

Vinculin Is a Component of an Extensive Network of Myofibril-Sarcolemma Attachment Regions in Cardiac Muscle Fibers

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ABSTRACT Immunofluorescent staining of bovine and avian cardiac tissue with affinity-purified antibody to chicken gizzard vinculin reveals two new sites of vinculin reactivity. First, vinculin is organized at the sarcolemma in a striking array of rib-like bands, or costameres. The costameres encircle the cardiac muscle cell perpendicular to the long axis of the fiber and overlie the I bands of the immediately subjacent sarcomeres. The second new site of vinculin reactivity is found in bovine cardiocytes at tubular invaginations of the plasma membrane. The frequency and location of these invaginations correspond to the known frequency and distribution of the transverse tubular system in bovine atrial, ventricular, and Purkinje fibers. We do not detect tubular invaginations that stain with antivinculin in avian cardiocytes and, in fact, a transverse tubular system has not been found in avian cardiac fibers.

Apparent lateral Z-line attachments to the sarcolemma and its invaginations have been observed in cardiac muscle by electron microscopy in the same regions where we find vinculin. On the basis of these previous ultrastructural findings and our published evidence for a physical connection between costameres and the underlying myofibrils in skeletal muscle, we interpret the immunofluorescence data of this study to mean that, in cardiac muscle, vinculin is a component of an extensive system of lateral attachment of myofibrils to the plasma membrane and its invaginations.

A frequently observed feature of cardiac muscle fibers is a periodic array of electron-dense plaques associated with the sarcolemma (1-6). The Z lines of myofibrils that are subjacent to the sarcolemma abut on these dense plaques, and fibrous connections have been seen between the Z lines and the sarcolemmal densities (6). Since the cardiac sarcolemma in the contracted fiber is scalloped or punctuated regularly with indentations precisely in register with the Z lines, the dense plaques seem to be sites where the plasma membrane is mechanically coupled to the underlying myofibrils.

Little is known regarding these apparent attachment regions of cardiac fibers. The three-dimensional organization of this sarcolemmal specialization has not been appreciated because reconstruction from serial electron micrographs is lacking, the attachment regions are not visible in the light microscope using phase-contrast optics, and no specialization that might correspond to these regions has been observed in freeze-fracture studies of the sarcolemma. Also, none of the molec-

ular components of this sarcolemmal specialization has been identified.

Using affinity-purified vinculin antibody as a probe for membrane-associated cytoskeletal structure, we have recently described a novel sarcolemmal specialization in skeletal muscle, the costamere (7). Costameres (Latin *costa*, rib; Greek *meros*, part) are transverse, rib-like bands at the sarcolemma which are precisely in register with the underlying I bands. Several observations suggest that costameres are regions of association of myofibrils with the sarcolemma. First, the costamere overlies the I band regardless of sarcomere length, and adjacent but misaligned bundles of myofibrils exhibit corresponding costamere discontinuities. Second, since vinculin has been found only at sites where the cytoskeleton appears to associate with the plasma membrane (8-10), the presence of vinculin in costameres supports the idea that they represent sites of myofibril-sarcolemma association. Third, ultrastructural data have demonstrated the association of

myofibrils with the sarcolemma at sites generally consistent with the observed costamere pattern (11, 12).

In this paper we present the results of immunofluorescence staining of avian and bovine cardiac tissue with affinity-purified antibody to vinculin. We confirm the presence of vinculin at the intercalated disk (8, 9). In addition, we report two new locations of vinculin—at costameres and at sarcolemmal invaginations. All three locations are known sites of myofibril-sarcolemma attachment regions defined by electron microscopy. These results have been presented previously in preliminary form (13).

MATERIALS AND METHODS

Vinculin was purified from frozen (-20°C), glycerinated central muscles of chicken gizzards (Pel-Freeze Biologicals, Rogers, AR) as a by-product from the purification of α -actinin. A low salt gizzard extract was fractionated by ammonium sulfate precipitation and DE52 chromatography. Vinculin elutes as peak No. 2 of the DE52 column profile as described by Craig et al. (14). Such a preparation is at least 93% homogeneous.

Antibody to vinculin was purified from rabbit sera by affinity chromatography as described previously (15). In Western blots of SDS-soluble proteins from cardiac and skeletal muscle tissues, the affinity-purified antivinculin reacted strongly with 130,000-mol-wt vinculin and weakly with 150,000-mol-wt meta-vinculin (7). The affinity-purified antibody was also screened by Western blotting for reactivity toward components of the vinculin preparation used in the affinity purification procedure. The vinculin antibody detected only a 130,000-mol-wt component in the vinculin preparation. The immunofluorescence staining presented in this paper could be abolished by prior incubation of the affinity-purified antibody with 130,000-mol-wt vinculin. For use in absorption, the 130,000-mol-wt vinculin was prepared as above and further purified by preparative SDS PAGE (16). Thus, all the staining in these sections is due to reaction of the antibody with antigenic determinants present on 130,000-mol-wt vinculin.

Freshly excised cardiac tissue from adult or 10-d-old chickens was dropped into freezing isopentane. In some experiments, the chick was given a lethal intravenous dose of calcium channel blockers, nifedipine (Pfizer Chemicals Div., Pfizer, Inc., NY, NY), and D600 (Knoll Pharmaceutical Co., Whippany, NJ), dissolved in perfusion buffer (0.134 M phosphate buffer, pH 7.4, containing 5.5 mM KCl, 0.5 mM EGTA) with 100 U/ml heparin. The pericardium was excised immediately after cardiac arrest, and the heart was perfused through the ventricle with perfusion buffer at 4°C to clear the blood. The tissue was then fixed for 5 min by perfusion under pressure with 2% (wt/vol) formaldehyde in cold perfusion buffer. The heart was chopped into small pieces and dropped into freezing isopentane. Bovine muscles, obtained as rapidly as possible after slaughter (30–60 min), were cut into small pieces and frozen immediately in isopentane.

The frozen tissues were transferred to powdered dry ice and frozen onto copper chucks with O.C.T. compound (Miles Laboratories Inc., Research Products, Div., Elkhart, IN). Ribbons of $4\text{-}\mu\text{m}$ -thick cryostat sections were thaw mounted onto gelatin-coated glass slides and fixed immediately for 3 min at room temperature in 3% (wt/vol) formaldehyde in perfusion buffer. The formaldehyde-fixed sections were quenched by incubation for 5 min at room temperature in Dulbecco's phosphate-buffered saline (PBS) containing 20 mM glycine. All sections were incubated in PBS for 10 min before staining, which was done at room temperature. For indirect immunofluorescence localization, sections were overlaid with $25\ \mu\text{l}$ of affinity-purified antivinculin or control antibody at $75\ \mu\text{g/ml}$ in PBS with 0.1% BSA and incubated for 30 min in a moist chamber. After three washes in PBS over 30 min, the slides were drained and overlaid for 30 min with $25\ \mu\text{l}$ of affinity-purified, fluorescein-coupled goat anti-rabbit (molar fluorescein to protein ratio, 7:1) at $50\ \mu\text{g/ml}$ in PBS with 0.5% BSA. The slides were washed as before and mounted immediately before being observed in 1 mg/ml of *p*-phenylenediamine dihydrochloride (J. T. Baker Chemical, Co., Phillipsburg, NJ) dissolved in mounting medium (0.1 M NaCl, 50 mM Tris-HCl, pH 8.9, 2 mM CaCl_2 , 10% (wt/vol) glycerol, and 0.02% NaN_3) which was readjusted to pH 9.0 with 1 N NaOH (17). For an indirect immunofluorescence control, affinity-purified anti-keyhole limpet hemocyanin was substituted for the antivinculin. There was no detectable nonspecific fluorescence except for some dim staining at occasional nuclei (see Fig. 6c).

The stained tissues were examined on an Ortholux II equipped for epifluorescence illumination with a 100-W Hg light source, and Leitz $25 \times 0.75\ \text{NA}$ and Zeiss $63 \times 1.4\ \text{NA}$ oil immersion objectives. Leitz filters KG1, BG23, K480, and cube H2 were used. Photomicrographs were recorded with a Vario-

Orthomat camera on Kodak Tri-X pan 400 and developed in Acufine to an exposure index of 1,000. Typical fluorescein exposure times were 1–3 min. Fluorescence photomicrographs were printed on Kodak F5 paper.

RESULTS

Vinculin Localization in Avian Cardiac Muscle

Vinculin localization is similar in both atrial and ventricular chicken muscle fibers. In cross section (Fig. 1a), the fluorescence is confined to the cell margins. No staining is observed intracellularly in those fibers that are sectioned transversely (see asterisk in Fig. 1a). Fibers that are sectioned more obliquely have some apparent intracellular staining, albeit indistinct, because some of the sarcolemma of these small fibers ($<10\ \mu\text{m}$ diam) is contained tangentially within the $4\text{-}\mu\text{m}$ section. Longitudinal sections of the interior of the fibers (see asterisk in Fig. 1d) lack internal staining, therefore the Z lines are not stained. Other longitudinal sections selected to contain the surface of the fibers oriented *en face* demonstrate that the vinculin at the sarcolemma is not distributed uniformly but is organized into an array of rib-like bands or costameres. The costameres encircle along the cells length with a periodicity corresponding to that of the subjacent myofibrils. The costameres are directly over the Z line and appear often to be narrower than the underlying I band (see asterisk in Fig. 1, b and c), but quantitative studies are necessary to clarify the precise relationship between the widths of these two bands.

As described previously (8, 9), the intercalated disks stain but are difficult to discern in these sections. The intercalated disks in chicken heart are poorly developed as compared to those found in ungulates (see below; 18). Intense patches of stain in Fig. 1 (see arrow in Fig. 1a) correspond to vessels (see also arrows in Fig. 4, c and d).

Vinculin Localization in Bovine Heart

ATRIAL FIBERS: In contrast to avian heart muscle, vinculin localization is not confined to the cell-surface regions of the plasma membrane in the three types of bovine cardiac fibers. In atrial muscle fibers sectioned transversely (Fig. 2a), vinculin fluorescence is intense at the cell margin. In addition, there is dimmer fluorescence at structures removed from the cell margin which appear to be membrane invaginations. As in avian heart muscle, longitudinal sections of these cells (Fig. 2, c and d) show concentrations of vinculin at costameres. By varying the focal plane, we observed that the costameres are not flat but seem to follow the contour of the membrane indentations overlying the Z lines. These sections also show prominent staining of intercalated disks (Fig. 2d) and are relatively devoid of internal stain except for the staining of convoluted structures similar to those observed in transverse sections (Fig. 2, d and e). Longitudinal sections substantiate the impression that the convoluted profiles are tubular invaginations of the cell membrane since fluorescent rings are seen whenever these structures are sectioned transversely (Fig. 2, c, e, and f). Usually, these tubes appear to arise from the sarcolemma overlying the Z lines at approximately the center of the costamere (Fig. 2, f and g). The fluorescent tubular structures appear to travel toward the cell center largely within the transverse planes containing the Z disks. These invaginations occur randomly along the length of the muscle cell and infrequently as compared to the number of sarcomeres. Most Z lines have no associated fluorescence.

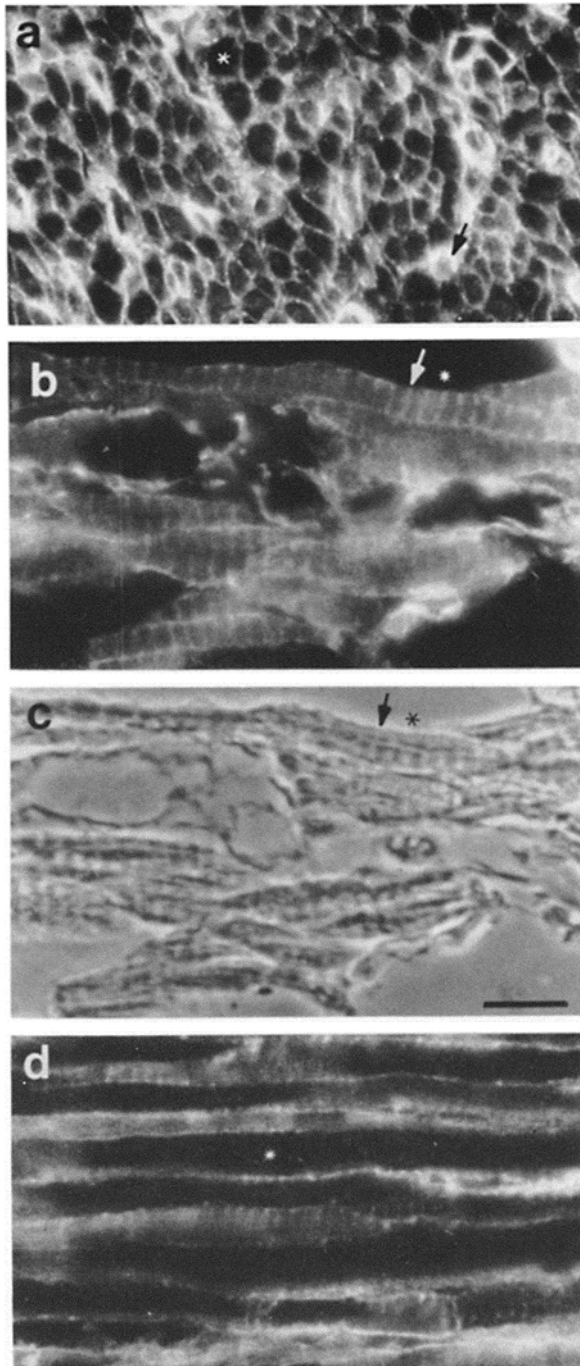


FIGURE 1 Immunofluorescence localization of vinculin in chicken cardiac muscle. Heart muscle was perfused under pressure with fixative containing calcium channel blockers to minimize contraction. (a) Transverse cryostat section demonstrating selective localization of vinculin at the cell margin. No vinculin is seen deep within the cell in those fibers that are sectioned most transversely (*). Occasional bright patches of stain (arrow) correspond to vessels. (b) Longitudinal cryostat section demonstrating a costamere (arrow) that overlies the I band (*). Often costameres appear narrower than the corresponding I band (*). (c) Phase-contrast micrograph of *b*; arrow at I band corresponds to costamere in *b*. I band (*) appears wider than corresponding costamere. (d) Longitudinal cryostat section demonstrating lack of internal staining (*). Bar, 10 μ m. *a*, \times 680; *b* and *c*, \times 1,070; and *d*, \times 950.

PURKINJE FIBERS: Although not studied in detail, Purkinje cells also have vinculin concentrated at costameres (not shown). Areas where Purkinje cells are closely apposed are

particularly bright (Fig. 3, *a-c*); these regions may represent intercellular junctions which have been described ultrastructurally (5). The interior of the Purkinje cell is totally devoid of stain except at branching, fluorescent tubular networks that can be observed to be continuous with indentations at the cell margin (Fig. 3 *b*). These networks probably represent vinculin localization along the intricate sarcolemmal invaginations that have been observed in these cells (5).

VENTRICULAR FIBERS: Bovine ventricular muscle cells display the most intricate pattern of vinculin immunofluorescence. These cells have intense vinculin fluorescence at the sarcolemma in the costamere pattern seen in the other cardiac fibers. The periodicity of the bands along the longitudinal axis of the fiber is always the same as the periodicity of the underlying sarcomeres (Fig. 4, *e* and *f*). The scalloped sarcolemma is sometimes visualized with intense vinculin fluorescence at the sites of indentation of the cell margin (Fig. 4, *a* and *i*; Fig. 5). The brightest fluorescence is clearly associated with the costameres but by adjusting the focal plane dimmer fluorescence is observed deeper within the cell (Fig. 4, *a* and *h*). In contrast to all other striated muscle cells examined, almost all ventricular muscle cells in longitudinal sections seem to have fluorescence, often distinctly punctate, associated with the Z lines (Fig. 4, *a*, *j*, *l*, and *n*). Whether the vinculin in any given area is associated with the sarcolemma is often difficult to assess owing to the significant amount of fluorescence coming from internal structures. Sometimes fragments of cells containing groups of myofibrils have vinculin associated with the Z lines (Fig. 4, *j-o*). When bundles of myofibrils are splayed apart, vinculin fluorescence can be observed associated with the inter-Z disk system (Fig. 4, *l* and *m*). In cross-sections (Fig. 4, *c* and *g*), a dense tubular network of vinculin fluorescence is surrounded by the more intensely stained cell margins. This pattern of vinculin staining which is removed from the cell margins is consistent with T tube staining, but immunoelectron microscopy will be needed to clarify the situation.

The presence of vinculin at sites removed from the cell margins of bovine heart muscle is probably not an artifact resulting from displacement of vinculin from the cell cortex during the long postmortem period (30–60 min) at the abattoir before the tissue can be processed for immunofluorescence. First, the vinculin localization is discrete and is observed consistently. Second, bovine skeletal muscle that is processed after a similarly long postmortem period had only sarcolemmal-associated vinculin, although this cell has similar internal structures as ventricular muscle, i.e., myofibrils, T tubes, etc. (data not shown).

The staining patterns reported in this paper were abolished when the affinity-purified vinculin antibody was preabsorbed with 130,000-mol-wt vinculin eluted from a preparative SDS gel (Fig. 6 *b*). As a control for nonspecific trapping of antibody in the tissue sections, affinity-purified rabbit anti-keyhole limpet hemocyanin was substituted for the affinity-purified antivinculin in the indirect immunofluorescence technique; no staining could be detected at the sarcolemma or internal tubules (Fig. 6 *c*). Also, staining of various skeletal muscles that have extensive T tubule systems have not displayed artifactual trapping of the antibody within the tubule (bovine muscle, unpublished data; avian muscle, 7).

DISCUSSION

We found that the distribution of vinculin in cardiac muscle cells is far more extensive than the previously reported local-

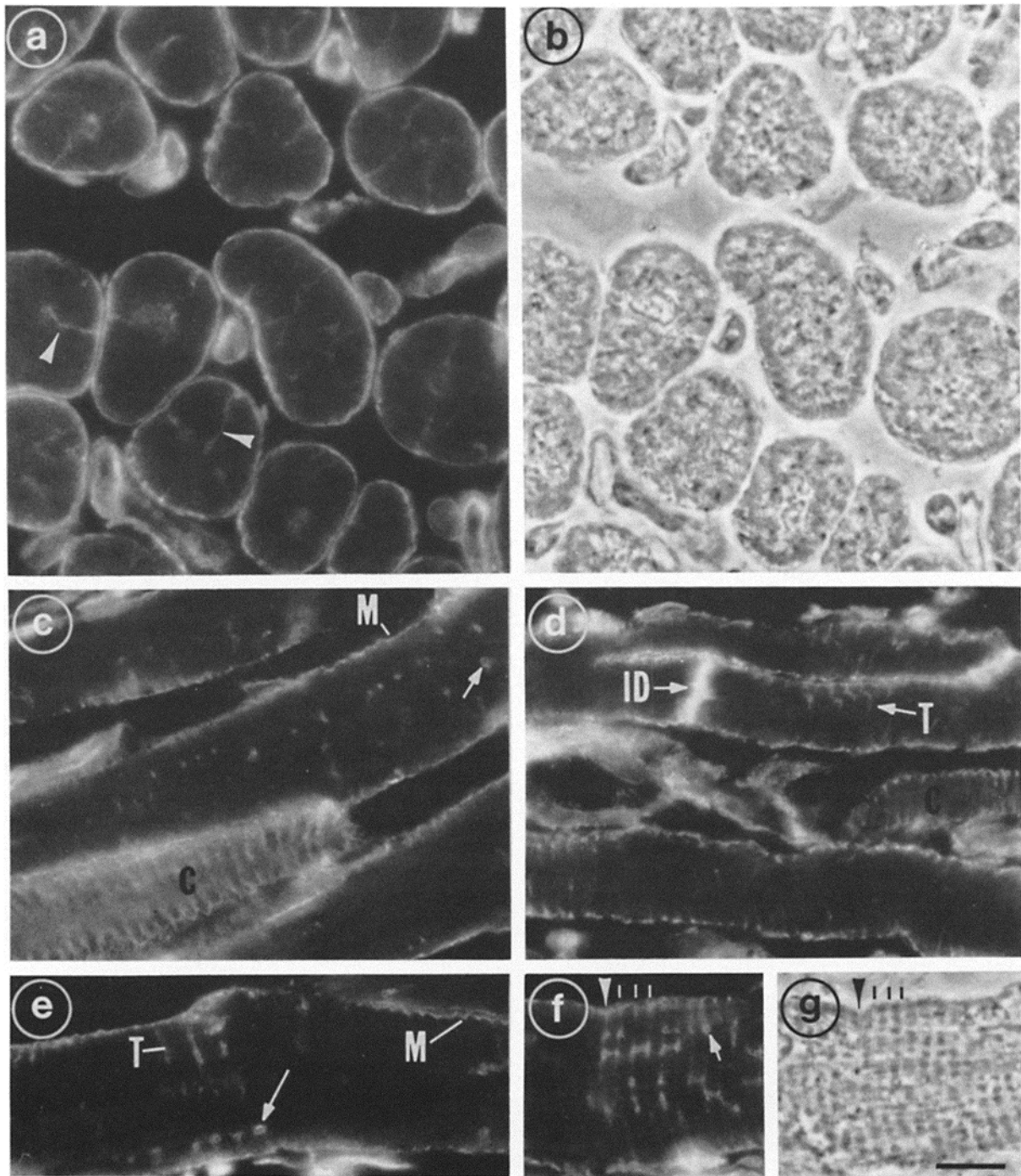


FIGURE 2 Immunofluorescence localization of vinculin in bovine atrial muscle. (a) Transverse cryostat section of atrial fibers demonstrating concentration of vinculin at the cell margins and along convoluted, tubular structures (arrowheads) which appear to be sarcolemmal invaginations. Most of the cell's interior is unstained. Dim staining of the nucleus is also seen in occasional controls (see Materials and Methods). (b) Phase-contrast micrograph of a; sarcolemmal invaginations are not evident. (c-e) Longitudinal sections showing intensely fluorescent costameres (C) at the sarcolemma. Vinculin is associated with cell margins (M), intercalated disks (ID), and tubular structures (T). When the tubular structures are sectioned transversely, fluorescent rings are evident (arrows). Most of the cell's interior is unstained. (f) Glancing section through cell margin showing *en face* localization of vinculin over the Z lines (arrowhead) and at an array of fluorescent circles (arrow) presumed to be the openings of the tubular invaginations. Note that all circles are situated over the Z lines. (g) Phase-contrast micrograph of (f); arrowhead at Z lines corresponds to costamere in f. Small bars indicate correspondence between Z lines in g and vinculin fluorescence in f. Large bar, 10 μm . a and b, $\times 1,150$; c-g, $\times 1,100$.

ization at the intercalated disk (8, 9). First, in all avian and bovine muscle fibers examined (Table I), vinculin is also found concentrated at the sarcolemma in a series of transverse, rib-like bands which overlie the Z lines and repeat along

the long axis of the cell with a periodicity corresponding to that of the underlying sarcomeres. These bands are analogous to costameres as defined in skeletal muscle (7). Cardiac muscle costameres appear somewhat different than those in skeletal

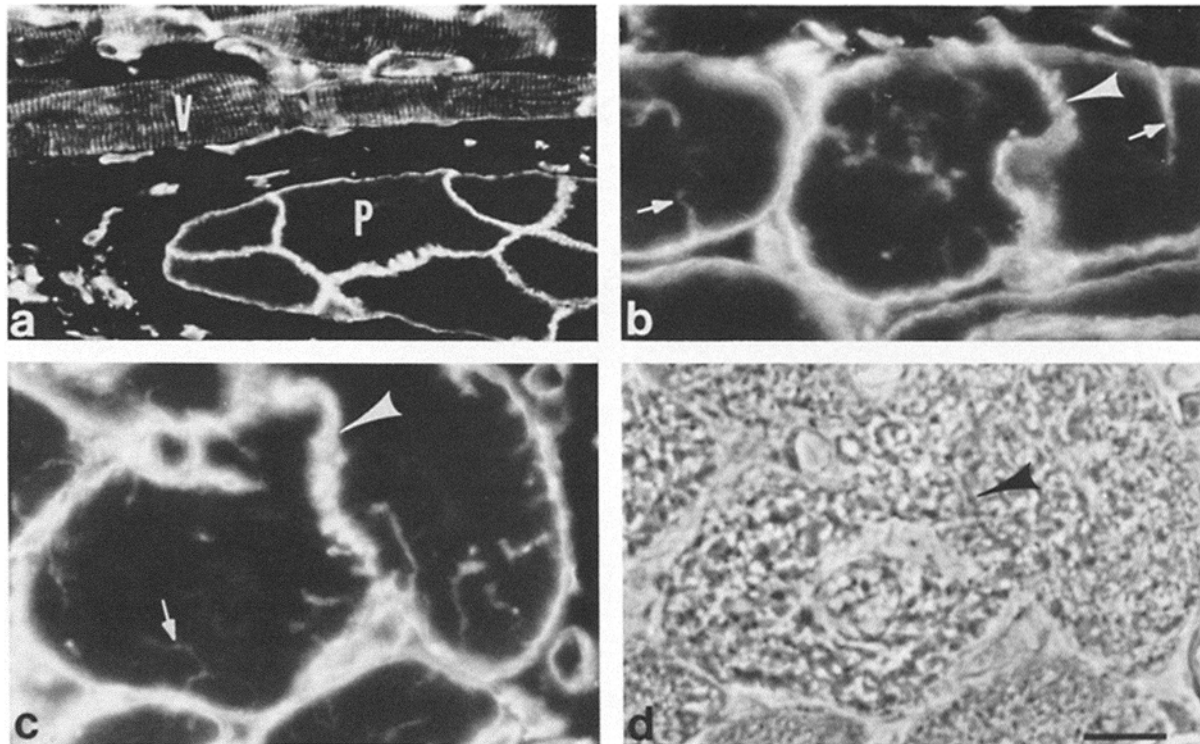


FIGURE 3 Immunofluorescence localization of vinculin in bovine Purkinje cells. (a) Low power micrograph of cryostat section showing vinculin in ventricular fibers (V) and Purkinje fibers (P). (b and c) Sections of Purkinje cells showing localization of vinculin along the cell margins, at convoluted tubular invaginations (arrows), and at intercellular junctions (arrowhead). (d) Phase micrograph of e; note intercellular junction (arrowhead) and large open nucleus characteristic of Purkinje cells. Bar, 10 μ m. a, \times 410; b-d, \times 1,100.

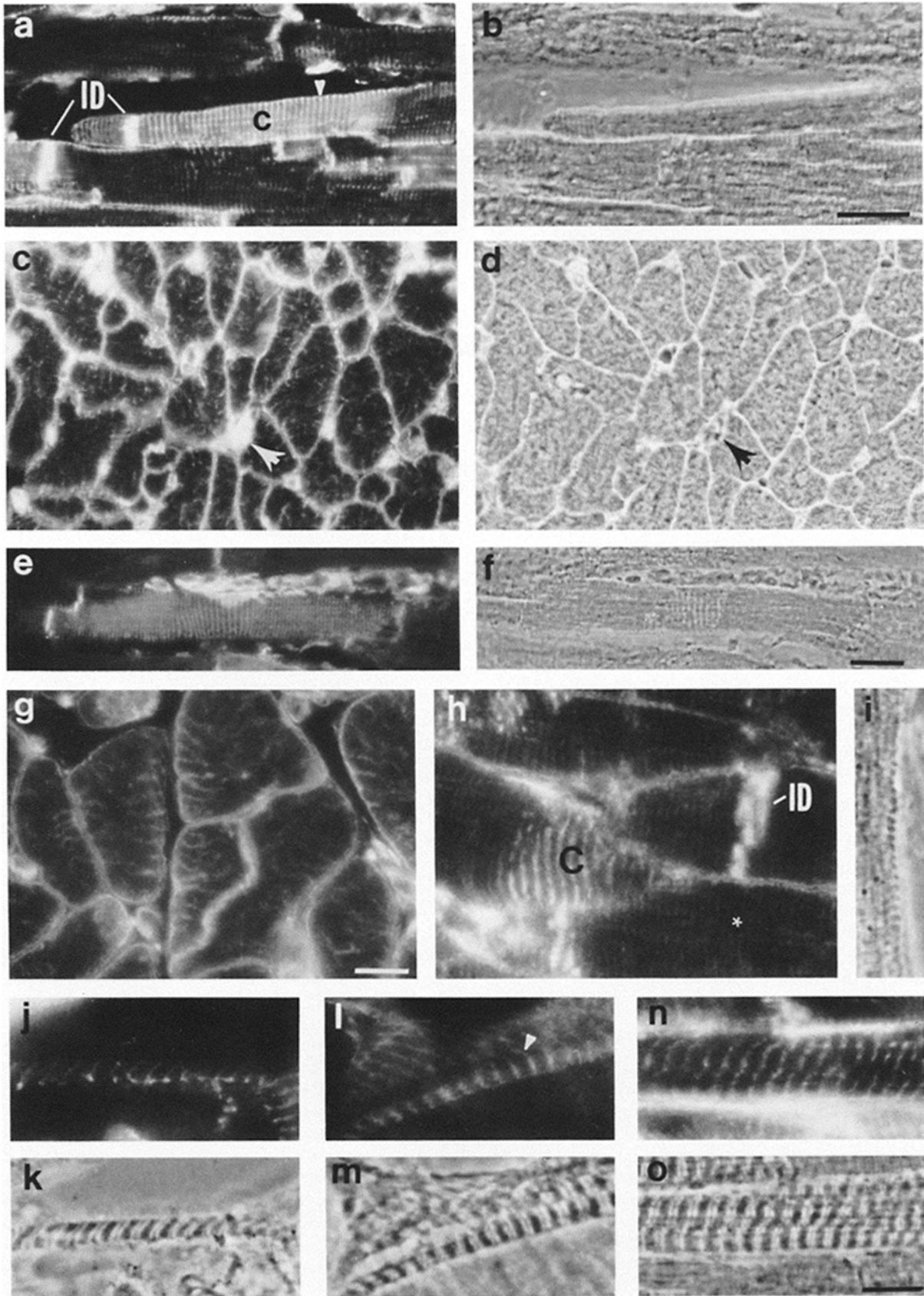
muscle because cardiac costameres: (a) do not seem to consist of doublet bands flanking the Z line but instead appear as one band over the Z line (although this may be an artifact due to the difficulty in keeping cardiac muscle well stretched), and (b) are generally narrower than the underlying I band with greater staining intensity directly over the Z line. Second, in Purkinje fibers vinculin is localized along tubular and cleft-like structures that appear to be invaginations of the sarcolemma. In atrial fibers, infrequent tubular structures appear to originate from the sarcolemma over the Z lines and travel toward the cell interior predominantly within transverse planes containing the Z disks. Third, vinculin localizes in longitudinal sections of bovine ventricular muscle along the Z lines. Transverse sections of ventricular fibers display, besides costameres, an intricate, tubular network extending throughout the interior of the cell.

Discovery of these additional sites of vinculin localization in the same tissue that was previously examined by other investigators using a different antivinculin (9) might mean that when vinculin is incorporated into different subcellular structures, dissimilar sets of antigenic determinants are exposed for reaction with antibody. Detection of the various structures would then reflect the spectrum of antigenic determinants recognized by the particular antivinculin used in the experiment. Alternatively, individual antisera to vinculin might recognize different isoforms (19) or cross-reactive vinculin-like proteins (20, 21). Preliminary experiments suggest that there are higher molecular weight cross-reactive proteins in avian and bovine cardiac tissue (20). In our experiments, absorption of the staining patterns by preincubating the antivinculin with 130,000-mol-wt vinculin (prepared by excision from an SDS gel of the purified vinculin protein) establishes

that the fluorescence patterns reported herein are due to reaction of the vinculin antibody with antigenic determinants present on 130,000-mol-wt vinculin.

In view of vinculin's proposed role as a membrane anchor site for the cytoskeleton (22) and its selective presence at a variety of cytoskeleton-membrane specializations (8, 23-25), we propose that cardiac costameres, as in skeletal muscle (7), represent regions of attachment of the myofibrils to the sarcolemma. The vinculin-rich bands in cardiac fibers appear to be mechanically coupled to the underlying myofibrils since the periodicity of the costameres is always the same as the periodicity of the subjacent myofibrils, regardless of the sarcomere length (e.g., Fig. 4, e and f). Also, the vinculin bands, which always overlie the Z lines, occur precisely at the sites of indentation of the sarcolemma of contracted cardiac muscle. Thus, a physical connection between the Z lines and sarcolemma at the site of vinculin localization seems likely. In fact, there is ample ultrastructural evidence in cardiac muscle for a structural link between the Z discs and the sarcolemma. Since the early 1960's, sarcolemmal dense plaques associated with the Z lines of the superficial myofibrils have been noted (1-5). More recently, Chiesi et al. (6) have prepared cardiocytes that clearly demonstrate Z-line attachments to sarcolemmal dense plaques. Since the cardiocytes used by these researchers are swollen and hyperpermeable, most of the cytosol is cleared allowing excellent visualization of some of the filament systems. Fibrous connections, mostly intermediate filaments, appear to connect the Z lines to sarcolemmal densities at the site of indentation of the sarcolemma.

The presence of vinculin at sites removed from the cell margin, as observed in bovine cardiac muscle, is unusual.



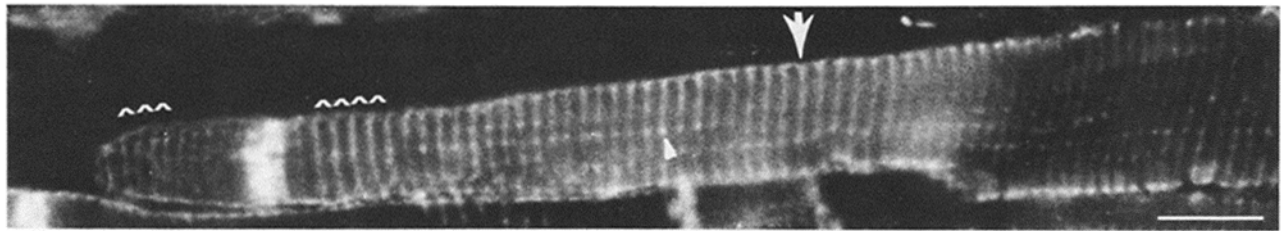


FIGURE 5 Detail of fiber marked with arrowhead in Fig. 4 a. In some areas (marked by zigzag lines) there is a suggestion of scalloping of the sarcolemma with regular indentations at costameres. The fluorescence of the costamere at the indentations often appears wedge-shaped (arrow). The staining of these costameres is sometimes distinctly punctate (arrowhead). Bar, 10 μm . $\times 1,360$.

Vinculin has been found exclusively at sites of cytoskeleton-membrane interaction and its striking absence at intracellular sites has been noted (8). However, ungulate Purkinje fibers, which lack T tubules, are known to have deep tubular sarcolemmal invaginations (5). Thus antivinculin appears to be a probe allowing visualization of these structures at the light microscope level. Similarly, the internal vinculin fluorescence observed in bovine atrial and ventricular muscle is consistent with T tubule staining. The extensive, fluorescent tubular profiles extending throughout the ventricular cell and associated with the Z lines would correspond to the known extensive T tube system of ventricular fibers; the precise alignment of T tubules with Z lines of cardiac muscle cells has been observed repeatedly (26). Apparent attachments of the anchor fiber system to dense plaques on T tubules have been observed ultrastructurally in cardiac muscle (3, 6, 27) and specifically in bovine ventricular fibers (28). Similarly, the relatively infrequent fluorescent tubular profiles observed in bovine atrial fibers would correspond to the T tubule system of bovine atrial fibers which in general is less developed than the T system of ventricular fibers; some bovine atrial fibers have few, in any, T tubules. Thus vinculin appears to be a useful probe to visualize sarcolemmal invaginations such as the T system at the light microscope level. These results, although requiring immunoultrastructural verification, suggest that the T tubule to Z line attachments in cardiocytes are similar to those on the sarcolemma and comprise an extensive system of myofibril-membrane attachment.

In summary, Fig. 7 diagrams our current ideas on the localization of vinculin with respect to the known ultrastructural features of the sarcolemma at the cortex of the cell. A similar cartoon would illustrate where vinculin is with respect to sites of internal myofibril association with sarcolemmal

TABLE I
Summary: Sites of Vinculin Localization in Striated Muscle

	Costa- meres	Sarco- lem- mal in- vagi- nations	Inter- calated disk
Skeletal muscle (7)			
Avian: pectoralis, ALD, PLD, pul- monary aponeurosis	+	-	N.A.
Bovine: gastrocnemius	+	-	N.A.
Cardiac muscle			
Avian: atrial, ventricular fibers	+	-	+
Bovine: atrial, Purkinje, ventricular fibers	+	+	+

NA, not applicable; +, vinculin is present; and -, vinculin is not detectable.

invaginations. In the diagram, vinculin is presumed to be in the membrane-dense plaques overlying the Z lines in cardiac muscle, in analogy to the location of vinculin in the dense plaques of smooth muscle plasmalemma (8). As suggested by Chiesi et al. (6) on the basis of their electron micrographs, some filament system, such as intermediate filaments or actin, connects the Z disks to the sarcolemma. This system of myofibril-membrane association, perhaps mediated through vinculin, could serve to mechanically couple the sarcolemma and its invaginations to the contractile machinery during fiber lengthening or shortening.

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FIGURE 4 Immunofluorescence localization of vinculin in bovine ventricular muscle fibers. (a and b) Fluorescence, phase contrast. Low power micrograph of cryostat section demonstrating bright vinculin fluorescence at intercalated disks (ID), costameres (c), and dimmer cross-striation pattern within the cell. Intense wedge-shaped fluorescence at cell margin (arrowhead) corresponds to costameres at indentations of sarcolemma (see Fig. 5 for detail). Bar, 20 μm . $\times 630$. (c) Transverse (on right) and oblique (on left) section demonstrating localization of vinculin at the sarcolemma and along tubular sarcolemmal invaginations. At lower left, the tubular array appears as a cross-striation pattern when sectioned obliquely. Note intense staining of vessels (arrow). (d) Phase-contrast micrograph of c. $\times 640$. (e and f) Fluorescence, phase-contrast. Costameres overlie I bands regardless of sarcomere length and are somewhat narrower than the underlying I band at extended sarcomere lengths. Bar, 20 μm $\times 450$. (g) Transverse section. Higher power micrograph of region similar to that on right side of c. Vinculin is concentrated at cell margins and along tubular sarcolemmal invaginations. Bar, 10 μm $\times 990$. (h) Oblique section showing intense fluorescence at costamere (C), intercalated disks (ID), and dimmer fluorescence deep within the cell in a cross-striated pattern (*). $\times 1,000$. (i) Higher power phase-contrast micrograph of region similar to that marked by arrowhead in a showing membrane scalloping. $\times 1,200$. (j, l, and n) Vinculin is concentrated over Z lines in bundles of myofibrils. Note staining at apparent inter-Z disk region (arrowhead) where myofibrils are splayed apart in l. (k, m, and o) Phase-contrast micrographs of j, l, and n, respectively. Bar, 10 μm . j-o, $\times 1,000$.

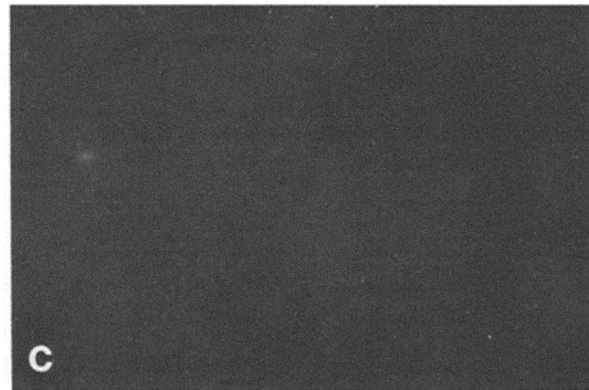
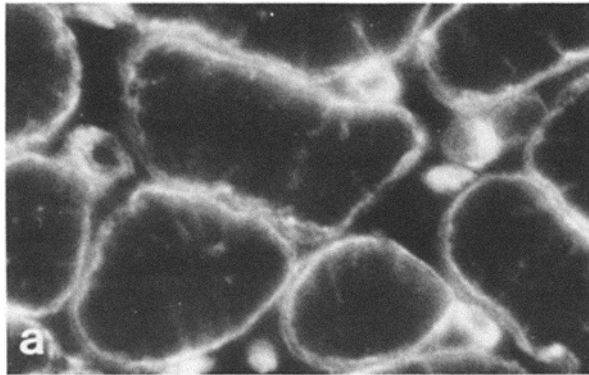


FIGURE 6 Controls for specificity of antibody. (a and b) Absorption of antivinculin staining with 130,000-mol-wt vinculin. The purified vinculin preparation was electrophoresed in a preparative SDS polyacrylamide (5%) gel. The gel was stained with cold KCl, and the protein from the 130,000-mol-wt region was eluted (16). The eluate was dialyzed against distilled water, lyophilized, and resuspended in PBS. The affinity-purified antibody to vinculin was preabsorbed with eluate from a gel without protein (a) or with eluate from a gel containing a large excess of the purified vinculin preparation (b) before staining transverse sections of bovine atrium. (c) Substitution of affinity-purified antivinculin with an equal concentration of rabbit affinity-purified anti-keyhole limpet hemocyanin (an antigen not expected to be present in this tissue) fails to result in staining of tubules or sarcolemma in transverse sections of bovine atrium. Therefore vinculin staining of tubules is not the result of artifactual trapping of the antibody within the tubule.

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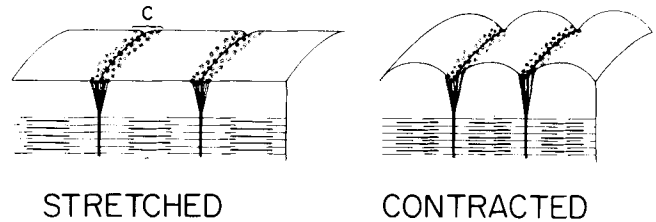


FIGURE 7 Cartoon illustrating proposed sites of vinculin localization with respect to the known ultrastructure of sarcolemma-myofibril associations. Areas associated with vinculin fluorescence are stippled. A costamere is marked by C.

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