THE Z LATTICE IN CANINE CARDIAC MUSCLE

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ABSTRACT

Filtered images of mammalian cardiac Z bands were reconstructed from optical diffraction patterns from electron micrographs. Reconstructed images from longitudinal sections show connecting filaments at each 38-nm axial repeat in an array consistent with cross-sectional data. Some reconstructed images from cross sections indicate two distinctly different optical diffraction patterns, one for each of two lattice forms (basket weave and small square). Other images are more complex and exhibit composite diffraction patterns. Thus, the two lattice forms co-exist, interconvert, or represent two different aspects of the same details within the lattice. Two three-dimensional models of the Z lattice are presented. Both include the following features: a double array of axial filaments spaced at 24 nm, successive layers of tetragonally arrayed connecting filaments, projected fourfold symmetry in cross section, and layers of connecting filaments spaced at intervals of 38 nm along the myofibril axis. Projected views of the models are compared to electron micrographs and optically reconstructed images of the Z lattice in successively thicker cross sections. The entire Z band is rarely a uniform lattice regardless of plane of section or section thickness. Optical reconstructions strongly suggest two types of variation in the lattice substructure: (a) in the arrangement of connecting filaments, and (b) in the arrangement of units added side-to-side to make larger myofilament bundles and/or end-to-end to make wider Z bands. We conclude that the regular arrangement of axial and connecting filaments generates a dynamic Z lattice.

KEY WORDS  cardiac muscle  Z bands  optical diffraction  image reconstruction

In canine cardiac muscle the Z band in longitudinal section varies in width from 80 to 160 nm and is a repeating filamentous structure with associated electron-dense material at the edge of each sarcomere. In cross sections the Z band is delineated from the surrounding sarcoplasm by vesicles of the sarcoplasmic reticulum (2), and the Z band has a repeating tetragonal unit cell (6). At higher magnifications, two distinct lattice forms are observed in association with this tetragonal unit cell (6).

We have recently studied the Z lattice and its relationship to the adjacent lattice of thin filaments at rest length in normal cardiac muscle using optical diffraction techniques (6). In this paper, we examine in greater detail small regions of the Z band extending for ~250 nm on a side. We have examined these details in successively thicker sections to include one, two, or three layers of lattice
units. We have used optical reconstruction techniques to clarify the substructure of the Z lattice and, more importantly, to preserve the irregularities that are consistently observed in the lattice. We have built and tested two different models for the mammalian cardiac Z lattice to deal with the possible similarities between cardiac and skeletal muscle and to test these models against our optical reconstruction data. A number of models based on thin Z bands in skeletal muscle have been proposed (4, 8, 10, 12, 15, 16, 21), and these have been reviewed recently (21). Several features from some of these models are also discussed in the light of the data from mammalian cardiac Z bands. A brief report of some of this work has been published (5).

MATERIALS AND METHODS

Preparation of Muscles

Four dog hearts were surgically removed and slit open to expose the left ventricle. Strips of superficial fibers of the papillary muscles were clamped to prevent shortening, then excised and immersed in 4% paraformaldehyde-5% glutaraldehyde in Millonig’s phosphate buffer at pH 7.4 for 1 h at room temperature (Group I). Then the muscles were cut into 1 mm cubes and placed in fresh fixative for an additional 2 h. The tissue samples were rinsed in buffer for 2–16 h and postfixed in 1% osmium tetroxide in phosphate buffer, dehydrated rapidly in ethanol, and embedded in flat silastic molds. For comparison, papillary muscles from three additional dog hearts were fixed at room temperature in a microtubule polymerizing medium consisting of 100 mM piperazine-N,N’-bis(2-ethane sulfonic acid) (PIPES) at pH 6.9 with 5 mM magnesium sulfate and 1 mM EGTA and 5% glutaraldehyde (Group II). En bloc staining with 1% uranyl acetate was carried out in one experiment for each fixative used.

Normal Z bands examined in a previous study (6) were analyzed in left anterior and posterior papillary muscle from four normal dog hearts and in anterior papillary (control) muscle from two other dog hearts, made partially ischemic.

Electron Microscopy

Thin sections were cut on a Porter-Blum MT-2B ultramicrotome (DuPont Instruments-Sorvall, Newtown, Conn.) by a diamond knife. The long axis of the myofibrils was parallel to the knife edge in the longitudinal sections. Sections were stained by immersion in a saturated solution of uranyl acetate in 50% ethanol for 5 min, followed by flotation on aqueous lead citrate for 3 min. Thin sections were examined in an RCA EMU-4 or a Philips 201 electron microscope. The microscope was calibrated with a Fullam carbon grating (Ernest Fullam, Inc., Schenectady, N. Y.) for each group of negatives. Measurements of Z band filament diameters were made with dial calipers (0.05 millimeter) on electron micrograph prints photographically enlarged 2–3 times to a final magnification of 200,000–300,000.

Optical Filtering and Reconstruction

EM positives (× 18,000) on Kodak high contrast lantern slide plates were used as diffraction subjects as described previously (6). When operating in the image reconstruction mode, we used both electron micrograph negatives and positives for filtering and reconstruction. We found that white on black images were easier to interpret. However, the original negative was a little too low in contrast and the second negative made from the positive had lost some critical information. Therefore, we used positives for reconstruction. An image reversed in the final printing stage is shown only for the longitudinal section. Image modification was carried out at the Fourier transform plane by placing a thin blackened brass plate in position. Holes drilled through the plate corresponded to the positions of the central laser beam and all the diffraction spots recorded from the original periodic structure. If a reflection was not intense enough to be observed, a hole was drilled in the location predicted by the lattice period. The filter was positioned optically to insure its precise alignment. Thus, only light rays due principally to the regular portion of the structure were allowed to pass through the retransformation lenses.

A different mask was prepared for each image of the Z lattice, and the hole sizes were adjusted to be larger than corresponding spot diameters. Typically, on our masks a distance of 1.3 mm corresponded to a reciprocal distance of 24 nm. The large diffraction spots were actually about 0.25 mm in diameter, each hole size was 0.63 mm in diameter except for the main beam hole which was twice this size. The local averaging distance was 0.63/1.3 (1/24 nm⁻¹) = 1/48 nm⁻¹ or the inverse of 2 × 24 nm; thus the averaging was calculated to be over two unit cells in all directions.

RESULTS

Optical reconstructions of the Z lattice are considered from three different orientations previously chosen to define the unit cell dimensions (6). In this study we examine the substructure of the lattice, paying particular attention to the arrangement of the individual filaments and the associated amorphous material.

Two different fixation procedures have been used and the overall appearance of the lattice is similar in both Groups I and II. Fig. 1 contains data from a longitudinal section of a normal dog cardiac Z band in the orientation referred to as the 24-nm orientation. Fig. 1 a is an electron micro-
graph of this muscle from Group II. The adjacent sarcomeres in this muscle preparation were measured to be 1.75 μm.

Fig. 1b, the unfiltered optically reconstructed image, reproduces the micrograph image and shows how much of the lattice is obscured by the amorphous dense material associated with the Z structure. Concentrations of amorphous material are apparent as four periodic transverse bands seen as one scans transverse to the myofibril direction. Cross-connecting filaments are not always visible, but may be obscured within the bands of amorphous material. The axial filaments when viewed along the myofibril direction appear to be continuous through the Z band and terminate on opposing sides. The thin filaments of the adjacent I bands are continuous with the axial Z filaments. The thin filaments become more electron dense about 38 nm before entering the Z band.

The optical diffraction pattern is shown in Fig. 1c and is similar to patterns previously recorded for this orientation of the Z lattice in cardiac muscle at rest length (6). The most prominent features of this pattern are the intense first- and second-order equatorial reflections corresponding to the 24-nm lattice vector transverse to the myofibril axis. A single layer line parallel to the equator is consistent with the 38-nm repeating lattice structure previously observed (6).

The diagram (Fig. 1d) indicates the positions of holes used in forming the mask for the filtered image reconstruction of Fig. 1a. Fig. 1f, a photographic reversal of filtered image Fig. 1e, is included to show that some features of this lattice are more readily apparent in a white-on-black image. Optically filtered images (Fig. 1e and f) show three layers of lattice units stacked end-to-end in the direction of the myofibril axis to give a Z band width of ~120 nm. The axial filaments appear most uniform in the middle layer of units. Twelve lattice units stacked side-to-side are seen clearly within a transverse distance of 288 nm. Even with the image enhancement, only a few connecting filaments are clearly visible. The axial filaments are aligned suggesting a regular end-to-end stacking. Apparent periodic discontinuities or disordered regions of the axial filaments occur every 38 nm, coincide with the amorphous regions seen in the unfiltered image, and span distances of ~13.5 nm along the filament axis.

Figs. 2 and 3 are images of the cardiac Z lattice from a cross section, silver in color, and estimated to be between 60 and 80 nm thick. These figures should be compared to Fig. 4, a much thicker section cut from the same block of muscle. Two lattice forms are visible in the electron micrograph Fig. 2a. The basket-weave pattern is seen best in the middle of this Z band (region 1), and the small-square pattern is seen most clearly at the edge of the Z band toward the top of the micrograph (region 2). Fig. 2b is a photographic enlargement of region 1 showing the details of the connecting filaments. The diagonal basket-weave appearance of the lattice is actually due to an accentuation of the spaces between the curving filaments, and one is recognizing the background rather than the actual filaments of the lattice.

Fig. 2c shows a typical optical diffraction pattern for the basket-weave appearance. Note the enhanced (11) reflections. In Fig. 2d, the arrangement of the connecting filaments from corner-to-center of the large square array gives a projected image which looks like a headphone. Since the section is 60–80 nm thick, we are looking at projections of at least two layers. A comparison of the enlarged electron micrograph (Fig. 2b) with the black-on-white filtered image shows an enhancement of structural details. Fourfold symmetry of the axial filaments becomes apparent in the filtered image. Several different curvatures of connecting filaments from straight to semicircular are also seen. This variation corresponds to the variation in the interstitial spaces from roughly rectangular to hourglass-shaped. The average brightness of the background shows apparently random variations over distances of 4–8 unit cells. This may be due to long-range variation in the amount of amorphous material masking the ordered structure.

Fig. 3a shows region 2 of the same micrograph with the associated diffraction patterns, Fig. 3b–d, and the optically filtered reconstruction, Fig. 3e. In the electron micrograph both lattice forms are visible. The small square pattern is visible at the top third of the micrograph, and its associated optical diffraction pattern is seen in Fig. 3b. In the bottom two-thirds of Fig. 3a, the basket-weave appearance predominates but the small square pattern does persist. This is confirmed by the optical diffraction pattern of this region shown in Fig. 3c. Note the enhancement of the (11) reflections. The optical diffraction pattern of the entire region 2 is shown in Fig. 3d. Fig. 3e is the filtered reconstruction of the entire region using the mask.
shown in Fig. 3f. Note that both the basket-weave and small-square patterns are faithfully reproduced. In Fig. 3e, an apparent step-wise top-to-bottom transition between the two lattice forms is enhanced. Yet, on closer inspection, features of both lattice forms are seen throughout. Stated in another way, the reconstructed image shows a change in the background shape (see in white) from a rectangular shape to a pinched hourglass shape to two distinct lobes, corresponding to the change from basket weave to small square.

The interfilament spacing between the axial fil-

![Image](image-url)
aments is fairly constant in region 2. This is seen as a regular side-to-side packing for the square arrays of crosscut axial filaments seen in Fig. 3a and e. Elsewhere in this same Z band the square array is rotating or distorting and conforming to the shape of the myofibril (or vice versa). This change in orientation is independent of the transition from basket-weave to small-square appearance. Note that the diffraction pattern for region 1 shows a different orientation for the \{10\} reflections. This change can also be seen by viewing the micrograph at a 45° angle to the page. The region to the right of region 1 shows a more abrupt change. The basket-weave appearance in this area is rotated 45° with respect to the adjacent basket weave in region 1. Inspection of an \times8 enlargement of the electron micrograph shows that in the border between region 1 and the region to the right the change occurs within a single square.

Fig. 4a shows an electron micrograph of a small portion of a Z band in a section 140–160 nm thick, also from the same block. Fig. 4b is the filtered optical reconstruction. Again, both lattice forms are visible. The extent of the persisting basket-weave appearance was about the same as in the intermediate and thinner cross sections of the Z band. The corresponding optical diffraction pattern in Fig. 4c is typical for the basket-weave appearance. The distinct profiles of the crosscut axial filaments suggests an end-on-end enhancement and a very regular stacking in the direction of the myofibril axis. The connecting filaments are also enhanced in this thicker section. Note that there is some variation in the distance between axial filaments and, again, this distance is independent of the apparent curvature of the connecting filaments. Note also that there is unequal enhancement between the axial filaments at the corners of the large square array and those at the center. This is probably related to the periodic discontinuities or disordered regions shown in longitudinal sections such as in Fig. 1.

In cross sections the knob-like appearances of the axial filaments are due to projections of the connecting filaments. We know from comparisons between thin sections and between intermediate sections of Z bands with and without en bloc staining that the loss of filament diameter in the middle of the connecting filaments is not due to a lack of stain penetration. If the plane of cross section is perpendicular to the axial filaments, as in this study, the connecting filaments appear more or less opaque and more or less thin depending upon two factors: (a) the level of sampling of the cross-sectional lattice (recall the abrupt shift in Fig. 1a) and (b) the tilt of the cross sectional lattice with respect to the plane of sectioning (recall the transition of connecting filament profiles in Fig. 3a in going from small square to basket-weave pattern). The axial filaments appear thicker than the connecting filaments regardless of section thickness.

In reconstructed images such as Fig. 4b, where amorphous material has been filtered out, the Z-

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**Figure 1** (a) Electron micrograph of a portion of a Z band in a longitudinal section of cardiac muscle fixed in PIPES-buffered glutaraldehyde (Group II). The Z lattice is at the 24-nm orientation and is between sarcomeres shortened to 1.75 μm. Note the thickened segment of each thin filament at the edge of the Z band in the sarcomere at the top. Note also occasional apparent connections between adjacent thin filaments at the edge of the thickened segments. ×182,000. (b) An unfiltered optically reconstructed image of the Z lattice shown in Fig. 1a. ×182,000. (c) An optical diffraction pattern of the region reconstructed in Fig. 1b is shown in the same orientation as the electron micrograph in Fig. 1a. The first-(10) and second-order (20) equatorial reflections correspond to the 24-nm lattice value we have reported previously (6). A single layer line parallel to the equator is consistent with the 38-nm repeating lattice structure also previously reported (6). (d) A diagram of the reciprocal lattice lines for the diffraction pattern in Fig. 1c indicates the positions of the holes in the mask used to make the filtered images shown in Fig. 1e and f. A filtered optically reconstructed image of the Z lattice is shown approximately at the same orientation and the same magnification as the corresponding electron micrograph and unfiltered image. The white background at the edge of the Z band appears prominent at first glance, but the fine black filaments in the Z lattice can be resolved easily upon closer inspection. ×182,000. (f) The filtered optically reconstructed image in Fig. 1c is shown photographically reversed for comparison with Fig. 1e. In both images three layers of lattice units are stacked end-to-end in the direction of the myofibril axis. Twelve lattice units stacked side-to-side in each layer are in close registration. An abrupt shift in the lattice is seen to the right in all four images (Fig. 1a, b, e, and f). ×182,000.
lattice axial filaments have minimum diameters of 8.5 nm and the connecting filaments have markedly different minimum diameters of 3.5 nm.

Fig. 5a is an electron micrograph of a thin, transparent section taken from a papillary muscle from another canine heart (Group I). The same type of abrupt shift in side-to-side orientation of adjacent groups of lattice units is seen here as in the thicker sections. The basket-weave appearance predominates as shown by the optical diffraction pattern Fig. 5b. Close inspection reveals aspects of the small-square appearance. Thus, both lattice forms are consistently seen in cross sections of canine cardiac Z bands regardless of section thickness.

Fig. 6a is an electron micrograph of a cross section of cardiac muscle (Group I) and is comparable to Fig. 2 in section thickness. Again, both lattice patterns are visible. A scan of successive diffraction patterns (Fig. 6b–d) across the top of the Z band transverse to the myofibril axis shows the gradual change in orientation of the lattice units. This orderly progression results in a general curvature of the lattice that conforms to the rounded borders of the myofibril lattice. This type of long-range ordering should be compared to Fig. 2a where at least two rows of 20 square arrays going across the top of the micrograph can be seen in near perfect side-to-side packing for a distance of ~500 nm. A third type of packing which consists of small regions of 6–10 units arranged in almost random ordering was observed in the same Z band from which Fig. 4a is taken and is also shown in Fig. 6a in the region marked at the left. The small-square lattice pattern is most apparent in this region. The corresponding optical diffraction pattern in Fig. 7b is typical for a small-square pattern. The optically filtered image (Fig. 7a) made with the mask in Fig. 7c exhibits an enhanced small-square pattern.

Filament diameters in thin cross sections from three dog hearts fixed in phosphate-buffered paraformaldehyde-glutaraldehyde (Group I) were compared with those in three dog hearts fixed in PIPES-buffered glutaraldehyde (Group II). Thirty measurements from electron micrographs such as Fig. 2a photographed at 30,000, or 70,000 or 100,000 and enlarged to at least ×200,000 for each dog were obtained. In Group I, thin filaments measured in the I band were 10.16 ± 0.47 nm (x ± S.D.); thin filaments measured in the I-Z region, where the tetragonal array was apparent, were 10.66 ± 0.46 nm; and axial filaments in the Z lattice were 11.14 ± 0.66 nm. In Group II, thin filaments in the I band were 8.90 ± 0.12 nm; thin filaments in I-Z were 8.98 ± 0.26 nm; and Z axial filaments were 9.59 ± 0.35 nm. The filament diameters in the three regions appear 15% larger in Group I. The thin-filament diameters in the I band in Group I were larger than those in Group II (t < 0.05).

A survey of electron micrographs of the canine cardiac Z lattice reveals the diverse nature of the myofibril shape. It is not surprising, then, that one goes to a different level of organization in going from a limited cardiac Z lattice composed of five-
to-ten lattice unit cells with dimensions less than half a micron to the cross-sectional area of the largest myofibril.

Optical reconstructions from longitudinal sections strongly suggest that the thin filaments are continuous with the axial Z filaments. The I band axial repeat distance of 38 nm is conserved in the repeating structure of the Z lattice. The canine cardiac Z lattice is most often composed of three 38-nm repeat units in the axial direction (four
layers of connecting filaments), and both sets of axial filaments traverse the entire distance of ~120 nm across the width of the Z band. Optical reconstructions particularly of cross sections show that there are additional filaments which meet the two sets of axial filaments with projected fourfold symmetry. The arrangement of these filaments accounts for the two lattice forms observed in electron micrographs. Both lattice forms persist in two different preparations in sections 40, 80, 160, and 200 nm thick. The optical reconstructions strongly suggest that these forms are aspects of a single lattice. In the unfiltered reconstructed images of the cardiac Z lattice, the dense amorphous material aggregates along the filaments as seen in both longitudinal and cross sections. The amorphous material varies irregularly in density, and in many cases obscures the underlying structural features. This variation in the amount of amorphous material persists even in the filtered reconstructed images.

DISCUSSION

With the help of optical diffraction and optical reconstruction techniques, we have defined a lattice unit for the Z band 38 x 24 x 24 nm. Using these dimensions, we can make several model drawings. Consider first that of a longitudinal section of the simplest Z band (Fig. 8a). Axial filaments are divided into antiparallel sets which arise from the thin filaments approaching each side of the Z band. At a given 38-nm repeat, four connecting filaments meet each axial filament but in the projection of this lattice unit into the two-dimensional longitudinal section the four appear as two. This arrangement now gives the familiar zig-zag appearance of the simple Z band. If we add another 38-nm segment to each axial filament and a second layer of connecting filaments, two rows of chevrons are visible (Fig. 8b). It becomes clear at this stage of model building that there may be something special about the last 38-nm interval along the thin filament. The axial filaments that separate the two layers of connecting filaments are the same as or continuous with the ends of the thin filaments. If we continue to add lattice subunits in the direction of the myofibril axis, we will conserve the 38-nm repeat distance and add successive layers of connecting filaments until the typical canine cardiac Z lattice is formed (Fig. 8c).

It is important to review some of the assumptions we have made thus far in defining our lattice-generating structure. In constructing the axial filaments, we assume that (a) they have a constant cross-sectional diameter, (b) they have a uniform composition, (c) they do not exert torque and (d) they have a specific handedness. In constructing the connecting filaments, we assume that (a) they have a constant diameter, (b) they have uniform composition, (c) they can change curvature and (d) they also have a specific handedness. In putting the unit cells together side-to-side, we assume that (a) the lattice subunits are stacked perpendicular to the myofibril axis, and that (b) the lattice is regular at least for a distance of five unit cells.

Now, as we go to a cross-sectional projection

FIGURE 3  (a) A photographic enlargement of Region 2 from Fig. 2a shows several arrangements of connecting filaments in the Z lattice. The small-square pattern predominates at the top third of the micrograph and here the connecting filaments resemble those modeled in Fig. 10(A'). However, some of the connecting filaments resemble those modeled in Fig. 9(A + B). The basket-weave pattern predominates in the bottom two-thirds of the micrograph. The connecting filament profiles resemble those in Fig. 9(A and B) and also those in Fig. 9(A + B) as suggested by a two-layer superposition effect as in Model I. X 240,000. (b) Optical diffraction pattern of the region in the top third of Fig. 3a is consistent with the small-square pattern but shows some higher-order diffraction spots more typical of the basket-weave lattice appearance. (c) Optical diffraction pattern of the region in the bottom two-thirds of Fig. 3a is consistent with the predominating basket-weave appearance. (d) Optical diffraction pattern of the entire Region 2 should be compared to Fig. 3b and c. (e) Filtered optical reconstruction of the entire region using mask holes in the positions indicated in Fig. 3f. Both the basket-weave and small-square patterns are faithfully reproduced. Individual L-shaped filaments at the top third of the image are seen. Cross-cut axial filaments appear diamond shaped, and the connecting filaments meet the axial filaments with projected fourfold symmetry in this same region. In the bottom two-thirds of the image, curving connecting filaments are seen as headphone shapes. X 240,000. (f) A diagram of the reciprocal lattice lines for the diffraction pattern in Fig. 3d indicates the positions of the holes in the mask used to make the filtered images shown in Fig. 3e.

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(Fig. 9) of the simplest cardiac lattice unit containing two layers of connecting filaments, we add additional constraints to our model. Each axial filament is shown with four associated filaments of the connecting filament network. The arrangement of these connecting filaments must account for the cross-sectional appearance of the cardiac Z lattice. In the case of a single layer of connecting filaments, curving filaments from corner (of the 24-nm square array) to center generate the basket-weave appearance (Fig. 9, A or B). The small-square appearance, however, can be generated in a number of ways: (a) double array of connecting filaments, each array joining axial filaments from the same sarcomere (similar to the interpretation of Landon [11] or the matrix lattice of Kelly and Cahill [9] for skeletal muscle); (b) single array of L-shaped connecting filaments, each array joining the axial filaments of one sarcomere to the axial filaments of the adjacent sarcomere (as proposed by MacDonald and Engel [12] and shown in Fig. 10 A'); (c) single array of curving filaments such that connecting filaments (from corner-to-center) in two successive planes curve away from the axial filaments in opposite directions (shown in perspective drawing in Fig. 11) and superimpose to yield a small-square pattern as shown in projection in Fig. 9 (A + B). This third possible explanation comes from a three-dimensional model (Model I) we have developed from optical reconstructions of cardiac muscle (Fig. 12). This model differs significantly from previous models of the Z lattice because two or more lattice units in the direction of the myofibril axis are considered. When three such lattice units are considered, it becomes difficult to distinguish between explanations 2 and 3 for the small-square pattern. This is the case for cardiac Z bands.

Now, consider the Z lattice in terms of a three-dimensional model. Model I is composed of a system of axial filaments and a system of connecting filaments (Fig. 12). The axial filaments of the Z lattice can be divided into antiparallel sets which arise from the thin filaments approaching each side of the Z band. Each axial filament entering the Z lattice has two strands of actin and two double strands of tropomyosin (Fig. 12). The stoichiometry of this model requires two connecting filaments for each axial filament in each layer. In the model lattice the distances between filaments, the length of the connecting filaments, the predicted angles between axial and connecting filaments, and the ratio of the diameters of the axial and connecting filaments are presented to scale and reflect measurements from cardiac Z bands. For clarity, the actual diameter of the filaments in the model is reduced to 40% of the observed diameter.

The complete lattice is formed by an equal number of axial filaments from opposite directions forming similar arrays offset from each other by 17 nm, one-half the diagonal distance of the large square. Both sets of axial filaments traverse the entire distance of 3 x 38 nm across the width of the Z band. Figs. 11 and 12 show, from different aspects, each axial filament with four associated filaments of the connecting filament network at each end of the 38-nm repeat distance. The precise orientation of these connecting filaments with respect to the square arrays of axial filaments gives rise to three patterns of reinforcement: the longitudinal chevron pattern and the cross-sectional basket-weave and small-square patterns.

If the model lattice is viewed in a longitudinal section in the 24-nm orientation as shown in Fig. 8, the bends in the filaments emerging from the axial filaments are in register and the chevron pattern is reinforced. Fig. 1a shows such a rein-

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**Figure 4** (a) An electron micrograph of a small portion of a Z band in a section 140-160 nm thick taken from the same block as that for Figs. 2 and 3. The extent of the persisting basket-weave pattern is about the same as in the intermediate and thinner cross sections. X 240,000. (b) Filtered optical reconstruction of the Z band region shown in Fig. 4a. Both the crosscut axial filaments and the connecting filaments are enhanced in this thicker section. X 240,000. (c) Optical diffraction pattern for region shown in Fig. 4a is typical for the basket-weave appearance. (d) Diagram of reciprocal lattice lines for the diffraction pattern in Fig. 4c indicates the position of the holes in the mask used to make the filtered image shown in Fig. 4b.

**Figure 5** (a) Electron micrograph of a thin section (~40 nm) taken from a canine heart muscle (Group I). The Z lattice exhibits the basket-weave appearance, but close inspection reveals aspects of a small-square pattern. X 196,000. (b) Optical diffraction pattern of region marked in Fig. 5a is consistent with basket-weave appearance of the Z lattice.
forcement of the chevron pattern for the cardiac Z lattice. When the lattice is viewed from the 17-nm orientation (Fig. 12), the bends in the connecting filaments now are out of register. This means that there is little or no reinforcement of the connecting filaments, though there is reinforcement of the axial filaments. Thus, the thin filaments appear to pass through the Z band without very noticeable connections between the axial filaments in the region of the Z band.

We have observed that in longitudinal sections the axial filaments of the cardiac Z lattice appear to be continuous with the thin filaments in both orientations. Each thin filament extends to the opposite side of the Z band as shown in Fig. 1 and as observed in skeletal muscle (17). Whether the Z band has the appearance of an overlap pattern (11) or a chevron pattern (17) depends on the extent to which the connecting filaments are visible. When the connecting filaments are visible in longitudinal sections of cardiac Z bands, they are less than half the diameter of the axial filaments. These finer filaments have also been described in skeletal muscle (17). The prominence of the fine connecting filaments may depend on preservation and staining characteristics, but may also depend on the regularity of their arrangement for a superposition effect. In electron micrographs of longitudinal sections of cardiac muscle, the connecting filaments are consistently more apparent in the 24-nm orientation. This is probably true for skeletal muscle as well. One explanation is that advanced earlier for Model I where the bends in the connecting filaments are in register in the 24-nm orientation. Another explanation is that the connecting filaments can oscillate—bowing first in one direction and then the other. Apparent reinforcement to give the same projected appearance

**Figure 7** (a) Filtered optically reconstructed image of region marked at left of Fig. 6a shows the small-square pattern. The crosscut axial filaments show most clearly the fourfold symmetry associated with this pattern. The lattice intersections where two L-shaped connecting filaments touch are clearly distinguished from those where the connecting filaments meet the axial filaments. (b) Optical diffraction pattern from Z band region shown in Figs. 6a and 7a is typical for the small-square pattern and resembles those previously reported for the Z rod in cardiac muscle (6). (c) Diagram of the reciprocal lattice lines for the diffraction pattern in Fig. 7b indicates the positions of the holes in the mask used to make the filtered image shown in Fig. 7a.

**Figure 6** (a) Electron micrograph of a cross section of cardiac muscle (Group I) comparable in section thickness to Fig. 2. Both lattice patterns are visible. The region marked at the left shows the small-square pattern. Note the myofibril shape for this Z band compared to that in Fig. 2a shown at the same magnification. × 84,000. (b-d) Optical diffraction patterns taken from the regions marked left to right at the top of the Z band shown in Fig. 6a. The arrow indicates the horizontal reciprocal axis used to orient the two-dimensional lattice defined for cross-section images reported previously for the cardiac Z lattice (6). Note that as one scans across the myofibril the orientation of the lattice units gradually changes as the general curvature of the lattice conforms to the rounded borders of the myofibril lattice.
FIGURE 8  (a) Model drawing of a longitudinal section of the simplest Z band. Antiparallel sets of axial filaments 38 nm in length are shown. The four connecting filaments associated with each axial filament are shown in projection here as two filaments. This arrangement gives the familiar zig-zag appearance associated with the 24-nm lattice orientation. (b) Another 38-nm segment added to each axial filament and a second layer of connecting filaments give an appearance more typical of the mammalian Z lattice. (c) Two more 38-nm repeat units and two more layers of connecting filaments give the typical canine cardiac Z lattice observed in this study at the 24-nm orientation.

as that for Model I can occur or nonreinforcement more typical of the 17 nm orientation can also occur.

Optical reconstructions from longitudinal sections, however, reveal persisting irregularities in the arrangement of connecting filaments even in the 24-nm, orientation yielding the brightest (sharpest) diffraction pattern. These images are hard to reconcile with a uniform and static model for the Z band. The extent to which irregularities on this scale persist in skeletal muscle is unknown.

Fig. 1a shows a distinct thickening of each of the thin filaments at the edge of the Z band in the sarcomere toward the top and several thickened segments in the sarcomere at the bottom. Each thickened segment is \( \sim 38 \) nm in length. These thickened regions correspond to thickened segments of some of the thin filaments on either side of the zig-zag shown by Franzini-Armstrong (3) in Fig. 3a of a single Z line in a fish skeletal muscle. Using the published magnification for her Fig. 3, we measured the length of this region along the thin filament in Fig. 3a and c to be \( \sim 38 \) nm. Franzini-Armstrong (3) also reports that in cross sections the apparent diameters of the thin filaments are wider near the Z line \((10.8 \pm 0.9 \) nm close to the Z line compared to \(8.6 \pm 0.7 \) nm in the I band). These findings are consistent with the thickened segments we see in longitudinal sections and the values for the diameter of the thin filaments at the edge of the Z lattice in cardiac muscle.

The cross-sectional drawings of the model show the projected fourfold symmetry observed in optical reconstruction images of cross sections. The projection of a single plane of connecting filaments gives the basket-weave pattern. The woven appearance is due to the bend in the connecting filaments and an accentuation of the spaces between the filaments. This woven appearance was first described by Reedy (15) in skeletal muscle. However, he related the set of curving filaments (his Fig. 16b) to a set of straight filaments (his Fig. 16a) that go from corner to center and form a 17-nm square. In our model and the model of MacDonald and Engel (12) in skeletal muscle, the curving filaments go from corner to center and form two sides of a 12-nm square.

The superposition of two or more successive planes of connecting filaments forms the usual cross-sectional projection for cardiac muscle. If cross-connecting filaments in successive planes curve away from the axial filaments in opposite directions, their superposition will generate the small-square pattern as modeled in Fig. 9 (A+B). A feature of this alternating layer structure is that the connecting filaments in successive planes are at a maximal distance from one another. This is the minimum energy configuration if the connecting filaments are electrostatically repulsive.

We have measured the apparent angle between a single connecting filament and the axial filament to which it binds as \( \sim 35^\circ \). We assume that the cross-connecting filament subtends an arc of one
FIGURE 9  Two-dimensional projections of the Z lattice in Model I viewed in cross section showing the alternating layers of connecting filaments (A) and (B) and the superposition of the two (A + B).

FIGURE 10  Two-dimensional projections of the Z lattice in Model II. The connecting filaments can change their curvature in any or all layers from the arrangement typical of the basket-weave appearance as in A to the small-square appearance as in A'. Reinforcing superposition may or may not occur in Model II for two successive layers of connecting filaments.

FIGURE 11  Perspective view of a single Z lattice unit which shows the curving of the connecting filaments and the square array of the axial filaments. Open circles in Figs. 9-11 represent axial filaments from one sarcomere, and closed circles represent axial filaments from adjacent sarcomere.

quarter of a circle in traveling from one axial filament to the next, consistent with observed profiles of connecting filaments seen in cross section. On this basis, we have calculated the length of the connecting filaments to be ~30 nm. A two-degree variation in the angle corresponds to a change of as much as 3 nm in the calculated lengths.

Ullrick et al. (21) have proposed a model in which three strands from axial filaments of the same sarcomere generate the lattice seen in cross-sectional projections. We consistently see fourfold or twofold symmetry in cross sections, independent of section thickness. We do see, in cardiac Z band, single connecting filaments. We also occasionally see three filaments. But when connecting filaments are seen, they go from corner to center, that is, connecting axial filaments from opposing sarcomeres and not from corner to corner between
axial filaments of the same sarcomere as suggested by Ullrick and co-workers (21).

In cross sections of the cardiac Z lattice there are two distinct patterns associated with the tetragonal array of axial filaments as in skeletal muscle. We have previously shown that each lattice gives a characteristic optical diffraction pattern (6). In this study we have found, using optical reconstruction, cardiac Z lattices with aspects of both patterns. This strongly suggests either interconversion or superposition or both. We have used model lattices to examine the evidence for and against these possibilities. In the thinnest sections a basket-weave pattern predominates but a number of individual filament profiles are seen. Some go from corner to corner, sometimes one to a square array and sometimes several, but all in the same direction. Such profiles are seen before centering of the lattice occurs. In the centered lattice, individual filaments that go from corner to center and pairs that form an S shape are seen.

In the intermediate sections both basket-weave and small-square patterns are present. In the thickest sections both patterns were visible. The basket-weave pattern was visible more than 50% of the time. This value was higher than that expected for the superposition model (1). However, a rigorous test of this superposition phenomenon predicted for thicker sections requires: (a) that all the connecting filaments in all layers have the same sense but are able to oscillate or move in any given layer. This rearrangement (from basket weave to small square) for any given square array may be independent of connecting filaments moving in neighboring arrays or in neighboring layers. MacDonald and Engel (12) have proposed a conversion from basket weave to small square by changes in curvature but did not consider the extent of this change and dealt with only one layer of subunits.

We have observed that both arrangements of connecting filaments are seen in Z bands exhibiting any or a combination of three types of long-range ordering of the square arrays. In one type, all of the units line up evenly in two directions (Fig. 2 a). In the second type, some of the units line up or sometimes a progression exists or sometimes a rotational effect is seen (Figs. 2 a and 6). In the third type, the units line up in a recognizable direction only for 3 or 4 units so there is random organization (Fig. 6). Fig. 2 shows all three types of ordering particularly well when the micrograph is viewed from a number of different directions.

The rearrangement of the connecting filaments seems to be related to the length and orientation of the connecting filaments and independent of the changing interfilament distance. This change in interfilament spacing is seen as widening between square arrays that start out parallel or as square arrays that gradually curve (both shown in Fig. 2).

In examining the three-dimensional multi-layer cardiac model, we conclude that: (a) there may be another protein in the Z lattice besides alpha-actinin, actin and tropomyosin, perhaps associated with the axial filament and added on at the last 38-nm interval of the thin filament; (b) there can be interconversion of the two different lattice forms in any or all layers to give a changing array of connecting filaments; (c) the connecting filaments appear to move in groups and to be related
to the amount of associated dense material; and
(d) while Model II does not contain a second set
of connecting filaments, our data do not rule them
out.

Unanswered questions still remain. To what
degree can the connecting filaments change their
arrangement? Is there a morphological Z band
smaller than the myofibril? Do all the connecting
filaments within this "Z band" change together
but independently from the neighboring "Z band"
contained in the same myofibril? In going from
layer to layer, is the polarity of the axial filaments
reversible within any given group of subunits and
does this change affect the changing array of
connecting filaments?

A flexible coated wire construction of Model I
shows some rather interesting mechanical proper-
ties. First of all, the greatest resistance to change
seems to be along the myofibril axis, and consid-
erable tension can be exerted before very small
changes occur in the distances between the axial
filaments forming the large square. Secondly, the
Z lattice is most easily deformed in response to
compression or expansion perpendicular to the
myofibril axis—that is, approximately parallel to
the direction of the cross-bridge action. Although
Models I and II were constructed to portray struc-
tural features observed by optical reconstruction,
both models exhibit features that are suggestive of
specific proteins.

Biochemical data so far suggest that the most
likely proteins in the Z lattice are alpha-actinin (7,
19, 20), tropomyosin (18, 20), and actin (22). Two
of these proteins, tropomyosin and actin, co-po-
lymerize in vivo and in vitro with a 38-nm repeat
(13). The other protein, alpha-actinin, generally a
nonfilamentous protein, is considered to be the
dense amorphous material in the Z band. This
protein binds to actin and influences the binding of
tropomyosin to actin under certain conditions
(19).

The 8.5-nm diameter of the axial filaments in
the Z lattice is consistent with that of actin-tropo-
myosin polymers. The 3.5-nm diameter of the
connecting filaments is consistent with the diam-
eter of the tropomyosin coiled coil. In the model,
the connecting filament network occurs every 38
nm and coincides with the oblique filaments seen
in the chevron pattern in longitudinal section. The
38-nm distance is consistent with the head-to-tail
repeat of tropomyosin molecules along the thin
filaments. If a tropomyosin strand unwraps from
the thin filament as it enters the Z lattice, the free
end may attach to the adjacent thin filament pre-
serving the 38-nm repeat. In so doing, potential
alpha-actinin binding site(s) could be exposed on
the actin remaining in the axial filament.

A tropomyosin strand may unwrap at the edge
of the Z band before interpenetration with oppos-
ing axial filaments occurs. In this case, the tropo-
myosin strand may bind to a nearest-neighbor
filament from the same I band, thus connecting
two corners of the large square. The curvature of
these filaments is reduced to span the larger dis-
tance of 24 nm. If the tropomyosin remains along
the axial filaments, then the connecting filaments,
regardless of their composition, must interact with
tropomyosin at each 38-nm binding site. The esti-
mounted range of lengths (30–40 nm) for the con-
necting filaments is compatible with the long di-
mension of either the alpha-actinin molecule (14)
or the tropomyosin molecule (1).

In examining canine cardiac muscle, we have
started with thick Z bands and irregular myofi-
brils. We have found that to understand the car-
diac Z lattice we must consider the whole Z band
but at the same time break it down into some
smaller workable unit. Our optical diffraction
analysis has helped us define this unit, while keep-
ing track of the changing orientation. The optical
filtering techniques we have demonstrated provide
a nondestructive method of separating the obscur-
ing amorphous material from the filamentous lat-
tice and reveal the complex substructure of the
cardiac Z lattice. Optical reconstructions strongly
suggest two types of variation in the substructure
of the lattice. The arrangement of the connecting
filaments can change, and the number and ar-
rangement of units added side-to-side or end-to-
end can change. We have considered possible
models for the mammalian cardiac Z lattice. If the
Z lattice is assumed to be homogeneous and static,
the fit to a number of existing models, including
those we have proposed, is not good. We conclude
that the cardiac Z lattice is both variable and
dynamic.

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