ULTRA STRUCTURE OF THE Z LINE
OF SKELETAL MUSCLE FIBERS

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ABSTRACT

A new model of Z-line structure in skeletal muscle is proposed. Unlike previous models it is capable of explaining the two apparently inconsistent lattice arrangements seen in thin sections, i.e., the “basket weave” lattice and the smaller lattice recently reported in the literature. The model is based on four looping helical strands derived from the I filaments within the Z line. Each of these four strands form hairpin-shaped loops within the Z line and then join with an adjacent I filament in the same sarcomere. The two apparently different lattices represent a common structure viewed at slightly different levels of section.

INTRODUCTION

The ultrastructure of striated muscle has been extensively studied in numerous animal species. In accordance with the sliding filament model of contraction proposed by Huxley and Hanson (1954) and Huxley and Niedergerke (1954), all of this work shows the sarcomere to be the basic repeat unit of the myofibril. The Z lines situated at either end of the sarcomere are considered to form the fixed points to which the actin filaments attach and upon which they exert tension during contraction. The Z lines also serve as the links between successive sarcomeres within the myofibril. The Z line then is of prime importance, in that it enables the tension developed by the interaction of actin and myosin of individual sarcomeres to be transmitted along the length of the myofibril to the sites of insertion and origin of the muscle fiber.

There have been a number of ultrastructural studies aimed specifically at the Z line (Knappies and Carlsen, 1962; Franzini-Armstrong and Porter, 1964; Reedy, 1964; Kelly, 1967), and from these a number of models of the structure have been proposed. The test of these models is their ability to explain the pictures of the Z line obtained by electron microscopy. All of the models agree on the positioning of the I filaments on either side of the Z line. Here an en face view reveals a square lattice of approximately 22 nm. The lattice on one side of the Z line is displaced by half a repeat distance relative to the lattice on the other side of the Z line. When a section thick enough to include parts of both of these I filament lattices is viewed en face, the combined lattices give the spatial arrangement of I filaments found in the Z line (Fig. 1 A). There is some debate as to the nature and orientation of the Z filaments linking the I filaments together in the Z line. The models of Knappies and Carlsen (1962), Franzini-Armstrong and Porter (1964), Reedy (1964), and Kelly (1967) (see Figs. 1 B and 1 C) all propose a lattice of approximately 15.5 nm rotated 45° to the 22 nm lattice of the I bands. Landon (1970) shows micrographs of an 11 nm lattice parallel to the 22 nm lattice (Fig. 1 D), and Macdonald and Engel (1971) put
A mixture of 1% osmium tetroxide and 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.3) (Hirsch and Fedorko, 1968). Material was washed overnight in changes of 0.1 M cacodylate buffer at 4°C and dehydrated in graded ethanol solutions. The material was then embedded in Araldite. Thin sections cut on an LKB ultrotome (LKB Instruments, Inc., Rockville, Md.) with glass knives were examined in a Philips EM300 electron microscope after staining with uranyl acetate (saturated solution in 50% ethanol) and Reynolds's (1963) lead citrate.

RESULTS AND DISCUSSION

One of the striking features of the Z line is the diversity of its appearance under the electron microscope both in transverse sections and in longitudinal sections. The diversity in the en face views of a single fiber arises as a result of different levels of section through the Z line and also slight variations in section thickness and angle of the plane of section. Differences in the appearance of the Z line between different fiber types have an additional source of variation. The Z lines of "red" mitochondria-rich fibers are thicker than the Z lines of "white" mitochondria-poor fibers (Gauthier, 1969). Some species of animal at an early stage of development have Z lines with little additional material associated with the lattice, whereas at a later stage of development the lattice of the Z line can be almost totally masked by an abundance of such additional material (Kelly, 1967).

The divergent appearances in longitudinal sections also result in part from the variables listed for transverse sections. In addition, there is the fact that the Z line is made up of filaments in a square lattice array with two planes of symmetry at 45° to one another. This lattice can therefore be viewed at angles ranging from 0° to 45° relative to these planes of symmetry (see Fig. 8).

Transverse Sections

En face views of the Z line examined in this laboratory have provided pictures which at first appearance show two different structures. On the one hand a basket weave lattice structure is seen (Fig. 2) closely resembling that presented by previous worker (Reedy, 1964; Kelly, 1967). This would seem to support the Z-line models having the lattice displaced by 45° relative to the square array of filaments in the juxtaposed I

MATERIALS AND METHODS

Rat soleus muscle fibers were removed immediately after death and fixed for 2-4 hr at 0°C in a fresh mixture of 1% osmium tetroxide and 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.3) (Hirsch and Fedorko, 1968). Material was washed overnight in changes of 0.1 M cacodylate buffer at 4°C and dehydrated in graded ethanol solutions. The material was then embedded in Araldite. Thin sections cut on an LKB ultrotome (LKB Instruments, Inc., Rockville, Md.) with glass knives were examined in a Philips EM300 electron microscope after staining with uranyl acetate (saturated solution in 50% ethanol) and Reynolds's (1963) lead citrate.

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Figure 2: Transverse section of Z line from rat soleus muscle showing basket weave lattice (single arrow) at 45° to the square array of juxtaposed I-band region (double arrow). X68,500.

Figure 3: Transverse section of Z line from rat soleus muscle showing the small square lattice of the Z line (single arrow) parallel with that larger square lattice of the juxtaposed I band (double arrow). X76,500.
Figure 4. Transverse section of Z line at high magnification. The section is slightly oblique and shows regions of Z line and also a region of the juxtaposed I band (upper right). The basket weave appearance of the Z line is divided into a number of independently oriented areas with faults between them. ×183,500. The areas enclosed in the small squares are represented diagrammatically in the upper left and lower right corners. In these diagrams the solid lines represent Z filaments visible on the micrograph; dotted lines represent Z filaments not visible on the micrograph. The diagram in the top left corner (A) should be compared with Fig. 8 E, and the diagram in the bottom right corner (B) with Fig. 8 F. The arrows indicate I filaments within the Z line which appear to be four stranded.
bands (Fig. 1 C). On other occasions the Z line displays an 11 nm square lattice with its planes of symmetry parallel to those of the I-band filaments (Fig. 3). This is equivalent to the lattice reported by Landon (1970) and Macdonald and Engel (1971), and represented diagrammatically in Fig. 1 D.

The basket weave models proposed by Reedy (1964) and Kelly (1967) depict the Z filaments, which appear to link I filaments from both sides of the Z line, as smooth curves (Fig. 1 C). Close examination of the basket weave lattice found in Fig. 4 shows that the Z filaments, which still appear to be linking I filaments from both sides of the Z line, have a somewhat pointed structure. The point occurs approximately at the midpoints of these Z filaments (B of Fig. 4). Other filaments of the same Z line (A of Fig. 4) can be seen joining with I filaments from the same side of the Z line (i.e., at 22 nm separation). These Z filaments can be seen to have an S-shaped configuration (A of Fig. 4). In both cases the I filaments inserting into the Z line appear to be giving off four strands (four Z filaments). Some of the I filaments cut in transverse section in Fig. 4 appear to be made up of four small strands (arrows). However, this appearance could be artifactual and may be the result of lead precipitation or phase granularity.

Proposed Model of Z-Line Structure

The model of Z-line structure proposed here requires that the I filament be a four-stranded structure, or at least that it gives off four strands at its point of insertion into the Z line. The four strands could be the two actin helices together with the tropomyosin B filaments postulated to run down the grooves of the double actin helix (Hanson and Lowy, 1964). At the Z line the four-stranded coil of each I filament separates into its four strands and each of these joins with an adjacent I filament from the same sarcomere, forming a 22 nm square mesh. The two meshes, one from each side of the Z line, are interlocked by virtue of the fact that the two sets of I filaments interdigitate before forming the meshes (Figs. 6 and 9).

The apparent splitting of the I filament into four strands at its insertion into the Z-filament lattice could also be achieved in a situation where only the actin helices contribute to the formation of Z filaments. In this case each I filament would give rise to and receive two Z filaments.

Longitudinal Sections

Knapppeis and Carlsen (1962), Franzini-Armstrong and Porder (1964), Kelly (1967), and Landon (1970) pointed out that there are two appearances in longitudinal sections of the Z line which are striking because of the well-ordered spacing of the I filaments on either side of and within the Z line. These two views correspond to views along the two planes of symmetry of the square lattice (1 and 2 of Fig. 8). When the Z line is viewed in line with plane 1 of Fig. 8, the I filaments converge on the Z line in such a way as to display the half-stagger of the two adjacent 22 nm square arrays (Fig. 5, double arrows). This results in the interdigitating of the I filaments within the Z line. A view in line with plane 2 of Fig. 8 brings the I filaments from both sides of the Z line into line, and they appear as continuous structures running through the Z line (Fig. 5, single arrow). Other views of the Z line in longitudinal sections are of the transition from view 1 to view 2 and, although usually more numerous, are less striking. Close examination of Fig. 6 shows that the Z line also contains Z filaments in arrowhead configurations running between the I filaments.

If the tropomyosin component of the I filament is regarded as contributing two of the four strands converging on each I filament at its insertion into the Z lattice, then the model has to accommodate the smaller pitch of the tropomyosin (actin 73 nm, Huxley, 1963; tropomyosin 38.2 nm, Caspar et al., 1969). The en face appearance of three half-pitches of two such different sizes would be similar if the smaller of the two (tropomyosin) takes a shorter course through a less acute hairpin bend than the longer actin strand (see Figs. 9 D, E, and F).
Figure 5  Longitudinal sections of rat soleus muscle. The two most striking appearances of the Z line can be seen. The double arrow indicates Z lines in which the I filaments interdigitate, whereas the single arrow shows Z lines in which the I filaments from either side of the Z line appear as continuous structures running through the Z line. X58,500.
Fig. 8 shows the en face appearance of the Z-line model. The appearance varies depending on the section thickness relative to the Z-line thickness and on the level of the plane of section. Views A–G (Fig. 8) correspond to section thicknesses and levels depicted in Fig. 9 A for the all-actin Z filaments and Fig. 9 D for the actin and tropomyosin Z filaments. A and B represent views of the two large square lattices formed by the I filaments of the two I bands on either side of the Z line. Views C and D represent the same lattice, but here the tips of the looping Z filaments have not been included in the section thickness. E of Fig. 8 is the result of superimposing views A and B and clearly gives a small square lattice with side orientations parallel to those of the I bands. This is the orientation demonstrated by Landon (1970). The result of superimposing views C and D is view F (Fig. 8). In this case the basket weave lattice, larger than the square lattice of E and with side orientation at 45° to those of the I bands, is clearly evident.

Although this basket weave lattice compares very favorably with those of Reedy (1964) and Kelly (1967), the important difference is the sharp point at the midpoint of the Z filament (cf. Figs. 1 C, 4 B, and 8 F). The square array of the I filaments found in the I band juxtaposed to the Z line is shown in view G (Fig. 8).

The appearance of the model in longitudinal section is shown diagrammatically in Fig. 9. The three different appearances for both models are achieved by different amounts of overlap of the two sets of I filaments. Figs. 9 A and D show the situation in which there is a reasonable amount of overlap and which can give rise to the various en face views of Fig. 8. In the situation with the least amount of I-filament interdigitation the Z line would appear as in Figs. 9 C and F. This is the appearance which most closely resembles the micrographs of Knappeis and Carlsen (1962) and Franzini-Armstrong and Porter (1964), where the Z line appears to be a single zigzag line joining the I filaments. Figs. 9 B and E.
FiguRe 7 (A) Diagrammatic representation of two I filaments showing the 73 nm pitch of the helical arrangement of the two actin strands (the tropomyosin B strands have been omitted for clarity). Three half-pitches of an actin strand are shown making up the hairpin-bend link (Z filament) between adjacent I filaments. (B) En face view of Fig. 7 A. The positions of the tropomyosin B strands have been outlined by broken lines. This diagram should be compared with Figs. 8 A and B and with the inset at top left of Fig. 4.

The micrographs and model shown here clearly demonstrate that both lattices can be explained in terms of a common structure. There are, however, facts and arguments, pertaining to the Z line and I filaments, which need further clarification before the structure of the Z line can be stated emphatically. These are the same arguments as those put forward by Kelly in his paper on models of Z line structure (1967).

The present model, as did one of Kelly’s models (1967), would mean that actin strands (single helix) approximately 2 µm in length would have to be present in hairpin configurations. Although synthetic actin filaments (double helix) are found with lengths in excess of 1 µm, these are usually regarded as being straight filaments. Native actin filaments prepared by homogenization of muscle fibers generally do not exceed 1 µm (Allen and Pepe, 1965). Homogenization may break long native actin filaments at the Z line where they become single stranded. The ability of the muscle to lay down actin filaments with hairpin configurations receives some support from the evidence provided by Kelly’s work on the myotendinous junctions (1967), intercalated discs (1966), and myofibrillogenesis (1969). Desmosomes, intercalated discs, myotendinous junctions, and Z lines, all of which have been put forward as having loopings filaments, all have an electron-opaque matrix in common (which may or may not be chemically identical). Synthetic actin filaments are produced in a situation where this matrix is not able to exert any influence on the filament or strand. Therefore it could be that this matrix is a prerequisite for the formation of a hairpin bend. Kelly (1969) has presented a scheme for the differentiation of Z lines in which filaments with hairpin configurations are first laid down in Z bodies in the presence of electron-opaque amor-
FIGURE 8  En face views of the Z line model corresponding to section thicknesses and planes of section shown in Fig. 9 A. I filaments are represented by dots, and the lines represent the Z filaments. Views A and B are the individual large square lattices formed by the I filaments from either side of the Z line. Views C and D are these same lattices, but in these cases the section thicknesses are slightly less and do not include the tip of the hairpins shown in Fig. 7. View E is the result of superimposing A and B, giving a small square lattice with planes of symmetry (1) parallel to the square array of filaments in the juxtaposed I-band region (view G). F is the basket weave lattice produced by combining C and D with its planes of symmetry (2) at 45° to those of E and G.

Phosphorus material. Initially, the filaments of the two I bands are only loosely enmeshed and, as the Z line matures, the meshes are pulled into one another and held in their mature positions. Models of Z-line structure with direct continuity between successive I filaments do not have the required flexibility for such a scheme.

Actin filaments, both native and synthetic, display a characteristic polarity. In native actin filaments the polarity is such that the characteristic barb-like subunits of the heavy meromyosin/actin filament complex (Huxley, 1963) invariably point away from the Z line. The model of Z-line structure presented is such that both arms of the hairpin strand point in the same direction and, to be compatible with the polarity of native I filaments, would require the two arms to have polarity in opposite directions (see Fig. 10). Up to this time the nature of the polarity of single strand actin is not known. It may be that the Z-line matrix, as well as conferring an acute bend on the strand, also reverses the polarity in the same region. An alternative possibility is outlined diagrammatically in Fig. 10. Each actin monomer making up an F-actin strand combines with two actin monomers of the other strand in the double actin-stranded I filament (Moore et al., 1970). Each actin monomer also has a polarized site on its surface farthest away from the center of the double helix capable of combining with heavy meromyosin. If the two F-actin helices making up an I filament are labeled A and B (Fig. 10), then the two arms of the hairpin actin strand proposed here would be one A strand and a B strand. In other words, strand A of I-filament X is continuous with strand B of I-
filament Y (Fig. 10). From I-filament X, actin monomers of strand A have a heavy meromyosin binding site on surface 4 and the actin/actin sites on surface 2. If during the course of the hairpin bend the strand also twisted through 180° around its long axis (see Fig. 10), then actin monomers in strand B of I-filament Y would have the following binding sites. The heavy meromyosin site would now be on surface 2 and the actin/actin sites on surface 4, i.e., the reverse of monomers in strand A of filament X. This explanation then requires that each G-actin monomer has the potential for combining with heavy meromyosin on at least two surfaces (2 and 4 of Fig. 10) which have opposite polarities and two actin combining sites on both of these surfaces. The heavy meromyosin site on one surface would be blocked when the actin/actin sites on the same surface were engaged. This is purely speculative, as there are no data on the number or positioning of such sites in the G-actin molecules.

Attempts to localize both actin and tropomyosin in the Z line using labeled antibody techniques have so far failed to provide convincing evidence. The failure of this technique could, however, also be due to the “matrix substance” blocking the reactive sites. Ebashi and Endo (1968) have reason to believe that tropomyosin is excluded from the Z line. However, tropomyosin makes up 10–15% of the fibrous proteins of vertebrate striated muscle, and not all of this is accounted for by its presence solely in the I filaments (Huxley, 1963). The question as to
it has the advantage of being compatible with both the small lattice of Landon (1970) and the largest lattices of Reedy (1964) and Kelly (1967) without a need for a change in structure.

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