# MODELS OF MUSCLE Z-BAND FINE STRUCTURE BASED ON A LOOPING FILAMENT CONFIGURATION

DOUGLAS E. KELLY

From the Department of Biological Structure, the University of Washington School of Medicine, Seattle, Washington 98105

ABSTRACT

The fine architecture of skeletal muscle Z bands is considered in view of stereo electron microscopical evidence and current biochemical and immunological concepts, and a new Z-band model is proposed. This model is based on a looping, interlinking configuration, within the Z band, of strands which emanate from I-band (actin) filaments of adjacent sarcomeres. Two versions of the model seem presently feasible: one in which the Z-band lattice is composed of actin loops; and another in which the same pattern is derived from tropomyosin. Either version satisfies actual electron micrograph images as well as or better than prior Z-band models. Moreover, the principle of looping linkage in filament-to-filament attachment can be related to similar filament patterns seen in several adhesion sites where intracellular filaments insert on cell membranes.

## INTRODUCTION

Significant progress has been recorded in the last decade in regard to a fine structural and functional understanding of contractile filament systems in vertebrate muscle (see Hanson and Lowy, 1963, 1964; Huxley, 1963, 1964; Pepe, 1966), the majority of this information being focused on the structure and interaction of actin and myosin complements in striated muscle A- and I bands. Less certainty has been expressed, however, as to the fine structure of Z bands and the means by which actin filaments of one sarcomere are coupled to those of its neighbor.

This problem has been considered by Knappeis and Carlsen (1962), Franzini-Armstrong and Porter (1964), and Reedy (1962, 1964) who provided models conforming to electron microscope images taken of Z bands at various angles of view. Huxley (1963), pointing out the striking three-dimensional similarity between some of those images and images of crystalline tropomyosin under certain circumstances, suggested that this protein might be the Z-band structural backbone. Other workers have considered developmental implications of Z bands (Heuson-Stiennon, 1965; Allen and Pepe, 1965) or the involvement of this structure in certain myopathic conditions (Gonatas, 1966; Gonatas et al., 1965).

In longitudinal, thin sections of skeletal muscle, a zigzag appearance of Z bands (Figs. 1, 4) can be interpreted as suggesting that actin filaments of adjacent sarcomeres might be continuous across the Z band, in effect being shared on a disarcomeric basis. However, because evidence of actin in the Z band itself is scarce, and since such an arrangement would require bipolarized and nearly 2-$\mu$ long actin filaments, most investigators have favored models in which actin filaments are monosarcomeric, about 1 $\mu$ in length, and terminate at the edge of the Z band (the Z–I boundary). At that point some form of filamentous (Knappeis and Carlsen, 1962; Reedy, 1962, 1964) or membranous (Franzini-Armstrong and Porter,

FIGURE 1 Three moderately stretched larval newt skeletal muscle fibrils sectioned nearly longitudinally. Sarcomeres between the Z bands (*z*) display typical A (*a*) and I (*i*) regions. The cytoplasm lateral to each Z band contains apposed elements of the tubular T-system and sarcoplasmic reticulum (arrows); in one region these are sectioned tangentially (top arrow). Note not only the typical zigzag appearance of Z bands in some regions, but also the variety of other images discernible along this structure. Collidine–$OsO_4$ fixation; lead and uranyl acetate stain. × 39,500.

1964) interconnection has been envisioned as coupling, across the Z band, the actin tips of one sarcomere with those of its neighboring sarcomere. It is now generally agreed, in view of the biochemical evidence (Huxley, 1963, 1964; Hanson and Lowy, 1963, 1964; Depue and Rice, 1965) that actin filaments within the A- and I bands must exist as double-stranded, right-hand helices.

This latter fact has posed some concern because, in order for the above mentioned models to satisfy the patterns seen in electron micrographs, (Figs. 4–6) four strands, or the apices of four, joined, triangular membranes, would have to be attached at the Z–I boundary to the tip of each double-stranded actin filament. The mode of such a four part to two part attachment is not revealed by the micrographs.

In the course of an investigation on the fine structure of desmosomes and hemidesmosomes (Kelly, 1966 *a*) it was found that intracellular filaments which converge to be anchored at such firm cell-to-cell adhesion sites actually loop past the point of filament-to-cell membrane attachment and course back into the interior of the cell. When it was subsequently observed that looping filament profiles are also detectable at amphibian myotendinal junctions (Fig. 2) and at cardiac, intercalated disc junctions (Fig. 3), the possibility

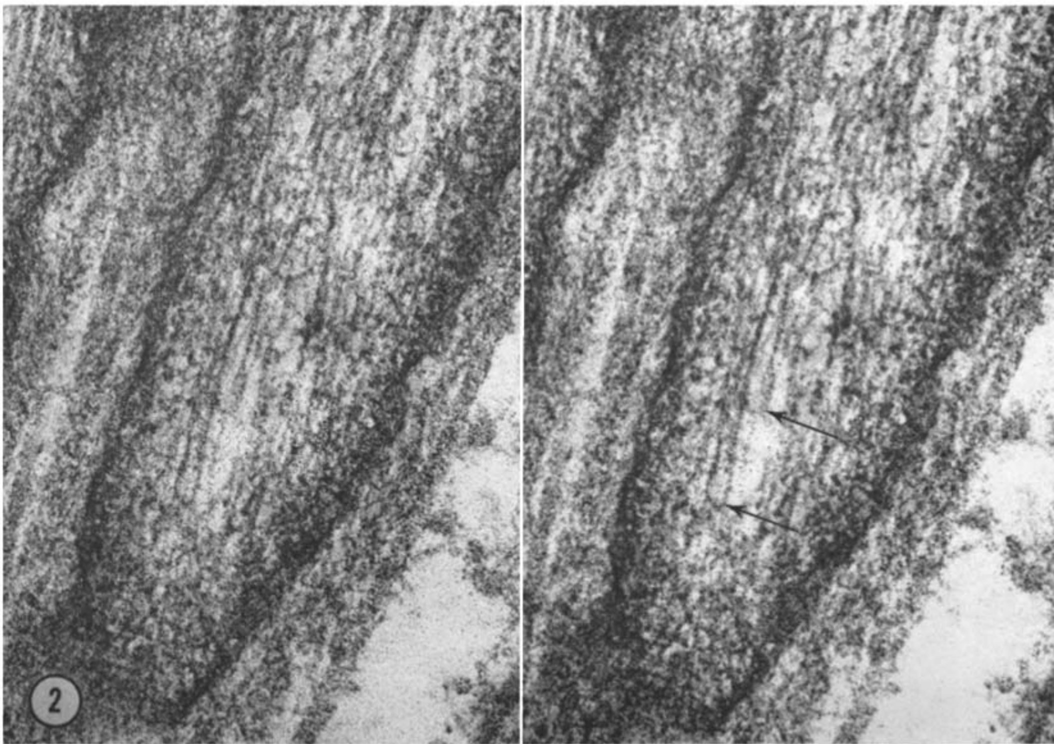



FIGURE 2 Stereo electron micrograph pair* showing a portion of a myotendinal junction from the limb of an older newt larva. A tongue of muscle cytoplasm (center) extends into a bed of collagen (sides and bottom). Filaments reaching into the tongue from the contractile system anchor along the plasma membrane. The possibility that such filaments loop into and out of the anchorage site is posed by the presence of several hairpin profiles (arrows). Collidine–$OsO_4$ fixation; lead and uranyl acetate stain. $\times$ 119,500.

* A three-dimensional image can be obtained by holding this page about 12–18 inches from the eyes and gazing at the micrographs with eyes crossed. By this means each eye comes to view only one micrograph of the pair in a fused image. Slight tilting of the head may aid in fusing that stereo image. Stereo micrographs of sectioned material on this and following pages may also be viewed with a cartographer's stereoscope, in which case the depth relationships are reversed. This method fails for the stereo photographs of models.

arose that such looping filament excursions might be a more general pattern of filament-to-membrane attachment (Kelly and Luft, 1966). At the intercalated disc junction, the looping filaments either are, or are related to, actin filaments of the cardiac, contractile, fibril system which terminates at that cell junction. In the sense that this fibril-to-membrane attachment occurs at one end of a sarcomere, the zone of looping filaments corresponds positionally to a Z band. If filament looping occurs at the junctional end of a terminal sarcomere, the question arises as to whether looping filament configurations might also be incorporated into intersarcomeric filament-to-filament junctions which Z bands represent.

This report analyzes electron microscopical data showing that a looping filament–Z-band concept is not incompatible with actual images, and that it explains some images better than do prior models. Two plausible, new Z-band models based on a looping filament pattern are outlined, of which one model has already been presented in a preliminary abstract and demonstration (Kelly, 1966 *b,c*).

## MATERIALS AND METHODS

Muscle tissue was obtained from larval stage West Coast newts, *Taricha torosa* (collected as embryos in the Stanford, California area). Most examinations were made on the superficial cervical rectus muscle, the lateral anconeus humeralis muscle, and ventricular myocardial cells from young (2–6 wk old) or nearly metamorphosing larvae. After being anesthetized with MS-222 (Tricaine-methanesulfonate obtained from Sandoz, Inc., New York), whole larvae or excised muscles were fixed for 1–2 hr with ice-cold 3.75% osmium tetroxide in 0.05 M *s*-collidine buffer (Bennett and Luft, 1959), or for 7 hr in one-half strength Karnovsky's (1965) paraformaldehyde-glutaraldehyde formula at room temperature. In the latter case, tissues were rinsed in 0.1 M phosphate buffer and were postfixed in osmium tetroxide for 1 hr. Tissue blocks were dehydrated in a graded series of ethanols and in propylene oxide and then were embedded in Epon 812 (Luft, 1961). Thin sections, cut on a Porter-Blum MT 2 ultramicrotome with diamond knives, were stained with half-saturated, aqueous uranyl acetate for 8–10 min at 55°C, followed by 8–10 min treatment with the alkaline lead-citrate procedure of Reynolds (1963). Micrographs were obtained using a Philips EM 200 electron microscope equipped with specimen cooling blades and a 12° tilting stage for stereo electron microscopy.

Filament models depicted in this report were constructed of size 12–14 plastic-insulated copper wire.

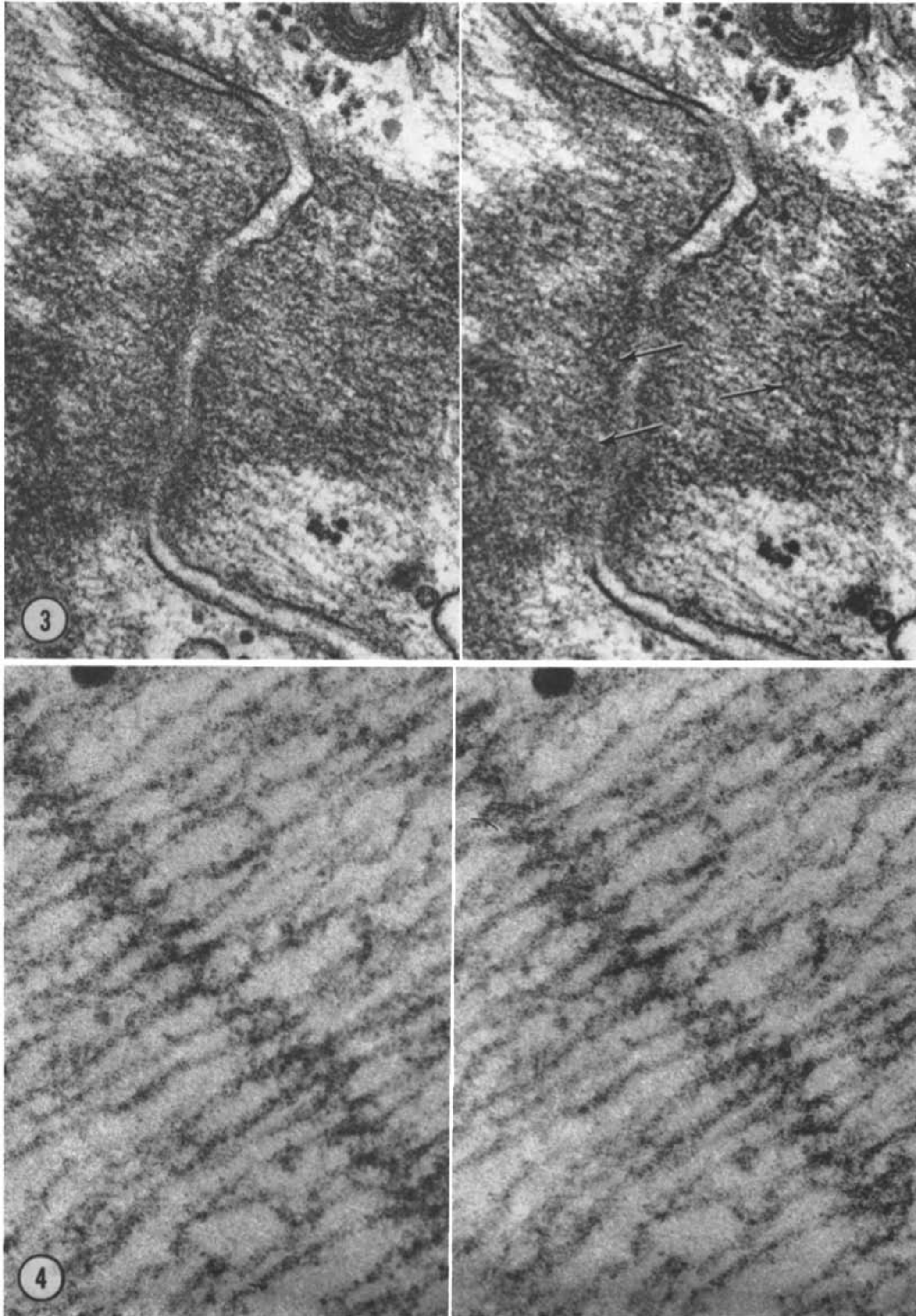
## OBSERVATIONS

Z bands of various muscles display divergent images in micrographs, in part owing to the quantity of densely stained, finely granular or "amorphous" material which may come to infiltrate the presumed filament network. In some cases, such as cardiac muscle and older larval skeletal muscle, this infiltration seems maximal, since it widens the Z band and almost totally obscures the network architecture. On the other hand, young larval skeletal muscle Z bands, especially if fixed with collidine–osmium tetroxide, are nearly devoid of this material, and, hence, are more suitable for this study.

Another type of diversity is also apparent, namely, the wide variety of Z-band patterns discernible in electron micrographs of any given muscle. In longitudinal sections of sufficient thinness, a most striking (and, therefore, perhaps most commonly portrayed) image is a zigzag line coursing across a given myofibril (Figs. 1, 4). The points of this line alternately appear to connect with thin actin filaments of adjacent sarcomeres; this provides a Z band of 400–500 A breadth.

FIGURE 3 Stereo electron micrograph pair of an intercalated disc junction between two myocardial cells of a young larval newt heart. Actin filaments approach each side of the junctional area where they are intermixed with a dense meshwork. Stereo examination of the meshwork region discloses numerous arching profiles (arrows) suggestive that thin filaments loop into and out of the attachment region. Collidine–$OsO_4$ fixation; lead and uranyl acetate stain. × 84,000.

FIGURE 4 High magnification stereo electron micrograph pair showing a portion of a skeletal muscle Z band sectioned in a plane longitudinal through the fibril. In the upper half of the picture, the Z-band image conforms predominantly to a zigzag pattern, while toward the lower right a series of looping profiles is visible. Collidine–$OsO_4$ fixation; lead and uranyl acetate stain. × 140,000.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Together with "face-on" images of Z bands taken from fibril cross-sections (Fig. 5), the zigzag pattern is, in large part, the basis for prior conceptual models of Z-band structure.

Close inspection of longitudinal sections, especially when one is employing stereo electron micrographs, reveals, however, that many other patterns can be found within Z bands. These are certainly due to the fact that each Z band is not perfectly regular or oriented to the section plane, and hence, portions of it will appear as if sectioned in slightly different planes. The previously proposed models can account for most of such image variations on this basis.

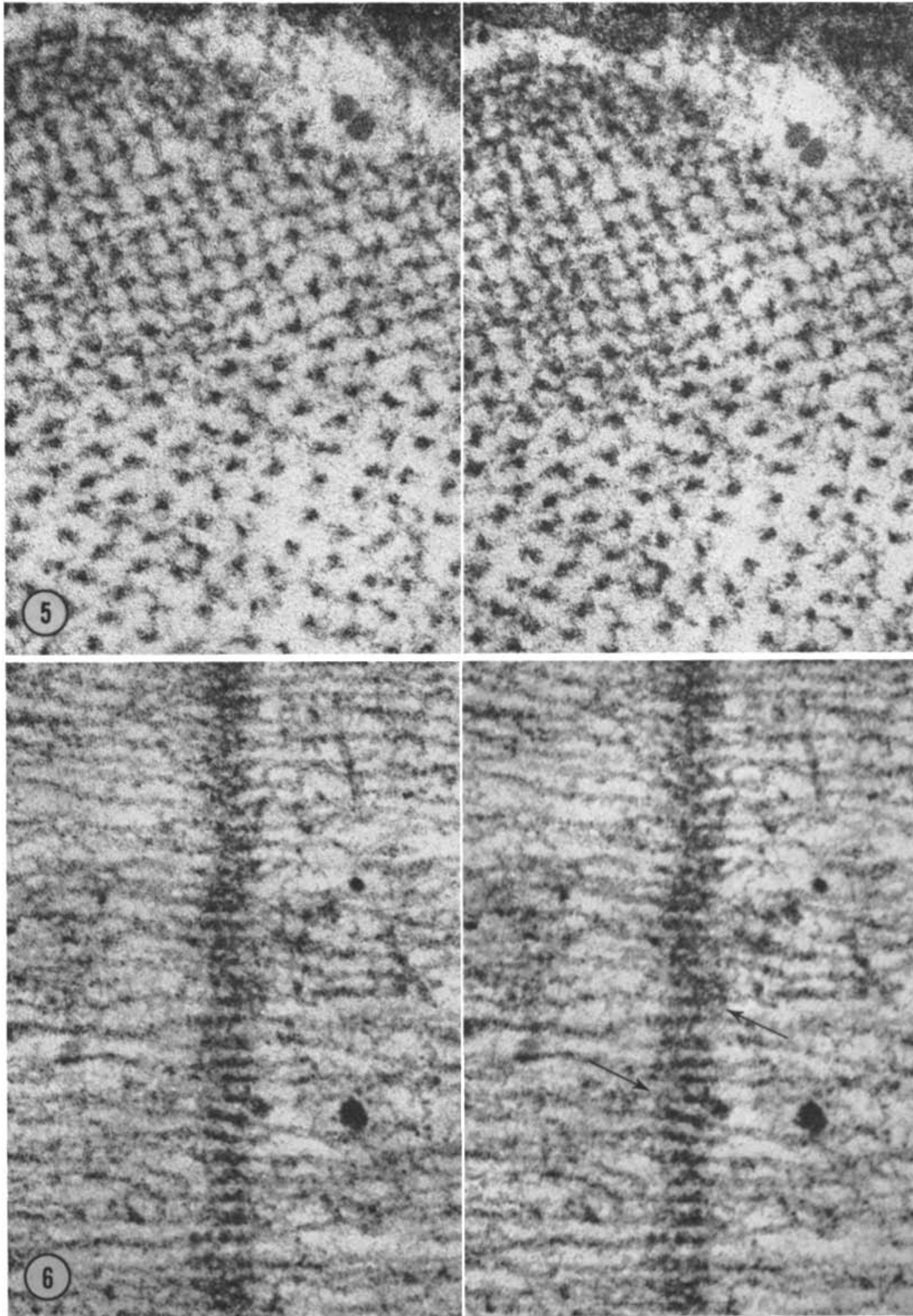
Of significance to the present proposal are interconnections between the Z band and actin filaments which do not seem to form angular joints, but rather appear as circular or arching densities. Fig. 4 provides a fairly striking comparison between angular and arching Z-band profiles. While the arching image can be explained speculatively on the basis of prior models as being due to distorting influences or deposition of extrafilamentous material, an equally plausible interpretation might be the presence of a "hairpin" conformation of filaments involved in the Z band. This would imply that filaments or strands of filaments entering the Z band from one side would loop within the Z band and return to the same sarcomere. Critical examination of many Z bands in longitudinal sections discloses numerous, looping filament images of varying size and shape to which such a concept might be applied.

Another variation of Z-band structure which presents difficulties for interpretation is shown in Fig. 6. Here a zigzag line is lacking. Rather, many filaments from one sarcomere appear to communicate directly through the Z band with filaments of the adjacent sarcomere; the only obvious demarcation of the Z band is an increased density or thickness over a short (600–800 A) segment of the *apparently* continuous filaments. Diagonal interconnections between neighboring filaments in the midst of the Z band, such as would be expected from the Knappeis and Carlsen or Franzini-Armstrong and Porter models, are not discernible. In fact, interconnections which do appear between adjacent filaments seem more common at the edges of the Z band rather than in its midst. This phenomenon has special implications for the models to be presented in this report.

Face-on, cross-sectional views of Z bands in this investigation have provided patterns which very closely conform to those presented by previous workers (Fig. 5). When the thickness of the section includes the whole breadth of a Z band, a tetragonal lattice array is visible. In some areas, the pattern has a "basket-weave" appearance which apparently, as first described by Reedy (1962, 1964) is due to a left-hand, tangential departure of lattice filaments as they emerge in fours from the corners of each tetragon. Other areas display a regular array of squares instead of the woven pattern. Stereoscopic views of this lattice disclose that each corner is the junction of four radiating strands from a single actin filament, and that neighboring corners represent actin filaments approaching from opposite sarcomeres. What cannot be discerned, and, therefore, must be left to speculation, is the actual nature of the junction of each actin filament with its four radiating

FIGURE 5 Stereo electron micrograph pair showing the region of a Z band in a cross-section of the myofibril. The Z-band lattice face-on forms a woven image in the center of the picture but it is more regular in other areas. Each dense actin filament approaching the Z band from the viewer's side or the side opposite appears to separate into, or be connected with, four finer strands. The model shown in Figs. 7 and 9 illustrates how this image can be obtained on the basis of interlocking loops from double-stranded actin filaments. Collidine–$OsO_4$ fixation; lead and uranyl acetate stain. × 186,000.

FIGURE 6 Longitudinally sectioned Z-band. In the lower half of this stereo pair, the Z band seems to be delineated only by the dense appearance of filaments which course undiverted through the Z band. In this region, no interconnection is visible along the center of the Z band. Rather, images suggesting interconnection occur at the ends of the dense area (arrows). I-band filaments appear closer together than in Fig. 4 and the width of the Z band may be slightly greater. Fig. 11 shows a view of the model which might explain this image. Collidine–$OsO_4$ fixation; lead and uranyl acetate stain. × 125,000.



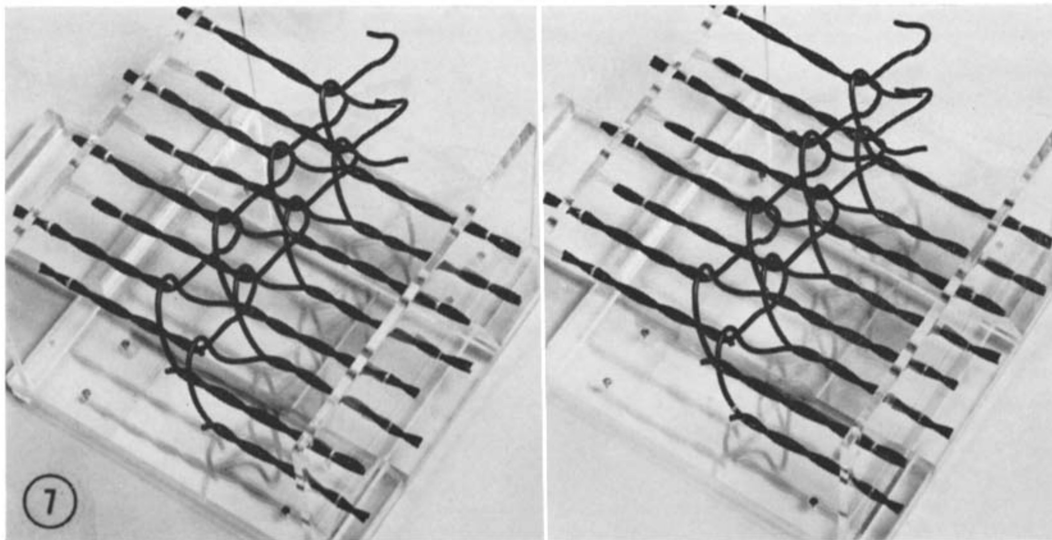


FIGURE 7 Stereo view of a model portraying two tiers of I-band filaments in which Z-band structure is represented as the interlinked loops of actin strands (dark wires). These loops arise as the uncoiled ends of I-band actin double helices. See Discussion in text concerning advantages and weaknesses in this arrangement.

strands. Do these points represent a splitting of each actin double-helix into four substrands? Is this the junction of four nonactin strands to the tip of the actin filament as proposed by Knappeis and Carlsen (1962)? Or might there exist at that point a looping of one strand over another set of two strands? High-resolution study of these small regions does not as yet allow any certain choice between the alternatives.

Nor do the observations made in this study allow a decision as to whether the Z-band lattice is membranous, as Franzini-Armstrong and Porter (1964) proposed, or filamentous. Stereoanalysis of young larval skeletal muscle seems to favor a filamentous fine structure,[1] and on this assumption an attempt was made to devise filament models that might account adequately for all the above observations while simultaneously incorporating the looping or hairpin filament configuration.

## Z-Band Models

LOOPING ACTIN STRAND CONFIGURATION: Figs. 7–9 and 11 illustrate a Z-band model constructed upon the assumption that the radiating fine strands of the Z band are strands of actin. Hence, each of the wires in this model represents an individual actin strand. I-band actin filaments on either side of the Z band are each composed of two such strands twisted into the right-hand actin double-helix now generally accepted from biochemical evidence (Hanson and Lowy, 1963; Depue and Rice, 1965). However, where a given I-band actin filament abuts the Z band, i.e. at the Z–I boundary, its strands separate, each coursing into the Z band to loop over an actin filament approaching from the opposite sarcomere. This looping-over occurs at the Z–I boundaries on either side of the Z band by the interplay of actin strand loops from apposing sarcomeres. Therefore, at the Z–I boundary each actin filament appears grossly to give off four radiating strands, although in actuality only two are its own unwinding actin strands, and two are the approaching and departing legs of a looping actin strand from the opposite side. After coursing through the Z band and looping over an opposite filament, each actin strand returns to its own sarcomere where it is incorporated into the

[1] In older larval skeletal muscle, and in cardiac muscle, where Z bands are infiltrated with a dense, amorphous material, face-on views of the Z bands provide images which seem interpretable as reflecting a membranous Z-band structure.

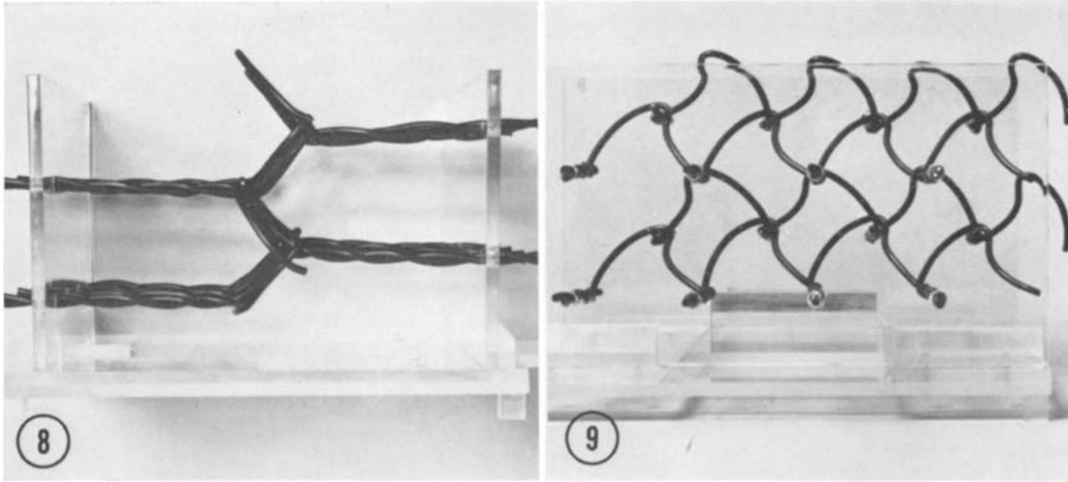


FIGURE 8 Lateral view of the all-actin Z-band model. This view corresponds closely to the zigzag Z-band image seen in the upper half of Fig. 4.

FIGURE 9 End-on view of the all-actin Z-band model. This view corresponds closely to the face-on view of Z-band lattice shown in Fig. 5. The looping wires of the model can be slightly curved to obtain the woven appearance seen in the center of Fig. 5. While they have been curved clockwise in this model, the same pattern results if the loops are curved in a counterclockwise fashion. If left uncurved, the looping wires provide a regular array of squares when viewed from this angle.

double helix of a neighboring I-band actin filament. Every actin strand according to this configuration is a hairpin, the legs of which are shared between two actin filaments, and the loop of which interlinks within the Z band.

The looping, interlinking relationship of this model is especially well displayed in Fig. 9 which corresponds to a cross-sectional, face-on view of the Z band, such as seen in Fig. 5. In the model portrayed, each looping portion has been slightly curved (this can be done clockwise or counterclockwise), and in this way the model comes to conform in this view to the basket-weave pattern which Reedy (1962, 1964) analyzed.

If the model is interlinked by the looping relationship just described, it is possible for its two adjacent sarcomeres to be slid together in a longitudinal direction (Fig. 11). This permits an expansion of the Z band, so that looping of actin strands over actin filaments occurs at some distance into the sarcomere from the former Z–I boundary; this is to say, the Z band is slightly broadened. When viewed at the proper angle, as shown in Fig. 11 an image is obtained which bears a close similarity to that in the micrograph in Fig. 6, wherein the Z band is slightly broader than normal, displays an apparent continuity of actin filaments through its breadth, and shows interconnections mainly or solely at its extremities.

LOOPING TROPOMYOSIN STRAND CONFIGURATION: A second model can be constructed combining the concept of looping Z-band components with the evidence suggesting that the Z-band lattice is composed of tropomyosin (see Introduction). In fact, such a model also incorporates a suggestion advanced by Hanson and Lowy (1963) that tropomyosin of the I band is located as two strands which lie within the spiraling grooves of the actin double-helices.

In this model, shown in Fig. 10, the dark wires represent actin strands, again arranged in double helices of the I band. However, in this case the actin filaments terminate at the Z–I boundary; the total length of any given actin strand is, therefore, about 1 $\mu$ compared with the 2 $\mu$ or more length required for each actin strand in the previous all-actin configuration. Tropomyosin strands (light wires in Fig. 10) course along the two grooves of each actin double-helix and continue past the Z–I boundary to make up the structure of the Z band proper. Within the Z band these tropo-

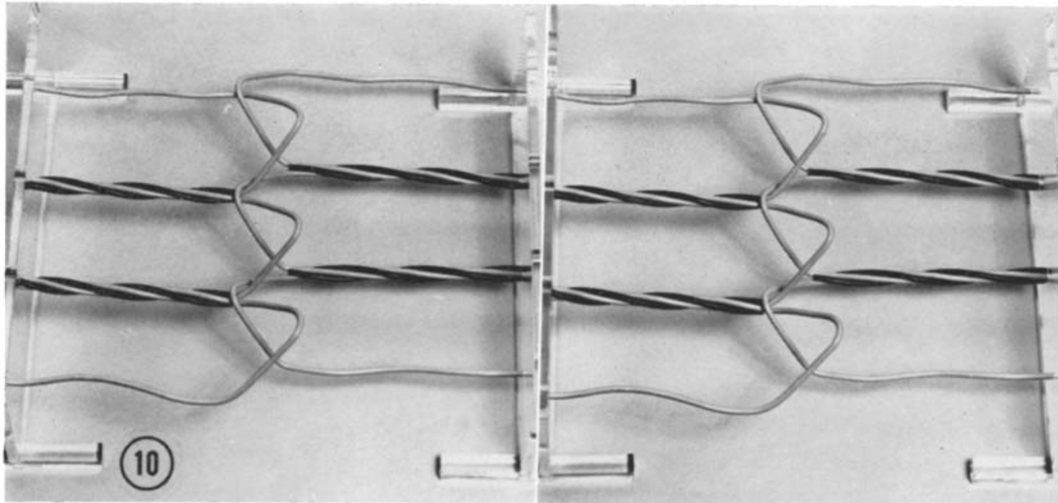


Figure 10 Stereo view of a Z-band model based on the same principle as that shown in Fig. 7, but in this case the looping interlinking strands (light wires) are formed of tropomyosin while actin (dark wires) is limited to the I-band actin double-helices. Tropomyosin not only forms the Z-band lattice but also resides as strands in the grooves of the actin double-helices. The model portrayed is constructed as a single tier of I-band filaments, but can be extended to multiple filament tiers just as was the all-actin model in Fig. 7.

myosin strands loop and inter-link in precisely the same manner as described for actin hairpins in the all-actin configuration.

## DISCUSSION

From any angle of view the two models proposed in this report are identical in arrangement, in concept, and in their compatibility with electron microscope images. Tropomyosin strands in the second model run a course which parallels that run by actin strands in the first model. However, certain facts known or suspected at the present time might be cited as militating against or in favor of one model over the other.

An all-actin looping model, for example, appears to require the presence of a very long (about 2 $\mu$) actin strand which is incorporated into two neighboring helical, I band, thin filaments over different parts of its length. It will be noted that synthetic and native actin filaments combined with heavy meromyosin display a characteristic polarized periodicity in the form of repeating barblike subunits (Huxley, 1963). In the case of native filaments, the barbs seem always to point away from the Z band. This polarized orientation of actin molecular substructure is believed to be related to its interlinking action in life with the A-band myosin filaments. It may be difficult to reconcile the all-actin Z-band model with these molecular concepts since the legs of the actin hairpins which are incorporated into neighboring helices might be required to display molecular polarization in opposite directions, a reversal occurring somewhere within the Z-band, looping portion of each strand. Without reversal of polarity, the two strands in every actin filament would possess opposite polarities. Such a reversal along the length of actin filaments or strands apparently has not been observed in isolated or synthetic preparations. Moreover, while actin filaments (not strands) exceeding 1 $\mu$ in length have been prepared synthetically, those isolated by homogenization of muscle fibers generally measure less than 1 $\mu$ (Allen and Pepe, 1965).

Because information about the polarity of single strand actin is lacking, such facts do not presently invalidate the looping, all-actin model. Homogenization may fragment long actin filaments, perhaps preferentially within the Z-band portions to give the characteristic filaments of less than 1 $\mu$ length. And the fact that synthetic actin filaments longer than 1 $\mu$ display no reversal in polarity does not exclude the possibility that such a reversal might exist along native strands.

Another critical question bearing on this model is whether actin does in fact reside within the Z band. Immunological approaches which might ascertain this point have given negative or equivocal results (Pepe, 1966). It is known, however, that Z bands are more susceptible to the action of trypsin than are I filaments (Ashley et al., 1951; Guba et al., 1960), a fact which might relate either to finer, more susceptible strands in that site, or to protein-containing, supportive matrices and junctions.

The looping tropomyosin model is not subject to the above criticisms laid against the all-actin model. Actin filaments in that scheme are on the order of 1 $\mu$, and are excluded from the Z band proper. Hence, no polarity reversal is required. Hanson and Lowy's (1963) suggestion on the location of tropomyosin as strands along grooves of I-band actin helices receives support from the immunological demonstration of I-band tropomyosin (Pepe, 1966; Ebashi and Kodama, 1966). And Huxley's (1963) demonstration of the similarity between face-on Z-band lattice patterns and crystalline arrays of tropomyosin certainly favors the reality of the Z band's containing tropomyosin. But again, a weakness in that reasoning at present is the fact that immunological tests for tropomyosin in the Z band have been negative, and therefore, silent on the question at hand (Pepe, 1966).

If the presence of Z-band tropomyosin is assumed, this model provides an easily visualized continuum between Z-band tropomyosin and the I-band tropomyosin. Since I-band tropomyosin is implied to be doubly helical (Hanson and Lowy, 1963), a looping model of the sort proposed here allows that continuum. A similar case might also be made for the all-actin model by saying that, if the Z-band lattice could be proven to be composed of actin strands, then a looping configuration of the type proposed is perhaps the only way of providing a continuum between Z-band actin strands and actin strands of the I-band actin double-helices.

Neither model is presumed to represent the total morphology of the Z-band region. As noted above, Z bands in the system under study become more dense with age, and Z bands in other skeletal and cardiac muscles appear so dense that filamentous fine structure is obscured. This property may be due to the deposition of matrix materials specifically around filaments or strands in the Z-band region.

Several forms of striated muscle myopathy, particularly *nemaline myopathy*, are characterized by the occurrence of dense, greatly expanded Z bands, often one or several microns in breadth. While the dimensions and periodicities within these giant Z bands are now described, their precise content remains obscure. Gonatas (1966) has proposed that they are formed from parallel stalks or discs of material resembling or identical with normal Z-band components, and that they attach at their borders to I-band actin filaments. He considered prior evidence equating such thickened Z-band material with tropomyosin accumulations as equivocal, and has suggested that Franzini-Armstrong and Porter's (1964) membranous Z-band model might present the most plausible basic subunit for such formations.

The models proposed in the present report might appear at first to be at odds with this concept of myopathic Z-band thickening, particularly since it is difficult to envision how interlinked Z-band units might be broken or otherwise expanded as the thickening takes place. However, if one considers the opposite motion, a sliding together of Z-band hairpins (as seen in Figs. 6 and 11, but to a greater extent), it can be seen how a given Z band might be widened at the expense of I bands or even degenerating A bands. Under such circumstances its full width might be invaded by some depositing periodic material, or filled by successive, longitudinally oriented hairpins of the fundamental type shown in the models, until the widened Z band was literally packed by their loops and legs. It remains to be seen how well such hypothetical, closely packed arrays might conform to the actual periodic dimensions observed in the diseased muscle.

In another myopathy, "central core" disease, some Z bands are similarly thickened while others are obscure or apparently lacking (Gonatas et al., 1965). It is enticing to speculate that here and in certain natural cases of Z-bandless sarcomeres, the effect might be due to a disperse arrangement of looping linkages over a broad area of I band in contrast to a preferential register or alignment of such linkages along a narrow and visible typical Z-band.

It might be argued that thickened Z-band myopathic conditions might occur more readily if the looping models were constructed in a nonlinking fashion where attachment of loops might occur *via* molecular bonding of parallel segments.

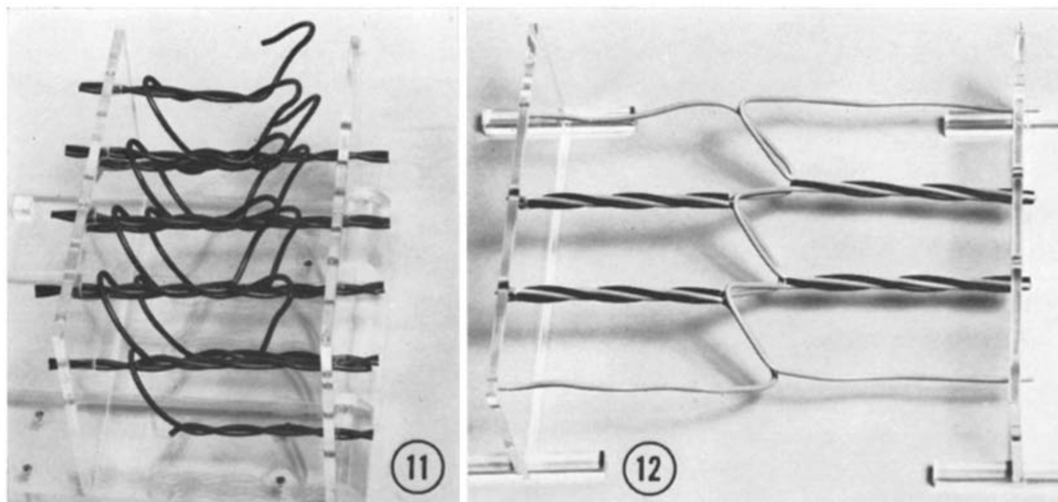


FIGURE 11 Oblique view of the all-actin Z-band model after sliding the wires of the two sides together. Such action widens the Z-band region, and when viewed from this angle, the model conforms closely to the Z-band image shown in the lower half of Fig. 6.

FIGURE 12 A variation of the tropomyosin-actin Z-band model shown in Fig. 10. In this case the loops of tropomyosin are unlinked in the Z-band region and lie apposed, presumably linked by intermolecular bonding. See text for discussion of the desirabilities of such a configuration and the reasons why it must be discarded.

Widening in this case would be accomplished by separations of the appropriate bonds. Such an arrangement is pictured in Fig. 12. When this arrangement is viewed in a one-tiered model, as is depicted, it seems that such a model is plausible. However, when additional filament tiers are added, it is readily found that the model no longer conforms to observed electron micrograph patterns, particularly cross-sectional, face-on Z-band views, and on that basis such a model must be rejected.

It is difficult to visualize a morphogenetic sequence that would integrate the fabrication of an interlinking, looping system with the concomitant organization of presumably the same strands and filaments into the rest of each sarcomere. Heuson-Stiennon (1965) has presented fine structural evidence that small, dense "Z bodies" arise along the cell membranes of developing myoblasts and are the precursors of the Z bands. This suggests that the foundations of Z-band organization are realized in relation to cell membranes, and oriented prior to the development of interposed sarcomere myofilament systems and myofibrils. Interestingly, Heuson-Stiennon pointed out the morphological similarity of the Z bodies, at their inception, to embryonic attachment sites such as young desmosomes, intercalated disc junctions, and insect myoepidermal junctions. She emphasized that, while the filament proteins of desmosomes are different from those of contractile proteins, they appear in a fashion strongly similar to that which she described for Z bodies. Analogy between Z bands and desmosomes has also been made by previous authors on somewhat different grounds (Franzini-Armstrong and Porter, 1964).

What is suggested in this report is that an even stronger basis for comparison between Z-band and desmosome structure exists by way of a fundamental looping filament configuration at both sites, and that such a configuration may be basic in nature to both filament-to-membrane and filament-to-filament attachment systems. If one deals with the tropomyosin model version, for example, it seems reasonable to postulate that in Z-band formation the initial production of a lattice of looping tropomyosin hairpins may be necessary in providing an orientation backbone along which actin and myosin filaments subsequently become aligned. Heuson-Stiennon's ob-

servations that such a precursor phase is initiated at the cytoplasmic surface of the cell membrane suggest that it is no coincidence if there is indeed a looping Z-band pattern akin to the well-known filament looping pattern at desmosomes (Kelly, 1966 *a*).

The advantages of the two models presented on these pages lie mainly along hypothetical lines linking Z-band fine structure to a pattern of filament arrangement known or strongly suspected to exist at several different cell-to-cell or cell-to-connective tissue adhesion sites. Both models seem to explain certain electron micrograph images somewhat more plausibly than former models and they correlate well to certain biochemical probabilities. But beyond this they stand at present as no more substantiated as representative of the living system than do prior Z-band concepts. Concurrent effort along biochemical, immunological, and developmental lines will help to ascertain which, if any, of the models now available most adequately represents Z-band organization.

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