

ULTRASTRUCTURE 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 (Knappeis 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 Knappeis 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

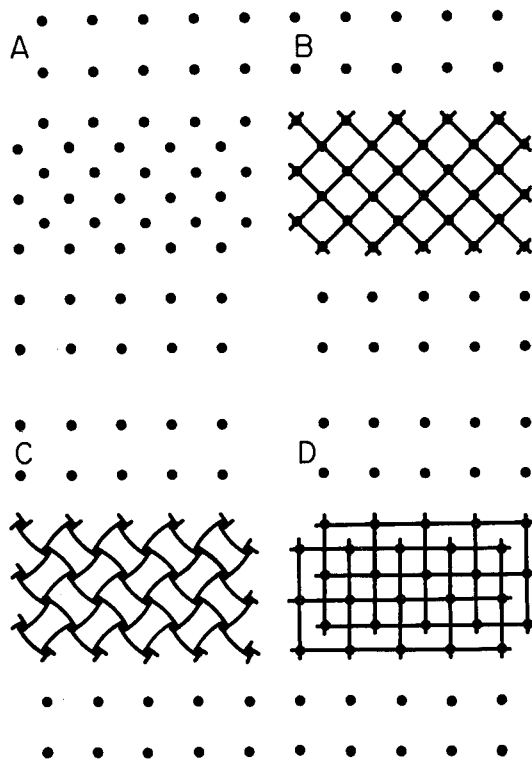


FIGURE 1 (A) Diagrammatic representative of the square array of I filaments in the I bands adjacent to the Z line which superimpose to give the Z line square array. (B), (C), and (D) The orientation of Z filaments linking the I filaments in the Z line in previous models of Z line structure after: (B), Knappeis and Carlsen (1962) and Franzini-Armstrong and Porter (1964); (C), Reedy (1964) and Kelly (1967); (D), Landon (1970).

forward a model to explain both lattices. Interpretation of the longitudinal appearance of the Z line is better explained by one model for some micrographs and by a different model for others. The models of Knappeis and Carlsen (1962), Franzini-Armstrong and Porter (1964), Reedy (1964), and Kelly (1967) cannot explain the 11 nm lattice of Landon (1970) without a change in structural organization. The model proposed here is capable of explaining all the appearances of the Z line seen in the electron microscope without a need for such a change.

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 ultratome (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

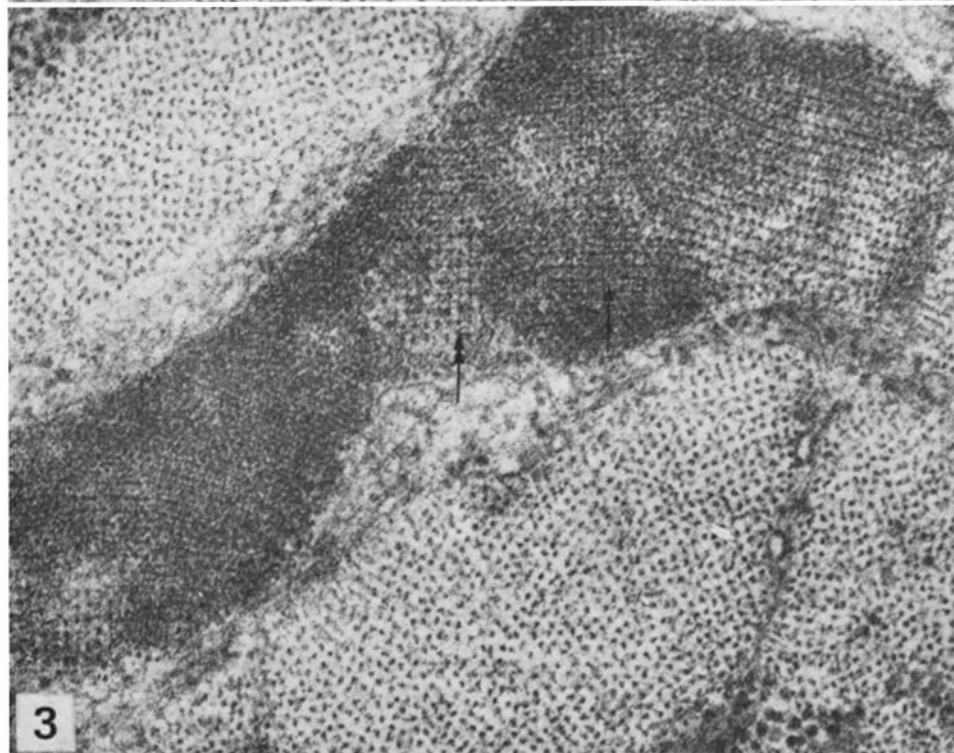
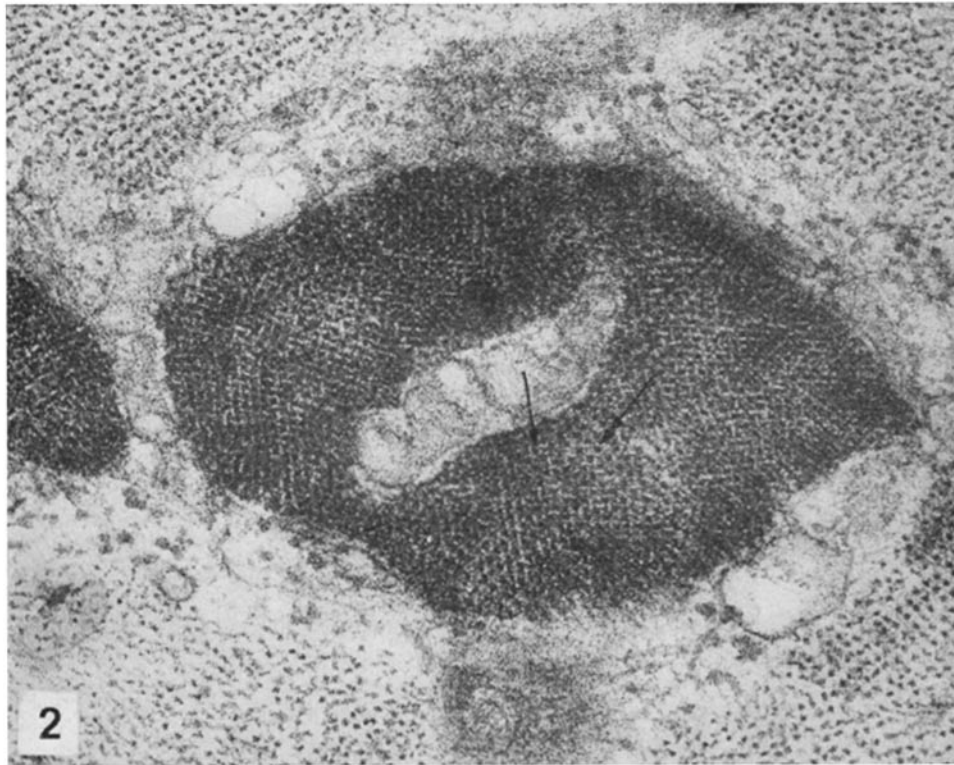


FIGURE 2 Transverse section of Z line from rat soleus muscle showing basket weave lattice (single arrow) at 45° to the square array of juxtapsed I-band region (double arrow). ×68,500.

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 juxtapsed I band (double arrow). ×76,500.

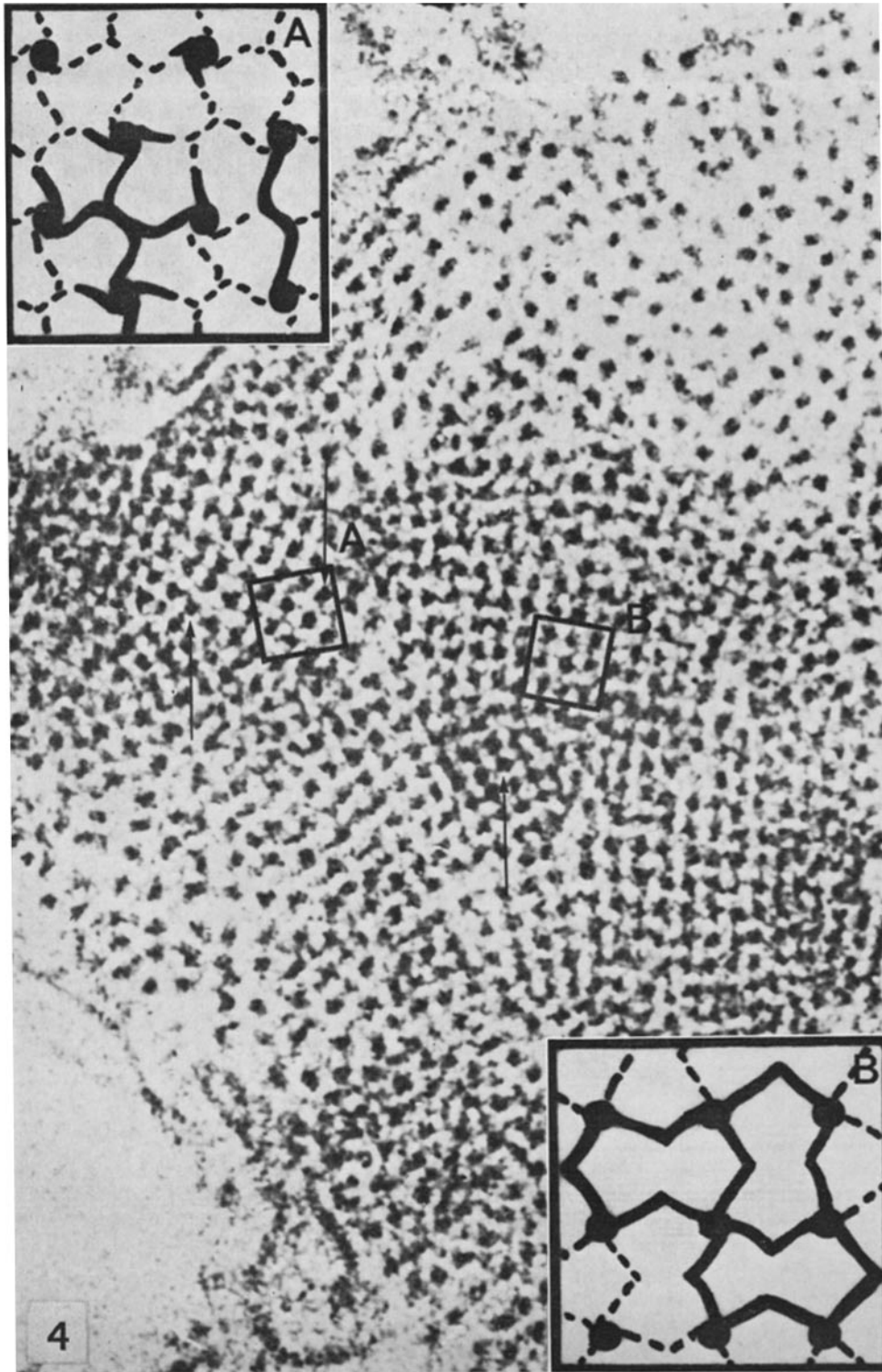


FIGURE 4 Transverse section of Z line at high magnification. The section is slightly oblique and shows regions of Z line and also a region of the juxtaposed I band (upper right). The basket weave appearance of the Z line is divided into a number of independently oriented areas with faults between them. $\times 183,500$. 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.

Longitudinal Sections

Knappes 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.

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.

The structural arrangement of the actin helices making up the I filament has been described by Huxley (1963). Each actin strand has a pitch of 73 nm. In the model of Z-line structure proposed here the actin strands are envisaged as maintaining this pitch when they separate at the Z line. It is proposed that one and one-half pitches of the helical strands make up one Z filament linking two I filaments (Fig. 7). When viewed *en face*, these three half-pitches would present exactly the same profile as the Z filaments in Fig. 7 (cf. Figs. 4 A, 7, 8 A and E).

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).

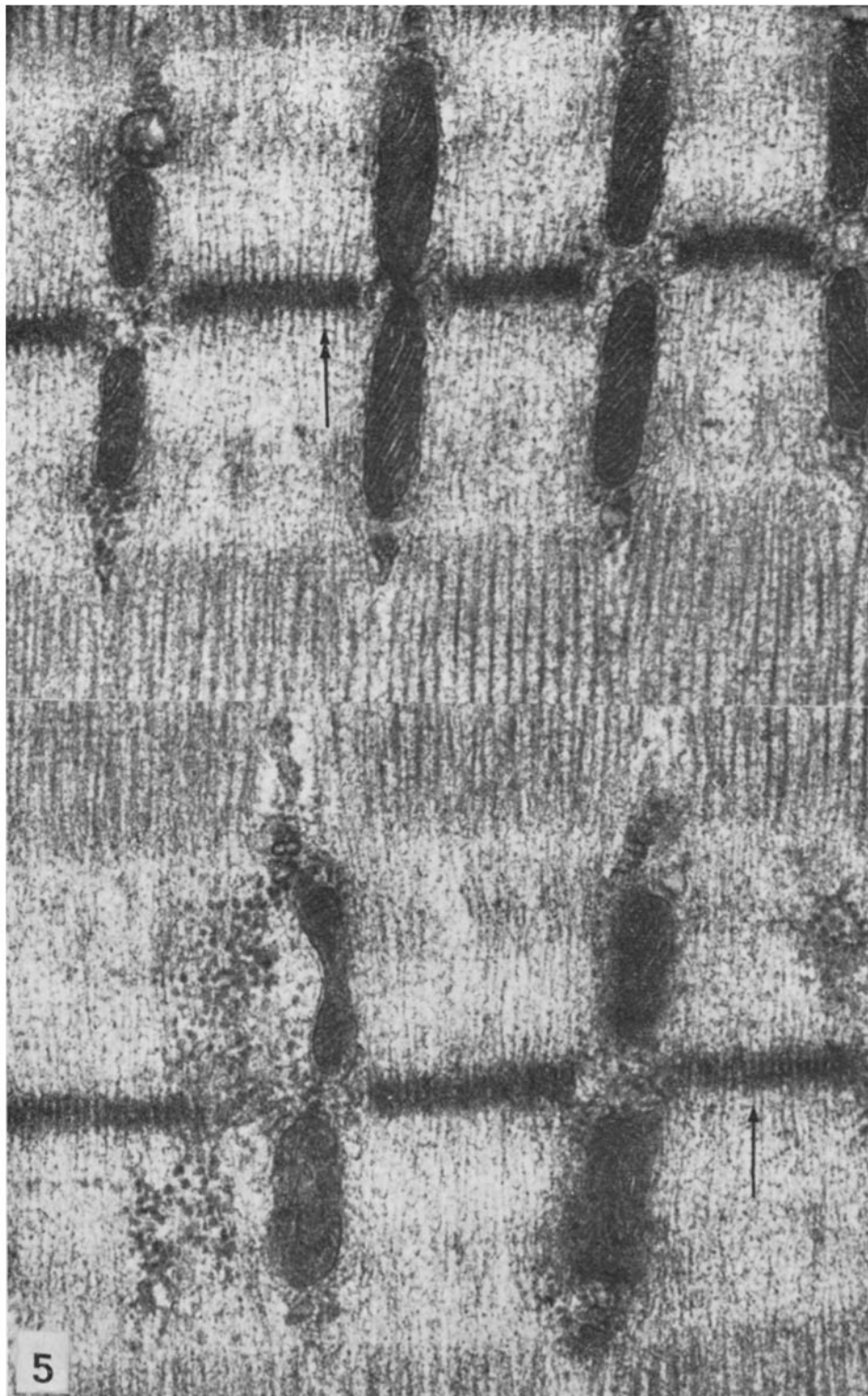


FIGURE 5 Longitudinal sections of rat soleus muscle. The two most striking appearances of the Z line can be seen. The double arrow indicates Z lines in which the I filaments interdigitate, whereas the single arrow shows Z lines in which the I filaments from either side of the Z line appear as continuous structures running through the Z line. $\times 58,500$.

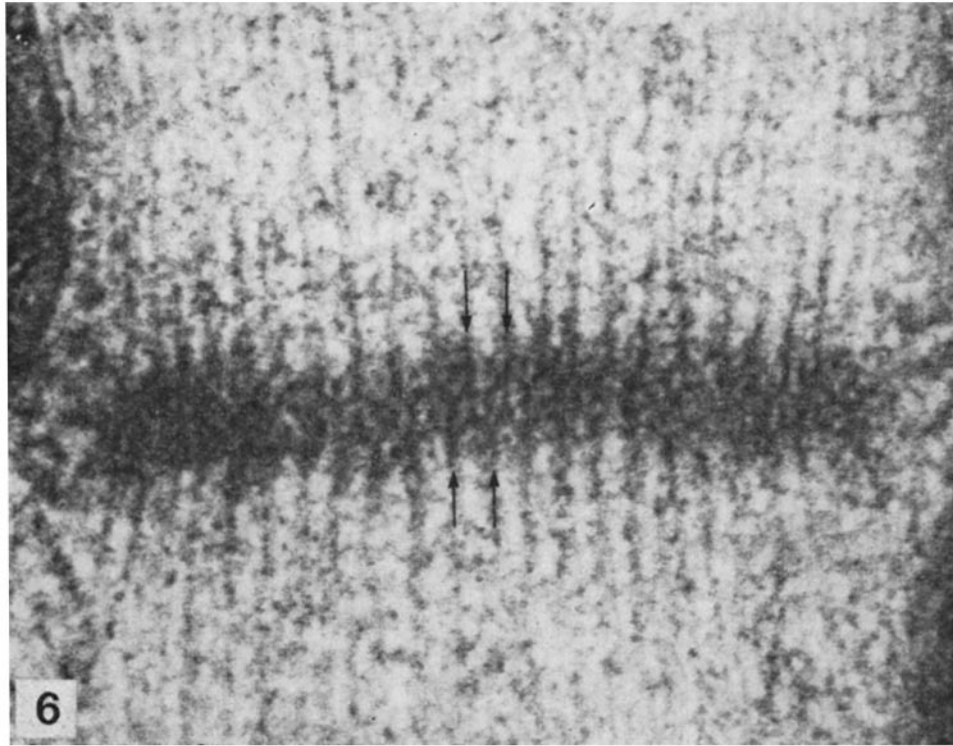


FIGURE 6 High magnification of a longitudinal section of a Z line. The I filaments interdigitate (arrows). The double chevron array of Z filaments linking the I filaments can be seen. This micrograph should be compared with Fig. 9 A. $\times 175,000$.

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 lattices, 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 Knappes 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

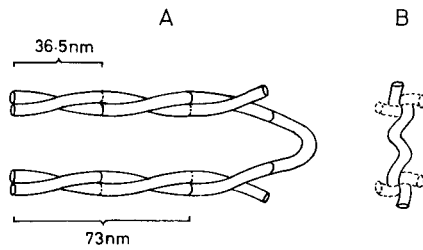


FIGURE 7 (A) Diagrammatic representation of two I filaments showing the 73 nm pitch of the helical arrangement of the two actin strands (the tropomyosin B strands have been omitted for clarity). Three half-pitches of an actin strand are shown making up the hairpin-bend link (Z filament) between adjacent 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.

give the appearance intermediate between Figs. 9 A and C, and Figs. 9 D and F, respectively.

There is a high degree of compatibility between the appearance of the Z-line model proposed here and the appearance of the Z line under the electron microscope. At first sight the basket weave lattices of Reedy (1964) and Kelly (1967) and those shown in Fig. 2 appear to be structures requiring a configuration of Z filaments completely different from that required to explain the small lattice of Landon (1970) and Macdonald and Engel (1971) shown in Fig. 3. Indeed, Landon's explanation of the occurrence of the small lattice was one in which the Z filaments disengaged from one I filament and reengaged with another after moving through 45°.

Macdonald and Engel (1971) offer two possible explanations for the occurrence of the two different Z lattices. The first is the existence of two sets of Z filaments in two different configurations, each showing up as the dominant pattern under different fixation conditions. Their alternative explanation is one in which the Z filaments making up the lattices exist in either an acutely or less acutely bended state. In both states Z filaments join I filaments from both sides of the Z line, making the structure incapable of interdigitating to any great extent.

The model proposed here and those of Kelly (1967) employing interlocked meshes of looping filaments raise the question as to what provides the attachment between the filaments of successive sarcomeres. Without direct continuity of the I

filaments of successive sarcomeres, under conditions of tension development by the muscle the two meshes would be pulled together rather like Figs. 9 A-C and 9 D-F. However, the amorphous material present in the Z lines is envisaged as providing the necessary rigidity enabling the meshes to maintain their relative positions. The bonding between the Z lattice and the amorphous material is sufficiently strong to accomplish this if it is the same as the bonding found at the terminal sarcomeres in myotendinous junctions and intercalated discs.

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 looping 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-

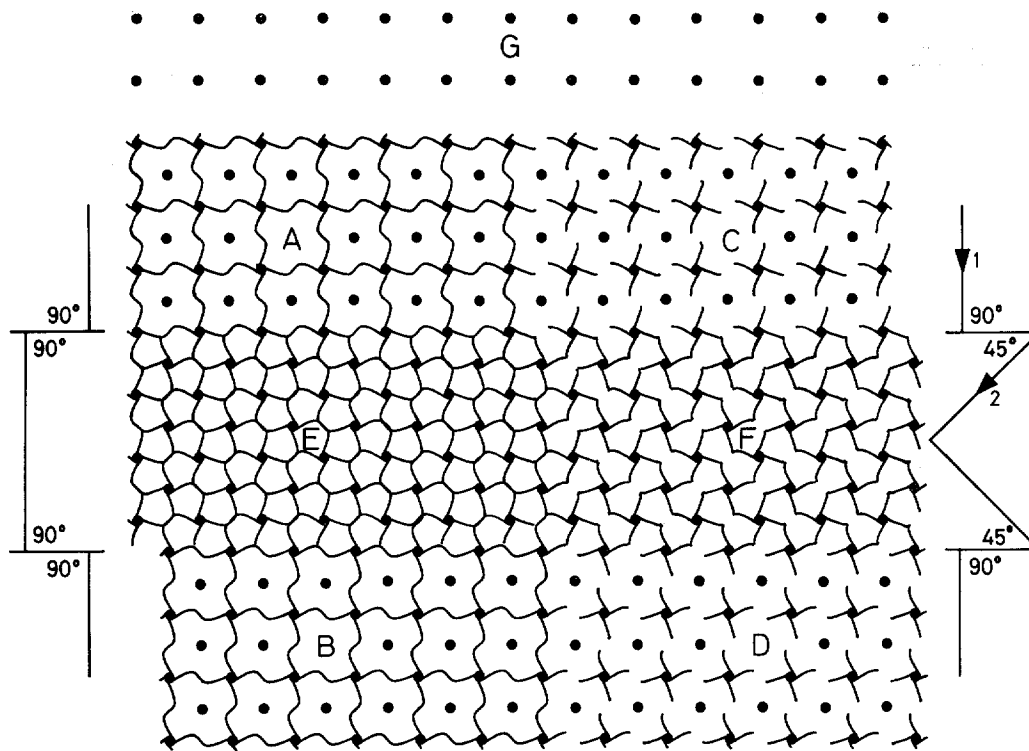


FIGURE 8 *En face* views of the Z line model corresponding to section thicknesses and planes of section shown in Fig. 9 A. I filaments are represented by dots, and the lines represent the Z filaments. Views A and B are the individual large square lattices formed by the I filaments from either side of 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.

phous 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-

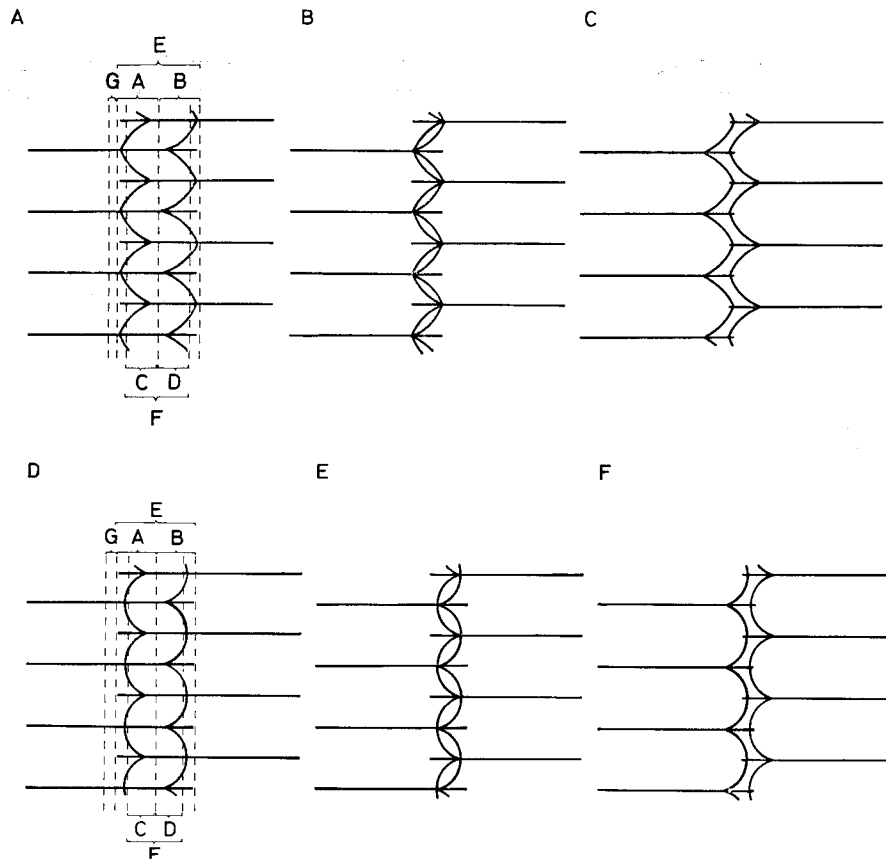


FIGURE 9 (A) Longitudinal sectional view of the proposed Z line model with all actin Z filaments. (B) and (C) show the model at different amounts of I filament interdigitation. (D), (E), and (F) correspond to (A), (B), and (C) but are for the model with both actin and tropomyosin Z filaments; note the shorter and more gentle curve of the tropomyosin Z filaments. A-G of Figs. 9 A and D represent section thicknesses and planes of section corresponding to the views presented in Fig. 8.

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 hair-pin 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

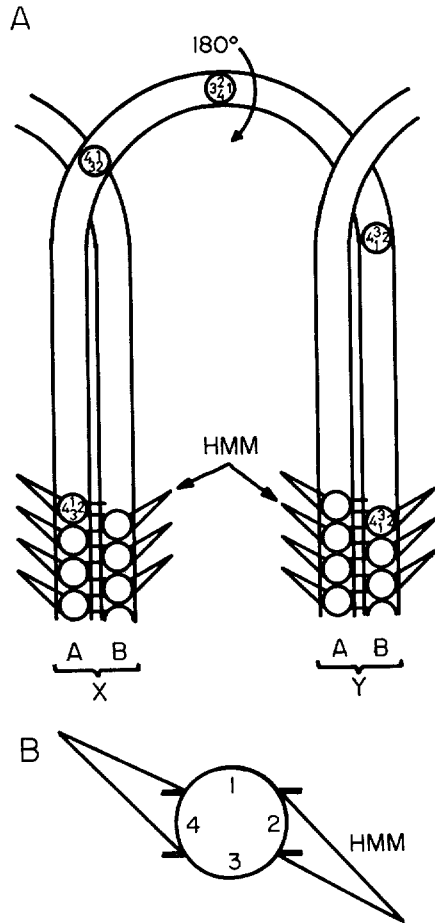


FIGURE 10 (A) Diagrams showing two I filaments *X* and *Y*; each has two actin strands *A* and *B* (for clarity the double helix has been straightened out). The hairpin configuration of three half-pitches (see Fig. 7) forming the Z filament links *A* of *X* with *B* of *Y*. Heavy meromyosin (*HMM*) combines with the I filaments showing the polarity of the *HMM*/actin complex. Individual actin monomers making up the actin strands are shown as small circles with their surfaces numbered 1-4 (see text for further explanation). (B) One G-actin monomer with its surfaces numbered 1-4 showing the two *HMM* binding sites on surfaces 2 and 4 with opposite polarities. The four actin binding sites are shown by the horizontal bars.

whether or not actin and tropomyosin exist in the Z line remains open.

The Z-line model presented stems purely from an attempt to find a structural configuration capable of explaining the varied arrangements seen by electron microscopy. Although it can be substantiated no more than other Z-line models,

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.

I wish to thank Mrs. Sue Tucek and Mr. John F. Weidemann for their skilled assistance in preparing the tissue for electron microscopy.

This work was supported in part by the Australian Meat Research Committee.

Received for publication 16 April 1971, and in revised form 14 June 1971.

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