arch. Histol. Cytol. 55: 117-124.

Ohmori T (1984) Three-dimensional architecture of sarcotubules of rat skeletal and heart muscle cells observed by scanning electron microscopy. J. Yonago Med. Assoc. 35: 241-251. (in Japanese with English abstract)

Padykula HA, Gauthier GF (1967) Morphological and cytochemical characteristics of fiber types in normal mammalian skeletal muscle. In: Exploratory Concepts in Muscular Dystrophy and Related Disorders, Milhorat AT (ed), Excepta Medica Foundation, Amsterdam, 117-131.

Peachey LD (1965) The sarcoplasmic reticulum and transverse tubules of the frog's sartorius. J. Cell Biol. 25: 209-231.

Popescu LM, de Bruijn WC (1982) Calcium in the sarcoplasmic reticulum of smooth muscle. X-ray microanalysis of oxalate-treated muscle fibers. Proc. 10th Int. Cong. Electron Microsc. Hamburg, Electron Microsc. 3: 385-386.

Porter KR, Palade GE (1957) Studies on the endoplasmic reticulum. III. Its form and distri-

bution in striated muscle cells. J. Biophys. Biochem. Cytol. 3: 269-300.

Rayns DG, Devine CE, Sutherland CL (1975) Freeze-fracture studies of membrane systems in vertebrate muscle. I. Striated muscle. J. Ultrastruct. Res. 50: 306-321.

Sawada H (1981) Scanning electron microscopy of guinea pig taenia coli. Biomed. Res. Suppl. 2: 153-158.

Segretain D, Rambourg A, Clermont Y (1981) Three dimensional arrangement of mitochondria and endoplasmic reticulum in the heart muscle fiber of the rat. Anat. Rec. 200: 139-151.

Tanaka K, Naguro T (1981) High resolution scanning electron microscopy of cell organelles by a new specimen preparation method. Biomed. Res. Suppl. 2: 63-70.

Tanaka K, Mitsushima A (1984) A preparation method for observing intracellular structures by scanning electron microscopy. J. Microsc. 133: 213-222.

Yoshikane H, Nihei T, Moriyama K (1986) Three-dimensional observation of intracellular membranous structures in dog heart muscle cells by scanning electron microscopy. J. Submicrosc. Cytol. 18: 629-636.

Discussion with Reviewers

Y. Shimada: What are the indentations observed on the surface of the terminal cisternae?
Authors: The indentations are the cone-shaped depressions of the terminal cisterna. They were also demonstrated by freeze-fracture (Dulhunty, 1983). The function of the indentations is unknown.

Y. Shimada: Is it possible to observe the internal surface structure of the SR and T- tubules with the method you are using?
Authors: Yes, it is possible to observe the internal surface structure of the SR and T-tubule by our method. This will be the subject of another study.

M. S. Forbes: Because of the focal depth available with the SEM and the size of specimen it can accommodate, stereoscopic micrograph pairs have been used virtually since the introduction of the instrument to reveal three dimensional architecture of countless subjects, including the interiors of cells. Have the authors made profitable use as yet of specimen tilting in their studies of the various muscles they illustrate in this review, and if so have they been able to enlarge upon observations and conclusions heretofore made with, and limited to, selective staining and thick-section analysis in the TEM or HVEM?
Authors: We used the stereoscopic SEM for the observations of the rat intrafusal fibers (Ogata and Yamasaki, 1991). Stereo pair micrographs of the rat twitch white fiber (Fig. 8) are presented in this review.

M. S. Forbes: The second question I have is in regard to quantitative investigation of SR (and other muscle cell components) by SEM examination. What results do the authors see resulting from the marriage (though perhaps manage a trois is the more apt term for this union) of the A-ODO technique, scanning electron microscopy, and stereology?
Authors: In the process of A-ODO treatment, a few sarcotubules are inevitably damaged and the perfect preservation of the SR is difficult. Therefore, the accurate stereological analysis of the SR is difficult at the present time.

R. S. Hikida: Although it may be difficult to distinguish between avian slow fibers by mitochondrial size and distribution, I suspect that one might be able to do so on the basis of the sarcoplasmic reticulum. Figure 3 shows very clearly that the pattern of SR is not twitch-like. Have the investigators observed intermediate type patterns of SR that might be classified as the other slow type (SR more prominent, thin Z-lines)?
Authors: We have not yet found the intermediate type pattern of SR. We think this will be the subject of further studies.

R. S. Hikida: It has been shown that different myosin ATPase activities may occur on opposite sides of the sensory region of mammalian intrafusal fibers. Because identification of the intrafusal fibers appears to depend on its fibers types, I wonder whether the authors have observed differences in the fibers on opposite sides of the sensory region in their studies?
Authors: We have not yet observed the opposite sides of the sensory region.