THE STRUCTURE OF A SIMPLE Z LINE

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ABSTRACT

The structural simplicity of the Z line in fish muscle fibers allows direct visualization of its basic geometry. Models which postulate termination of the I filaments at the edges of the Z line and the direct linkage of I filaments belonging to the two adjacent sarcomeres by Z filaments crossing the whole width of the Z line give the best fit to the electron micrographs. The structure of fish Z lines is not significantly altered by the use of different fixation procedures and by changes in sarcomere length.

INTRODUCTION

The Z line of vertebrate twitch fibers has recently been the subject of a number of investigations. The numerous models derived from these studies fall into two categories. One group of authors (Franzini-Armstrong and Porter, 1964 *a*; Reedy, 1964; MacDonald and Engel, 1971) favor the idea, originally worked out by Knappeis and Carlsen (1962), that each thin (I) filament, upon reaching the Z line, connects to four Z filaments and that these in turn directly link it to the four nearest thin filaments belonging to the adjacent sarcomere.

The exact structure of the Z filaments, their mode of anchorage to the thin filaments, and their course within the Z line are variously interpreted. Franzini-Armstrong and Porter (1964 *a*), for example, visualize the connections across the Z line as diffuse ridges, remnants of an original membrane, rather than well-defined filaments. Disagreement in interpretation of details is mostly due to the fact that shape and visibility of the Z filaments vary in various fiber types (MacDonald and Engel, 1971) and that they are largely dependent on fixation (Landon, 1970). In the following description and discussion the term Z filament will be used to indicate a direct link joining I filaments of opposite sarcomere. The exact shape and/or origin of the links will not be discussed.

Models by a second group of authors (Kelly, 1967, 1969; Rowe, 1971) are based on the assumption that filamentous loops (or hairpins) arise from each thin filament and join it to adjacent filaments from the same sarcomere. The loops either run across the entire Z line and wrap around the thin filaments from the opposite sarcomere (Kelly, 1967), or they traverse only a variable portion of the Z line and return to the same sarcomere, interlocking but without direct contact with thin filaments and loops from the opposite sarcomere (Rowe, 1971). Looping filament models assume that at least one of the major thin filament proteins (actin and tropomyosin) continues into the Z-line loops.

All models agree on the disposition of the thin filaments at the Z-line edges: the filaments are disposed in a regular tetragonal lattice, the thin filaments occupying the corners of the squares. The lattices of the two adjacent sarcomeres which abut on an individual Z line are parallel and displaced by a half-period along the two major axes. This detail of the structure is readily visible in all types of preparations, and it will be used as a landmark for the description of orientation of section plane relative to the Z line.

The course of the Z filaments, on the other hand, is difficult to define, due to the small di-

mensions of the filaments and to their superimposition even in very thin sections. The latter factor is very limiting in wide Z lines. A fish muscle was chosen for this study, because it offers a distinctive advantage: the Z line is very narrow, less than 300 Å (Franzini-Armstrong and Porter, 1964 *b*), so that individual Z filaments can be traced without ambiguity in cross sections (see Franzini-Armstrong and Porter (1964 *a*), Kelly (1967), and Kelly and Cahill (1972) for other studies on narrow Z lines). Here advantage is taken of a recently described modification of the en bloc application of uranyl acetate (Locke et al., 1971) to obtain images of the Z filaments and their geometry. The results indicate that only models of the first group account for the appearance of the Z line in longitudinal and cross sections of fish muscle fibers.

MATERIALS AND METHODS

Tails from a small fish, the guppy (*Lebistes reticulatus*) were fixed by three alternate procedures, after the removal of the skin. (*a*) 1% osmium tetroxide in 0.1 M cacodylate buffer, pH 7.2, at room temperature for 1 h, dehydration in ethanol. (*b*) 3% glutaraldehyde in 0.1 M cacodylate buffer pH 7.2, for 2 h, rinse in the same buffer, postfixation in 2% OsO_4 in the same buffer for 2 h, dehydration in acetone. (*c*) 3–6% glutaraldehyde-H_2O_2 (Peracchia and Mittler, 1972) in 0.1 M cacodylate or phosphate buffer, rinse in the same buffer, postfixation in OsO_4 as for method *b*, en bloc treatment with 2% uranyl acetate in H_2O overnight at 60°C (Locke et al., 1971), dehydration in ethanol. All embeddings were in Epon. Sections were cut on a Cambridge Instrument microtome of A. F. Huxley design (Cambridge Instrument Co., Inc., Ossining, N. Y.), stained either with uranyl acetate in 50% alcohol and lead (Sato, 1968) or with the latter only, and examined by an AEI EM 801 microscope. The microscope was calibrated against a diffraction grating replica (2,160 lines/mm) at the beginning and end of the research period. The magnification was very constant, provided that hysteresis of the lenses was cancelled as suggested by the manufacturer. Measurements were made on pictures taken after hysteresis was concelled and thus the magnification error is no more than ±2%.

Remarks on Methods

Overall preservation of muscles fixed in glutaraldehyde and osmium tetroxide and stained en bloc with uranyl acetate (method *c*) is the same as that of material similarly fixed, but not contrasted en bloc, particularly with regard to the topology of the filaments (Fig. 1). The contrast is excellent and all qualitative description and most of the illustrations were derived from this material. On the other hand, there is a variable amount of shrinkage in both longitudinal and transverse directions, as indicated by the fact that the thick filaments are up to 11% shorter than the presumed in vivo length (1.5 μm) and the Z-line lattice dimensions are more variable than can be accounted for by changes in sarcomere length (see below).

Method *b* most faithfully preserves filament lengths (see Page and Huxley, 1963). Z-line thickness was thus measured in longitudinal sections from this type of material, cut with the knife edge parallel to the long axis of the fibrils to minimize changes due to compression (Carlsen et al., 1961), even though these are minimal in Epon-embedded material sectioned with a sharp diamond knife. Interfilament distances were obtained from material fixed by methods *a* and *b*. In cross sections, measurements were always taken along two directions at right angles. Longitudinal sections were cut with the knife edge perpendicular to the long axis of the fibrils. Contrary to what has been reported for frog fibers (Brandt et al., 1967), interfilament spacings were slightly wider in material prefixed in glutaraldehyde, relative to that treated with osmium tetroxide only (see Table I).

The diameter of the thin filaments was measured in cross sections after preparation with method *c*, in which the outlines are most clearly visible. The measured diameter, approximately 85 Å, is noticeably larger than the most commonly quoted value of 50–70 Å. The difference may be due to a combination of the following factors: (*a*) better preservation and contrast than previously achieved, allowing measurements which are closer to the in vivo diameter (the diameter of actin- and native tropomyosin-containing filaments is now estimated to be 80 Å (Moore et al., 1970); (*b*) on the other hand, overestimation of the diameter is likely to result from a slight longitudinal shrinkage which may be accompanied by thickening of the filament.

RESULTS

Structure of the Z Line

Since the Z line is very narrow in fish muscle white fibers (Fig. 2), its whole thickness is included in cross sections of the fiber at the appropriate level (Fig. 1 *a*). The two square lattices of the I filaments on either side of the Z line are seen to be identical, but staggered by a half-period relative to one another. This is particularly evident when one follows the image from one side to the other of a Z line (i.e., from top left to bottom right in Fig. 1 *a*): initially there are the profiles of one set of filaments lying at the corners of squares; then the filaments from the other side appear as faint outlines, at the center of each square; finally, the

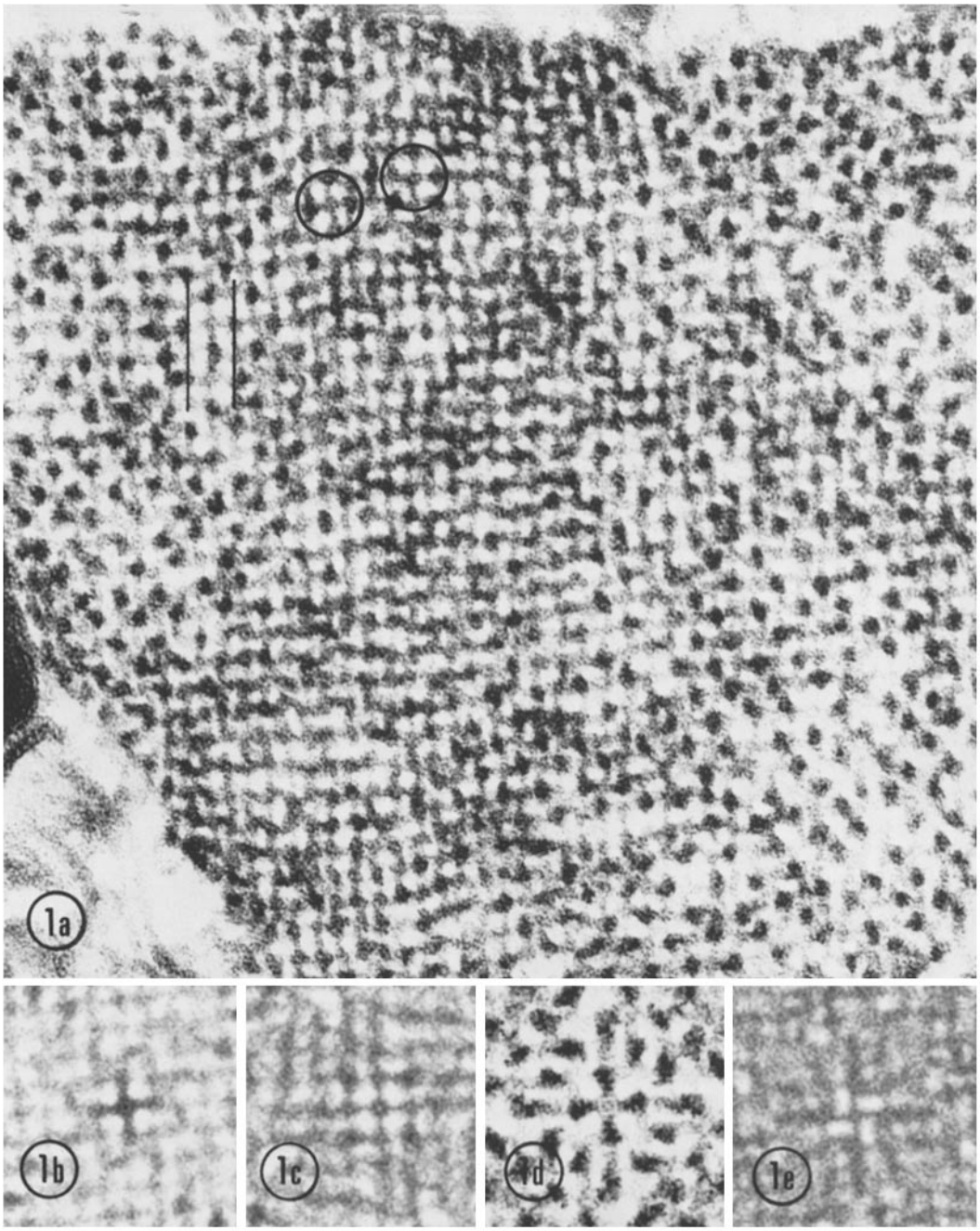


FIGURE 1 *a* Cross section of a fibril at the Z-line level. The thin filaments upon joining the Z line are disposed at the corners of squares (top left and bottom right of the image). The Z-line region, in the center, is occupied by a lattice at 45° relative to the thin filaments' lattices. Slight irregularities in the lattice are attributable to preservation artifacts. Circles surround thin filaments' projections around which the image was rotated to obtain Fig. 1 *b* and *c*. The two parallel lines give orientation and thickness of a section capable of giving pattern 3 (see text). × 200,000.

FIGURE 1 *b–e* Fourfold Markham rotations centered on a thin filament projection. Notice course of Z filaments and shape of light areas in the Z network. × 250,000.

first set fades, while the second becomes more prominent. I filaments from the opposite sarcomeres can thus be unequivocally identified and the course of the Z filaments can be easily related to them.

Z filaments occupy the diagonals of each square, running from the four I filaments at the corners to the one located in the center, which belongs to the opposite sarcomere. The pattern thus formed at the center of the Z line is a smaller square lattice with a repeat having the same value as half the diagonal of the original square lattice, and oriented at 45°. The relative orientations of Z- and I-band lattices are more visible in Figs. 4 and 5. In the Z-line lattice, Z filaments occupy the sides of the squares and alternate filaments from adjacent sarcomeres define the corners.

The basic symmetry of the structure is best put in evidence by applying the image rotation technique (Markham et al., 1964) to micrographs of material treated as in method *c* (Figs. 1 *b–e*). Before describing the results of this procedure, I would like to emphasize that it is used here only to enhance relatively gross details, already clearly visible in the original print (Fig. 1 *a*). The small asymmetries which are cancelled by the rotation technique are not of importance in the following description and discussion. For example, in the two circled areas of Fig. 1 *a* the thin filaments are not located exactly at the center of the squares. Such slight asymmetry, which is totally lost in the rotated image (Figs. 1 *b* and *c*), is present at random throughout the Z line and it is probably due to slight disarrangement of the structure during preparative procedures.

The Z filaments lie on an almost perfectly straight line joining the center of neighboring thin filaments. Each thin filament projection into the Z-disk plane is the center of fourfold rotational symmetry. Four axes of bilateral symmetry can also be drawn through the center of each thin filament projection, since the electron-transparent areas or "holes" in the Z network are almost perfect squares, with all four corners rounded off.

A slight variation of the above pattern occurs when the Z filaments, rather than being centered with the thin filaments, attach tangentially to them. As a result, the "holes" are elongated and the bilateral symmetry is lost, but the rotational symmetry is maintained. The loss of bilateral symmetry is evident even after rotation of the image (Fig. 1 *e*). Many Z filaments join the I filaments in a position which is intermediate between those described above, so that the holes in the network are not always perfect squares.

The spacings of the square lattice formed by the thin filaments in close proximity to the Z line, measured in cross sections of sarcomeres showing evident I and H bands (sarcomere length probably around 1.7–1.9 μm), are 253 $\pm$ 11 Å (n = 75) and 257 $\pm$ 9 Å (n = 120) for fibers fixed by methods *a* and *b*, respectively. After fixation by method *c*, the spacings are more variable and less than those just quoted: 225–240 Å. For a square lattice with a unit cell of 260 Å the distance between the filaments along the diagonals is approximately 366 Å. Half of that distance (183 Å) is the center-to-center separation between the thin filaments of adjacent sarcomeres when projected on the Z-disk plane, i.e., as seen in cross sections of the fiber.

The shape of the Z filaments has not been accurately determined in this study because the appearance in cross section cannot be correlated with that in longitudinal sections, where many profiles overlap in the image and obscure the details (see below). The apparent diameter of the Z filaments in cross sections is 50 $\pm$ 7 Å (n = 33). The thin filaments are wider near the Z line. Their diameter is 86 $\pm$ 7 Å (n = 57) in the I band and 108 $\pm$ 9 Å (n = 57) close to the Z line.

In longitudinal sections, the Z line takes three configurations (here called patterns 1–3) depending on section thickness and, more significantly, on the orientation of the section relative to the thin filaments' square lattices (Figs. 2 and 3). Two of these appearances have been defined previously (Knappeis and Carlsen, 1962; Franzini-Armstrong and Porter, 1964 *a*). In pattern 1, the Z line is occupied by a clear zigzag whose tips alternately join thin filaments from opposite sarcomeres. In pattern 2, the Z filaments run across the Z line parallel to the thin filaments, and the spaces between them are occupied by a poorly defined structure. Finally, in pattern 3, described here for the first time, a zigzag occupies the Z line, as in pattern 1, but its lines are not so clearly defined. In addition, longitudinally oriented Z-filament profiles bisect each branch of the zigzag. Intermediate areas of undefined structure usually connect the different patterns.

Pattern 1 is the most readily noticed, because of its visibility at low magnifications (Fig. 2); the zigzag image can be obtained over the length of several micrometers (Figs. 2 and 3 *a–c*), indicating that the pattern is not sensitive to small variations

in the relative orientation of square lattice and section plane. A reliable estimate of the separation between the thin filaments at their point of attachment to the Z line can thus be obtained. Measurements taken on fibers fixed by methods *a* and *b* are given in Table I, which also correlates the spacings with the sarcomere length. This point will be discussed below. Here it must be

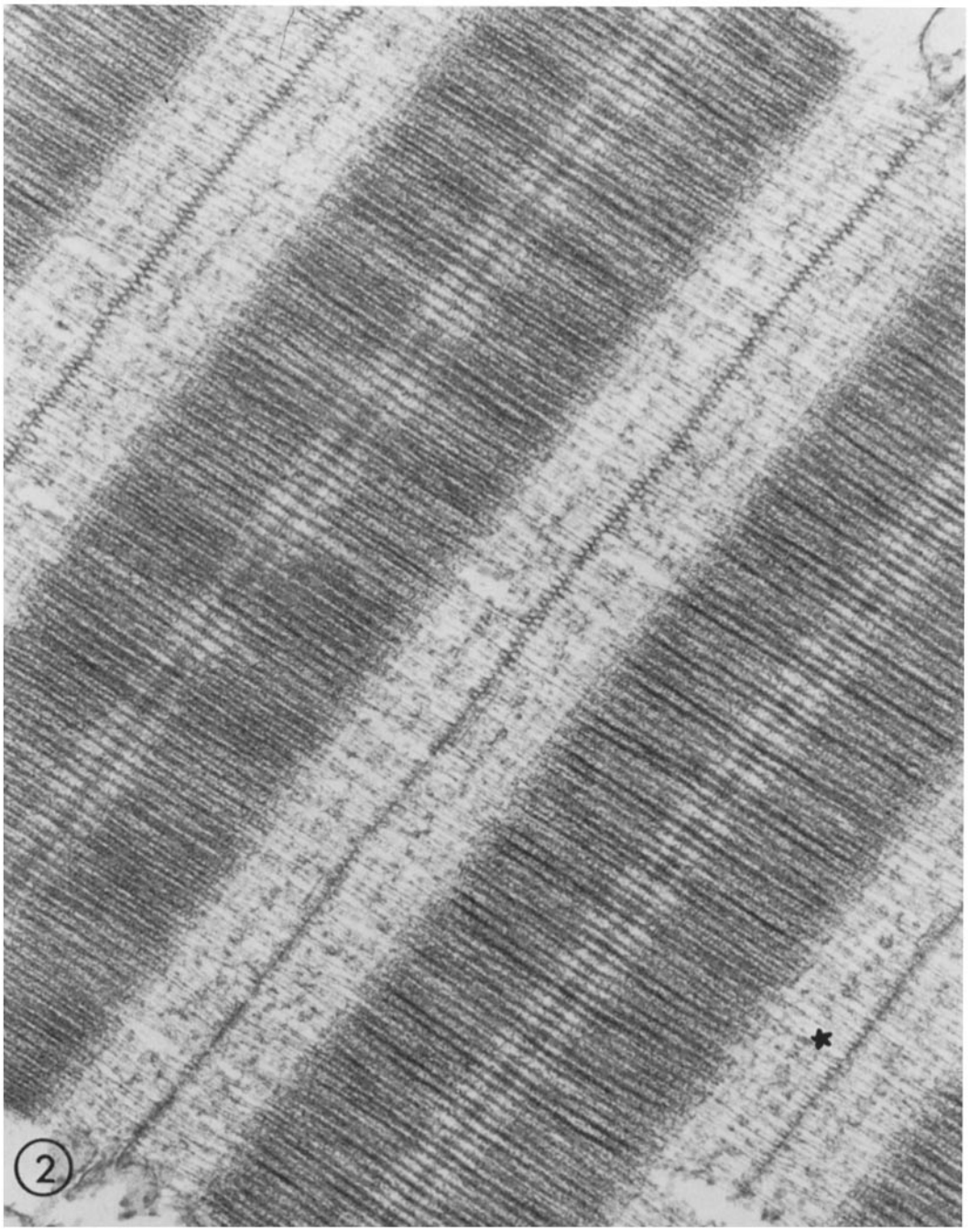


FIGURE 2 Longitudinal section parallel to one of the flat fibrils. Most of the Z line is in pattern 1 (zigzag). A short segment in the right bottom corner is in pattern 2 (asterisk). × 35,000.

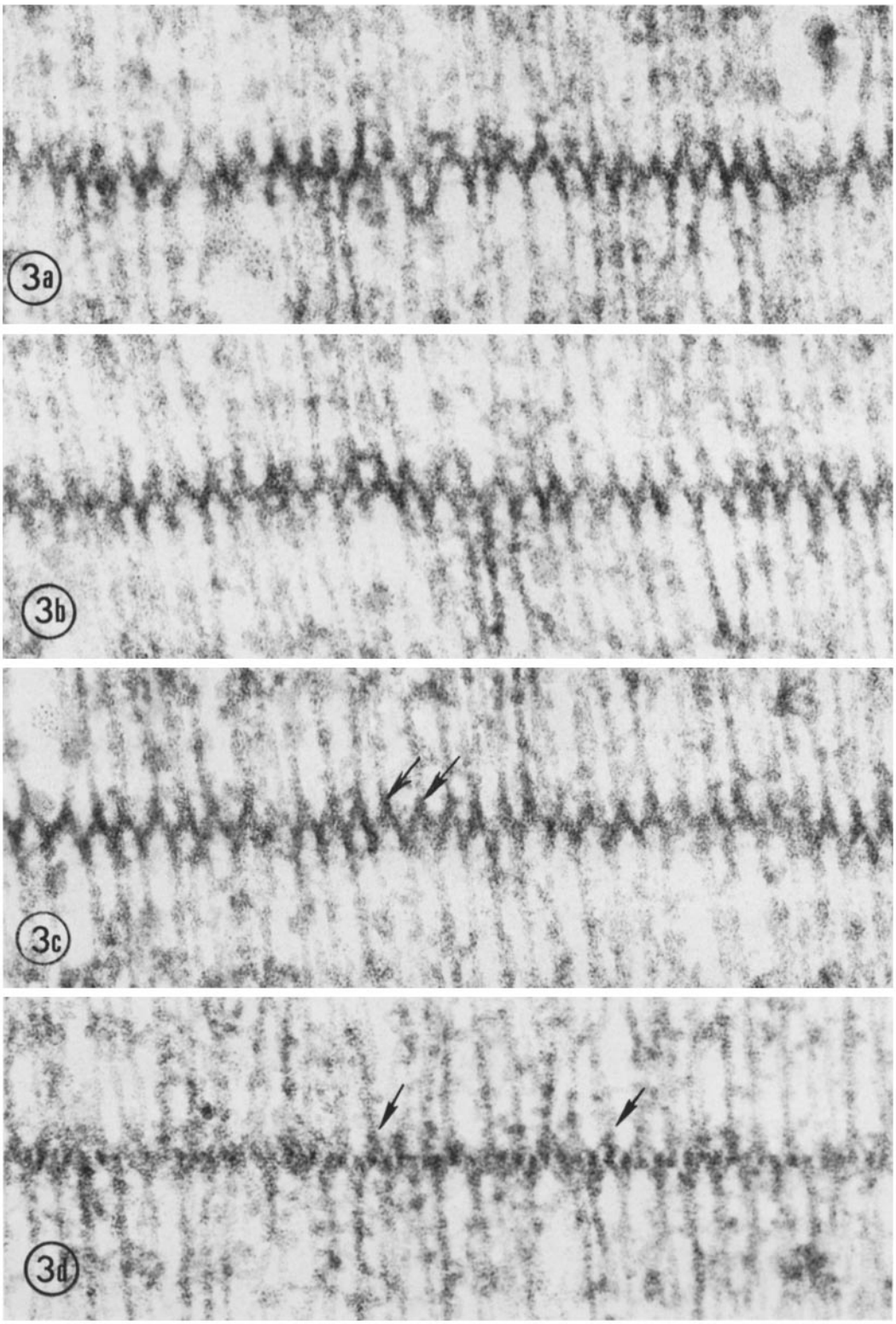


FIGURE 3 *a–c* Details of pattern 1. All figures are enlargements of a single Z-line image. Notice how pattern 1 can be obtained over a long distance. The thin filaments terminate at the edges of the Z line, and their terminal portion has a larger diameter (arrows). Slight irregularities in pattern correspond to those noticeable in cross sections. × 150,000.

FIGURE 3 *d* Short segment of pattern 3 (particularly evident at arrows). Notice apparent continuity of thin filaments into Z disk. × 150,000.

noted that the spacings given in Table I are close to the separation between the thin filaments in the I band square lattice as quoted earlier for cross sections, confirming previous evidence that pattern one arises when the section plane is parallel to the sides of the squares (Knappeis and Carlsen, 1962; Franzini-Armstrong and Porter, 1964 *b*). In Fig. 1 *a*, the viewer would obtain pattern 1 when looking at the Z line from any of the corners of the micrograph.

At least one square and, in most cases, more are included within the thickness of an ordinary section, so that each line of the zigzag is the image of at least two, usually more, Z-filament projections in the section plane. Minor variations in the appearance of the zigzag as one travels along an individual Z line (Fig. 2 *a–c*) may be attributed to the more or less accurate superimposition of the Z filaments into the image: the thinner, best-defined profiles arise from a nearly perfect overlap.

In pattern 1, the thin filament arrays from the two adjacent sarcomeres are staggered by a half-period and the filaments clearly do not penetrate the Z line. The apparent points of termination of the I filaments and the beginning of the Z filaments are not aligned along straight lines. This is probably due to two factors: (*a*) slight disarrangement of the structure, and (*b*) sensitivity of the image to variation in the tilt of the section plane relative to the Z-disk plane: when the two planes are perfectly perpendicular, the zigzag has the shortest segments. An estimate of the width of the Z line was obtained by marking the tips of the zigzag on both sides and then measuring the average separation between two lines joining the marked spots. The measurements were taken on images projected from negatives at a final magnification of 160,000. The value thus obtained is 281 ± 29 Å (n = 124). The large standard deviation is an indication of the variability in position of the marking spots.

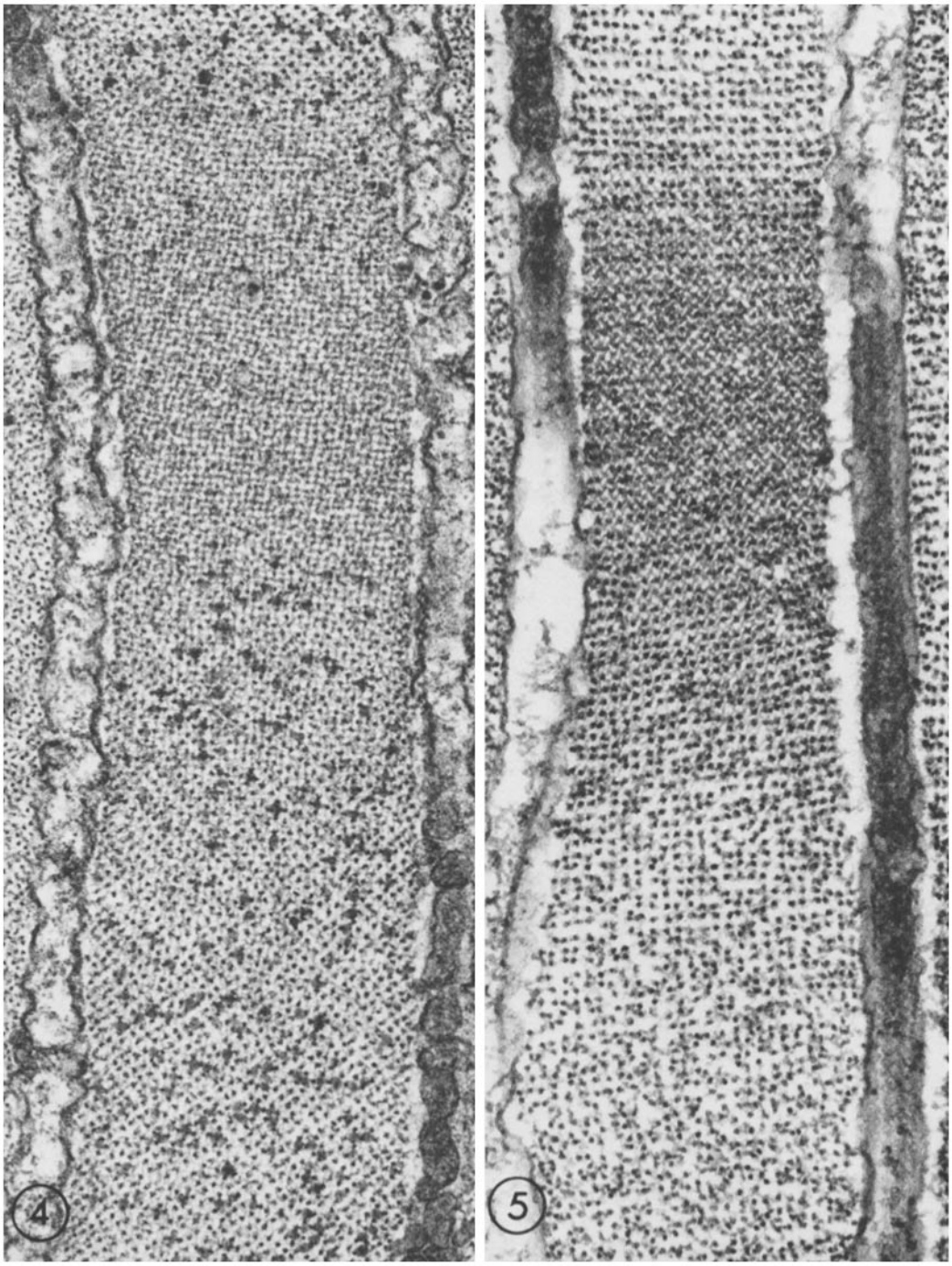
The enlargement of the terminal portion of the thin filaments is quite noticeable in pattern 1, where it extends over 200–300 Å. More accurate measurements were not attempted because of the variation in the apparent point of termination of I filaments (see above) and the difficulty in detecting the precise point at which the change in diameter occurs.

In pattern 2, the thin filaments reach the Z line in register and with a separation considerably less than in pattern 1. The plane of the section is thought to be parallel to the diagonal of the square lattice (Knappeis and Carlsen, 1962; Franzini-Armstrong and Porter, 1964 *a*). The continuity of the thin filaments into the Z line is only apparent, the longitudinally oriented filaments in the Z line being images of Z filaments which lie in planes perpendicular to the section and parallel to the fiber axis. Pattern 2 does not provide any relevant information and thus it is no longer discussed.

In pattern 3, the thin filament arrays are out of register, as in pattern 1. The configuration of pattern 3 is observed over very short distances and this introduces a large uncertainty in the measurements of the interfilament spacings. In areas where the pattern is most clear (e.g., Fig. 3 *d*, left), the spacings vary between 330 and 360 Å, values not significantly different from those of the length of the diagonals in the thin filaments' square lattice. Pattern 3 is thought to arise in thin sections which are parallel to the diagonals (see parallel lines in Fig. 1 *a*, separated by a distance of 250–300 Å). The arms of the zigzag are thus individual Z filaments and the longitudinally running profiles are the superimposition of two Z filaments running towards the observer and towards the back of the page, respectively. The longitudinal profiles give the false impression that I filaments penetrate into the Z line. A slight change in orientation and/or thickness of the section is sufficient to obscure the pattern (e.g., notice that similarly oriented but thicker sections produce pattern 2). Previous lack of notice of pattern 3 is probably due to low contrast of the preparations, which necessitated the use of thicker sections.

Variations in Z-Line Structure due to Changes in Fixation and in Sarcomere Length

In fish muscles, fixation in osmium tetroxide (Fig. 4) produces Z lines whose geometry is identical to that described just above for fibers fixed in glutaraldehyde and osmium tetroxide (Fig. 5). In both cases, the Z filaments define a lattice which is at 45° relative to the lattice of the thin filaments; they are almost perfectly aligned with the thin filaments' center and, finally, when projected into the Z-disk plane, they limit electron-transparent areas, which are approximately



FIGURES 4 and 5 Cross sections of Z line in fibers fixed by osmium tetroxide only and glutaraldehyde plus osmium tetroxide, respectively. Notice similarity in orientation of Z filaments relative to I filaments' lattices. After fixation with osmium tetroxide only (Fig. 4), the thin filaments have a smaller diameter. × 63,000.

square. The only minor difference between the cross-sectional images of Figs. 4 and 5 is in the diameter of the thin filaments, which is considerably smaller after fixation by osmium tetroxide alone. This gives the false impression that the Z lattice in Fig. 4 is expanded relative to that of Fig. 5. In reality, the spacings are almost identical and the small difference is attributable, as mentioned earlier, to the fact that Fig. 5 is from material treated en bloc with uranyl acetate.

The double hexagonal array of thin and thick filaments in the A-band expands, as the sarcomere takes shorter lengths (either passively or during active contraction), in such a way as to maintain a constant volume of the sarcomere (Huxley, 1953; Elliot et al., 1965). It is of considerable interest to know whether the Z-line lattice follows this variation or maintains a rigidly fixed spacing through changes in sarcomere length.

Sarcomeres of different lengths were obtained by two different methods. (*a*) A tail was bent as far as possible and then fixed in glutaraldehyde (method *b*) while held in this position. The longest sarcomere lengths obtained in the stretched portion of the tail were only about 2.1 μm, not much longer than the "rest length" of 1.9–2.0 μm that most sarcomeres have when the tail is straight. Fish tail myotomes thus function in vivo within a limited range of sarcomere lengths, near the maximum overlap. (*b*) After fixation in osmium tetroxide (method *a*), most fibers have contraction waves, usually close to either end, near the neuromuscular junctions (see Franzini and Porter, 1964). These provide a spectrum of sarcomeres fixed at different lengths, supposedly while in the process of contracting. Table I summarizes the results obtained. Despite some variability, it is clear that longer sarcomeres have smaller interfilament spacings at the Z line.

The general appearance of the Z line in pattern one, which is indicative of the geometry of the Z filament, is not affected by changes in sarcomere length, regardless of how they are obtained. In some fibers fixed in glutaraldehyde at short lengths (in the bent portion of the tail), "wavy" fibrils were produced. In frog fibers it has been demonstrated that this occurs when sarcomeres are passively pushed in towards lengths shorter than those at which the thin filaments meet in the center of the sarcomere (Brown et al., 1970). It is here assumed that the same occurs in fish muscle and thus that sarcomeres with wavy fibrils had been passively obliged to shorten just before fixation. Even in these sarcomeres the appearance of the Z line is unaltered.

TABLE I

Comparison of Sarcomere Length and Z Spacing using Preparation Methods a, b, and c

Preparation	S	Z spacing*	No. of spaces
	μm	Å	
a	1.68	250	40
	1.75	275	15
	1.86	244	23
	2.00	239	40
b	1.65	280	100
	1.70	265	40
	1.80	250	75
	1.90	264	50
	2.10	255	50
c	1.90	245	50
	2.05	237	50

Sarcomere length was in all cases normalized against a 1.5-μm A-band length. H zone disappears at $S < \sim 1.70$ μm.

* Separation between adjacent thin filaments joining the Z line in pattern 1. Cases where the measured interfilament distance is large (290 Å or more) have not been tabulated, because they represent areas of pattern 3, sometimes partially mixed with pattern 1. Each measurement represents one micrograph.

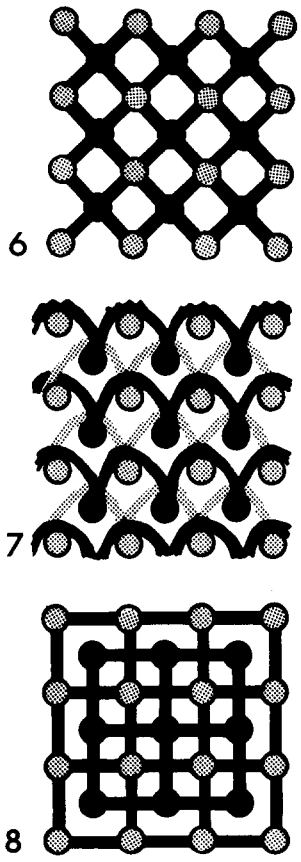
DISCUSSION

The Z line of white fibers in fish muscle is very narrow and it clearly contains only one layer of Z filaments. The thin filaments terminate at the edges of the Z line, so that, within the Z disk, Z filaments are the only visible structural components. This architectural simplicity makes the Z disk of fish muscles ideal for a study of the basic geometry of Z and I filaments, particularly when one considers cross sections in which there is no overlapping of the components in the image. The course and disposition of individual Z filaments can be directly visualized and an estimate of the fitting of different models can simply be done by a point-to-point comparison between appearance of the Z line and of the projection of each model into the appropriate plane.

A schematic top view of a Z line of the general type envisaged by Knappeis and Carlsen (1962)

and Franzini-Armstrong and Porter (1964 *a*) is illustrated in Fig. 6. The model assumes that each thin filament terminates at the edges of the Z line and there it is attached to four Z filaments. These cross the whole width of the Z line and join the four nearest thin filaments in the opposite sarcomere. In Fig. 6 the projections of the thin filaments into the Z plane are represented as circles; for clarity, filaments from one sarcomere are black, the other stippled. Dimensions of the filaments and their spacings are in accurate scale at an approximate final magnification of 700,000. The dimensions used are: 100 Å for the thin filaments diameter at the Z line, 260 Å for their spacing and 40 Å for the Z filaments. The latter is slightly smaller than the measured diameter. The same dimensions are used in Figs. 7 and 8.

Notice that in Fig. 6 the Z filaments occupy the diagonals of the squares defined by the thin filaments of one sarcomere and thus constitute a second square lattice at 45°, with a spacing correspondent to one-half the diagonal of the first lattice. The Z filaments define lines which cross each other over the center of the thin filaments. Four axes of bilateral symmetry can be drawn through the center of each actin filament, and the latter is also a center of fourfold rotational symmetry. The empty areas in the network are squares with the corners rounded off by the profiles of the thin filaments. As described above, all these features are represented in the image of the Z line in cross section, particularly following the Markham rotation technique. Lateral views of the model of Fig. 6 give images that are very similar to patterns 1 and 2, when the model is viewed from either the sides or the corners of the page, respectively. To obtain pattern 3, one should cut a thin slice of the model. The conclusion follows that a Z-filament type model adequately represents the geometry of the Z-line structure.



FIGURES 6–8 Top views of different Z-line models. For details, see text.

Production of a Z line with a "woven" appearance in cross sections (Reedy, 1964; Landon, 1970) requires only two slight modifications in the geometry, that is, tangential attachment of Z to I filaments and a sinuosity of the Z filaments. In most fibers, osmium tetroxide fixation (method *a*) produces Z lines with a woven appearance. In fish muscles this occurs only occasionally. The difference may be due to the fact that the Z filaments are very short and they are less likely to be distorted by osmium fixation.

Fig. 7 is a top view (analogous to Fig. 6) of a model built essentially as proposed by Kelly (1967), with two loops, arising from each thin filament, wrapping around filaments from the opposite sarcomere and returning to form part of other filaments from the same sarcomere. Thin filaments belonging to the sarcomere above the page and the loops arising from them are stippled, the others black, to help the reader in visualizing the structure. One major change has been introduced in Fig. 7, relative to the original proposal, i.e. Z loops, I filaments, and their spacings are depicted in scale relative to their observed dimensions, whereas in Fig. 9 of Kelly (1967) the thin filaments' spacing-to-diameter ratio is three times that in electron micrographs. Apart from this change in dimensions, which has been introduced in order to make the model comparable with the electron micrographs and with Fig. 6, care has been taken to preserve the original geometry as faithfully as possible. The drawing of Fig. 7 is not

necessarily acceptable to the author of the original model.

Fig. 7 differs from Fig. 6 and from actual micrographs of the Z line in three details. (*a*) The thin filaments are not centers of rotational symmetry and no axes of bilateral symmetry can be defined in the structure. The asymmetry of Fig. 7 differs both qualitatively and quantitatively from the slight random asymmetry which was described in Fig. 1. The quantitative difference is best illustrated by fourfold rotation centered on a thin filament. In the case of Fig. 7, this produces a blurred image, whereas in the actual photographs a very distinct image is retained (Fig. 1 *b–c*). The qualitative difference is due to the fact that in Fig. 7 the crossover point of the Z loops is consistently located on the same side of the thin filaments (towards the top of the page), whereas in actual micrographs the thin filaments' projections are displayed at random relative to the Z-filaments' profiles. (*b*) The asymmetry mentioned above is due to the fact that the crossover points of the loops belonging to different thin filaments do not coincide with the center of the thin filaments but they are at their periphery, that is, at a distance of slightly more than a thin filament radius from the center. The same is true in Fig. 9 of Kelly (1967), but the resultant asymmetry is hardly noticeable there because the thin filaments' diameter is much smaller. (*c*) As a result of the above points, the openings of the Z network are approximately rhomboidal, with a thin filaments' profile projecting from one of the corners. Such asymmetric openings are never visible in the electron micrographs and it must be concluded that this type of looping model cannot account for the observed appearance of the Z line of fish muscle in cross sections.

A discrepancy also exists between lateral views of Fig. 7 model and the appearance of the Z line in longitudinal sections, particularly when pattern 1 is considered. When viewed from either the top or the bottom of the page, so that the line of view is parallel to the sides of the thin filaments' square lattice, the model produces an image very similar to pattern 1. Viewing the model from either side of the page should also produce pattern 1. Instead, in this case the tips of the zigzag are not superimposed on the apparent point of termination of the thin filaments, but they lie on one side of it. Slight variations in the position of the viewer are reflected in a change of the position of the zigzag tips relative to the thin filaments, an effect which would be noticeable in sections of a Z line even at fairly low magnifications and which is obviously absent from recorded images of the real Z line.

In the model of Rowe (1971) four loops arise from each thin filament and connect it to the four nearest in the same sarcomere. When projected on the Z-disk plane, the loops occupy the sides of the squares defined by the two thin filament lattices. The two sets of filaments from opposite sarcomeres interdigitate and two sets of loops thus form the Z line. In fish muscle both sets of loops would be included within a thickness of about 300 Å and would always appear superimposed even in a very thin cross section of the fiber at the Z-line level. When projected into the Z-disk plane the two sets of loops define a small square lattice parallel to the thin filaments' lattice and with half its period. This is illustrated in Fig. 8. It is evident that no image of the Z line of fish muscle resembles Fig. 8, and that this model fails to account for the structure of such a simple Z line.

The above discussion clearly indicates that in fish muscles, and very likely in all fibers where the Z line is very narrow, the structure of the Z line is better explained assuming that the thin filaments terminate at the edges of the Z line and that Z filaments directly connect I filaments from adjacent sarcomeres. This view is also in close agreement with experimental findings indicating that (*a*) at least one protein different from the two major components of the thin filaments (actin and tropomyosin) can be extracted from the Z line and it is capable of interacting with the thin filaments and of reconstituting a Z line (Drabikowski et al., 1968; Goll et al., 1969; Stromer et al., 1969; Stromer and Goll, 1972). This protein is called α-actinin. (*b*) Anti-α-actinin but not antiactin or antitropomyosin react with the Z bands (Pepe, 1966; Masaki et al., 1967). (*c*) Finally, the thin filaments change polarity at the Z line (Huxley, 1963), a fact which introduces difficulties in looping models (See Kelly (1967) and particularly Rowe (1971) for a discussion of this point.)

One last point should be made about the unsuitablility of looping models, at least as originally formulated, to account for the structure of simple Z lines. No provision is made for attachment between elements belonging to adjacent sarco-

meres. Yet it is known that the full isometric tension (3–4 kg/cm²) is transmitted across the Z disk. One would expect this pull to produce some sliding of the two sets of loops arising from the adjacent sarcomeres and narrowing of the Z line in contracted sarcomeres. Conversely, passively pushing in the sarcomeres should broaden the Z line and produce a disarrangement of pattern 1 image. Such a slight broadening of the Z line has indeed been formulated by Kelly (1967) to explain the appearance of a simple Z line in some planes of sectioning. The observations reported here, showing that the structure of the Z line is unaltered during active and passive shortening, clearly indicate that some form of linkage between elements of the adjacent sarcomeres must exist at the Z line. Such linkage is most simply obtained by directly connecting I filaments from adjacent sarcomeres to one another, as in Z-filament type models. Of course, a similar linkage can also easily be introduced in looping type models.

In most vertebrates the Z line is considerably wider than in fish muscles, the width being in many cases correlated with the fiber type (Gauthier, 1969; Schiaffino et al., 1970). Mammalian red fibers have Z lines which are approximately 1,000 Å wide. After fixation in osmium, wide Z lines usually have a "woven" appearance in cross sections (Reedy, 1964; Landon, 1970; MacDonald and Engel, 1971) and it is thought that the thin filaments penetrate into the Z band, where they overlap with the thin filaments of the other sarcomere (Landon, 1970; MacDonald and Engel, 1971; Rowe, 1971). These details of the structure can easily be accommodated into a Z-filament type model. After glutaraldehyde fixation, on the other hand, the same Z lines have a structure closely resembling Fig. 8 (Landon, 1970). Such an image cannot be explained solely on the basis of a simple Z-filament model as described here for the fish fibers.

Attempts at constructing Z-line models capable of accounting for the two configurations of Figs. 6 and 8 are numerous and mutually exclusive. (*a*) Landon (1970) attributes the differences introduced by fixation to a complete transformation of the structure, requiring disconnection of Z from I filaments, rotation by 45° and attachment in a different configuration. The basic model chosen is a looping model, which is most readily capable of accounting for Fig. 8. (*b*) Interestingly, MacDonald and Engel (1971) succeed in producing the two images starting from a Z-filament type model. The advantage of this interpretation is that transformation from one pattern to another only requires a change in curvature of the Z filaments, without any detachment and reorganization. (*c*) Rowe's (1971) intriguing looping model is the first to combine in a single structure the capability of accounting for both patterns. The same model can also be stacked to produce a Z line composed of multiple rows of zigzag, such as occurs in rat red fibers (Rowe, 1973). Unfortunately, this model fails to account for the structure of simple Z lines, as described above. (*d*) Finally, Kelly and Cahill (1972) propose the existence in wide Z lines of a matrix component, which after glutaraldehyde fixation is clumped more densely (producing an image comparable to Fig. 8), whereas after osmium tetroxide fixation it is less visible, thereby unmasking the underlying structure, in the approximate shape of Fig. 6.

The stimulating search for a comprehensive model of the Z line is, thus, far from terminated. This study, limited as it is to a single type of Z line, one whose structure is not altered by the use of different fixatives, cannot provide an answer to the problem. It should be noted, however, that since a Z-filament type model, providing direct links between I filaments of adjacent sarcomeres, is demonstrably the only one capable of fully accounting for the structure of narrow Z lines, a more complex model must, to be of general use, incorporate these features. It is important to note that the general appearance of fish Z lines is not altered by fixation.

The thickening of the last portion of the thin filaments is not unique to fish muscles. It has also been observed in rabbit (Goll et al., 1969) and in frog (Franzini-Armstrong, 1970). In view of the fact that this thickening disappears when Z line proteins are extracted (Goll et al., 1969), it is logical to assume that the Z filaments do not terminate at the edges of the Z line, but that they penetrate into the I band, where they attach to the thin filaments over a length of about 200 Å.

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