A vinculin-containing cortical lattice in skeletal muscle: Transverse lattice elements ("costameres") mark sites of attachment between myofibrils and sarcolemma

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ABSTRACT We have found that vinculin is localized at the sarcolemma of skeletal muscle cells in a two-dimensional orthogonal lattice. Perpendicular to the longitudinal axis of the cell, bands of vinculin encircle the muscle cell and repeat along its length with a periodicity corresponding to the subjacent sarcolemmal elements. Because of their appearance and probable function, we call the transverse elements of the lattice "costameres" (Latin costa, rib; Greek meros, part). Costameres have a substructure consisting of densely clustered patches of vinculin; the patches are segregated into two rows which flank the Z line and overlie the I band of the underlying sarcomere. It is likely that the costameres are physically coupled to the underlying myofibrils because: (i) the costameres broaden and narrow in concert with the underlying band in stretched and contracted muscle, and (ii) adjacent but misaligned myofibrils are mirrored by corresponding discontinuities in the overlying costameres. We hypothesize that the sarcolemmal lattice, detected because vinculin is one of its molecular components, integrates the contractile apparatus with the sarcolemma during lengthening and shortening of the muscle cells.

Vinculin (1) has been implicated in cytoskeleton-to-membrane attachment because the smooth muscle protein binds actin (2, 3) and because, in a variety of cell types, vinculin is located at specialized regions where microfilaments abut the plasma membrane. Some examples of these specialized regions include the plasma membrane-associated dense plaques of smooth muscle cells (4), the fascia adherens of cardiocytes (5), focal adhesion plaques of fibroblasts (1, 6-8), and the zonula adherens of the junctional complex in epithelial cells (9).

In this paper, we report that vinculin defines a striking, two-dimensional lattice associated with the sarcolemma of skeletal muscle cells. Like the hoops and staves of a barrel, this cortical lattice has both longitudinal elements and transverse circumferential elements. We present evidence that the transverse elements of the sarcolemmal lattice ("costameres," from Latin costa, rib, and Greek meros, part) are coupled to the myofibrils.

MATERIALS AND METHODS

Vinculin was purified to at least 93% homogeneity from frozen (-80°C) glycerinated central muscles of chicken gizzards (10) and used to purify vinculin antibody from rabbit antiserum (11). The antibody was tested on immunoblots of total NaDodSO4-soluble muscle proteins separated by NaDodSO4/polyacrylamide gel electrophoresis (12, 13). Thaw-mounted 4-μm cryostat sections were fixed in phosphate-buffered 3% (wt/vol) paraformaldehyde for 5 min and stained as will be described elsewhere, except that p-phenylenediamine was used to delay fluorescence quenching (14). In control staining protocols, affinity-purified anti-keyhole limpet hemocyanin was used as the first-step antibody. Affinity-purified, fluorescein-coupled, goat anti-rabbit IgG was purchased from Boehringer Mannheim and used as the second-step antibody. Some 4-μm cryostat sections of tissue that had been fixed with formaldehyde and infiltrated with sucrose were treated with anti-vinculin and then staphylococal protein A-coated colloidal gold (gift of Douglas Murphy, Johns Hopkins). These sections were processed for electron microscopy; only the most superficial regions of the sections were used for thin sectioning.

RESULTS

Characterization of the Vinculin Antibody. A NaDodSO4 gel of the vinculin used to purify vinculin antibody is shown in Fig. 1A. Purified anti-vinculin recognizes only the 130-kilodalton (kDal) polypeptide in immunoblots of this same preparation of vinculin (Fig. 1A). This result argues against the presence of reactivity other than to 130-kDal protein (vinculin) in the affinity-purified antibody. When tested on electrophoretic gels of NaDodSO4 gels containing solubilized samples of chicken skeletal and cardiac muscle (Fig. 1B), the affinity-purified vinculin antibody reacts strongly with a 130 kDal polypeptide. The reactive material at the top of the blot is an artifact because it is also present in the control blot. The staining of structures in skeletal muscle by the affinity-purified antibody is abolished by preabsorbing the anti-vinculin with 130-kDal vinculin eluted from a preparative NaDodSO4 gel (Fig. 2). Therefore, all of the immunofluorescence shown in this paper is due to reactivity of the affinity-purified antibody with determinants present on 130-kDa vinculin. Recently, polypeptides larger than vinculin, but immunologically related to it, have been detected in chicken gizzards (15, 16) and to a lesser extent in cardiac and skeletal muscle (15). When we process muscle tissue rapidly, we can detect a higher molecular weight vinculin-like polypeptide in immunoblots of cardiac and skeletal muscle tissue. However, the important point for this report is that all of the fluorescence that we observe in cryostat sections of skeletal muscle can be absorbed by the 130-kDa vinculin (Fig. 2).

Localization of Vinculin in Avian Skeletal Muscle. Cryostat sections of chicken skeletal muscles were studied by indirect immunofluorescence using affinity-purified anti-vinculin and fluorescein isothiocyanate-conjugated goat anti-rabbit IgG. All skeletal muscles examined (pectoralis, anterior latissimus dorsi, posterior latissimus dorsi, and the pulmonary aponeurosis—the avian analogue of the diaphragm) showed the pattern of fluorescence described in the following text.

Intense vinculin fluorescence is associated with the cell cor-

Abbreviation: kDal, kilodalton(s).

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tex, whereas the interior of the muscle fibers lacks detectable fluorescence (Fig. 3A). Organization of the membrane-associated vinculin in a lattice having both longitudinal and transverse periodicity can be seen in sections that provide a surface (en face) view of the cell (Fig. 3B and Fig. 4). In Fig. 3B, which is an edge-on view of stacked fibers from the pulmonary aponeurosis, only the transverse periodicity is easily seen, but on the surface view of a large pectoralis fiber (Fig. 4), both longitudinal and transverse elements of the lattice are evident.

Parallel to the long axis of the cell, foci of vinculin are arranged in linear rows separated by a uniform spacing (Fig. 4). Individual foci might be connected in the longitudinal direction by fine fluorescent strands, but this is not certain. We have no further information on the longitudinal pattern of vinculin fluorescence at the present time.

Perpendicular to the long axis of the cell, periodic rib-like bands of vinculin (costameres) encircle the cell. The staining in the transverse bands is finely punctate. By using phase-contrast and fluorescence microscopy on the same specimen, we observed that in both contracted (Fig. 3 C and D) and stretched (Fig. 5 A and B) muscle the costameres always overlie the I band and exhibit the same periodicity as the underlying superficial layer of myofibrils (Fig. 3 C and D; Fig. 5 A and B). The width of the costamere is approximately the same as the underlying I band (Fig. 3 C and D). When adjacent superficial myofibril groups are staggered with respect to each other as seen by phase-contrast optics, corresponding displacements are evident in the overlying costameres (Fig. 3 C and D). Therefore, the costameres seem to be coupled in some manner to the subjacent layer of sarcomeres.

In contracted muscle (Fig. 3) it is difficult to determine by immunofluorescence whether the costamere is a single band overlying the entire I band (including the Z line), or a doublet band that flanks the Z line. In some cases (Fig. 3 C and D, arrows) a doublet band with the Z line in the middle can be seen, but in most instances the exact relationship of the costamere to the Z line is ambiguous. However, when the muscle fibers of the anterior latissimus dorsi, the posterior latissimus dorsi, or the pectoralis are stretched (as evidenced by wider I bands), the costamere becomes broader and less intense with an obvious discontinuity at the Z line (Fig. 5 A and B). Note that Fig. 5 A and B is half the magnification of Fig. 3 C and D.

To determine more precisely the location of membrane-as-

**Fig. 1.** (A) Specificity of affinity-purified antibodies tested against purified vinculin. A triplicate set of various loadings of purified vinculin was run on NaDodSO4/polyacrylamide gel electrophoresis. The proteins were electrophoretically blotted onto nitrocellulose paper and each set was processed individually as follows: lanes 1, 2, and 3, Coomassie blue (CB)-stained blot of vinculin (2, 1, and 0.5 µg, respectively); lanes 4, 5, and 6, immunosautoradiogram of identical blot overlaid with affinity-purified anti-vinculin (a-VINC) and developed with radiolabeled protein A; lanes 7, 8, and 9, similar immunosautoradiogram but overlaid with normal rabbit IgG (NRIgG); lane St, standards (β-galactosidase, 130 kDa). The antibody reacts only with a 130-kDa polypeptide in the purified vinculin preparation. (B) Specificity of affinity-purified antibodies tested against homogenates of chicken muscles. Triplicate sets of two loadings each of chicken pectoralis skeletal (Sk) muscle (lanes 1, 2, 5, 6, 9, and 10) and cardiac (C) muscle (lanes 3, 4, 7, 8, 11, and 12) tissue were run on NaDodSO4/polyacrylamide gel electrophoresis and electrophoretically blotted onto nitrocellulose paper. Each set was processed individually as follows: lanes 1–4, Coomassie blue-stained blot; lanes 5–8, immunosautoradiogram of identical blot overlaid with affinity-purified anti-vinculin and developed with radiolabeled protein A; lanes 9–12, similar immunosautoradiogram but overlaid with normal rabbit IgG.

**Fig. 2.** Cryostat section of chicken pectoralis stained with anti-vinculin that was previously absorbed on 130-kDa vinculin eluted from a preparative NaDodSO4 gel. The position of the 130-kDa vinculin on the preparative gel was detected by lightly staining the gel with Coomassie blue. The appropriate band was sliced out of the gel, electrophoretically transferred to nitrocellulose, and then used to absorb the affinity-purified anti-vinculin.
FIG. 3. Immunofluorescence localization of vinculin in chicken muscle. Muscle was from the avian diaphragm analogue. (A) Transverse cryostat section. (×1,500; bar represents 10 μm.) (B) Oblique section. Note rib-like bands at cell margin and absence of staining deep within the cell. (×1,380.) (C) Section demonstrating costamere discontinuities and displacements. (×1,380.) (D) Phase-contrast micrograph of C demonstrating misalignment of corresponding myofibril bundle. Arrows indicate regions in this contracted muscle where the costamere can be resolved into a doublet that flanks the Z line.

Associated vinculin with respect to the Z line in contracted muscle, we processed a set of cryostat sections for immunoelectron microscopy, using protein A-coated colloidal gold to detect the rabbit anti-vinculin. Labeling occurs in clusters at the periphery of the cell, with the greatest density of labeling on either side of the Z lines (Fig. 5C). The Z line itself is relatively devoid of vinculin. We conclude that, in both contracted and stretched skeletal muscle, the costamere is a doublet band that flanks the Z line of subjacent sarcomeres.

The sarcolemmal lattice and coregistration of the transverse costameres with the subjacent sarcomeres is not a peculiarity of avian skeletal muscle; we find a similar lattice in avian cardiac muscle and in bovine skeletal and cardiac muscles (data not shown).
DISCUSSION

Using antibody to vinculin, we have found an orthogonal lattice associated with the sarcolemma of skeletal muscle cells. This structure appears to be conserved across species and throughout postnatal development (data not shown). We have examined closely the transverse bands of the lattice (costameres) and find that they have the following characteristics: (i) they exhibit punctate vinculin fluorescence; (ii) they overlie the I bands at various sarcomere lengths; (iii) they have approximately the same width as the underlying I bands; and (iv) they are split into two parts with a transverse linear discontinuity over the Z line.

We think that the costameres are coupled to the underlying myofibrils in skeletal muscle because, whether the muscle is contracted or stretched, the costamere periodicity is identical to that of the underlying sarcomeres. Also, when a bundle of superficial myofibrils is staggered with respect to an adjacent bundle, corresponding costamere discontinuities and displacements occur.

There are three observations which show that the costamere is not related to the T tube (transverse tubule) system. First, there is no vinculin at sites removed from the cell periphery in the various avian skeletal muscles examined. Second, T tube openings, especially in chicken pectoralis, are often directly over the Z line, yet typically there is no vinculin concentration precisely at this site. Third, chicken anterior and posterior latissimus dorsi differ radically in the extent, periodicity, and sites of T tube insertions, yet both muscles have identical and completely regular costamere banding patterns (data not shown). The peripheral couplings (19) of the sarcoplasmic reticulum are found in the general area where vinculin is concentrated at the costamere. However, these couplings are seen less frequently in twitch muscles (20) and probably cannot account for the observed extensive costamere pattern. Because the costameres are intimately coordinated with the underlying sarcomeres, we think it is probable that the costameres mark sites at which the sarcolemma is connected to the internal bundle of myofibrils.

In a survey of the ultrastructural literature, we found that electron micrographs of cardiac tissue frequently show festooning of the plasma membrane with apparent fibrous attachments to the Z lines (21). In contrast, ultrastructural evidence for sarcolemma–myofibril connections in skeletal muscle is sparse. Most electron micrographs give no indication of such connections and widely read histology textbooks do not describe sarcolemmal attachments of skeletal muscle myofibrils. Nevertheless, there have been sporadic but excellent descriptions of sarcolemma–myofibril attachment regions in skeletal muscle. In 1859, Amici reported (22) a regular festooning of the skeletal muscle plasma membrane with apparent attachments at the Z line. This finding was confirmed by subsequent light microscopic studies, although there was confusion as to whether the membrane was attached to the Z line or whether the apparent attachments were merely sites of T tube origin (for historical review, see ref. 23). By electron microscopy, fibrous attachments from the M and Z lines were described in frog sartorius muscle (24), insect flight muscles (25), and the scallop adductor muscle (26). The infrequent observation of these connections might mean that the ultrastructure is poorly preserved by conventional fixatives or that significant clearing of the cytosol must be achieved before the structure can be visualized. In fact, this explanation has been proposed by Pierbon-Bornioli, who reported recently that fibrous connections of M and Z lines to the sarcolemma can be visualized in rat diaphragm under certain unconventional conditions of tissue preparation and fixation, albeit with significant compromise of the general ultrastructure of the cell (27). It will be important to determine if and how the two-dimensional lattice, visualized by antibody to one of its molecular components, is related to the one-dimensional, fibrous connections defined in the papers cited above.

If, as we suggest, the transverse costameres of the sarcolemmal lattice are connected to the underlying sarcomeres, this
structure could mechanically integrate the muscle contractile units to the plasma membrane. During contraction the costameres would be expected to pleat the membrane, causing it to form outward-directed festoons. The plasma membrane would thus be prevented from being grossly displaced from the body of the cell during the extensive shortening that occurs during contraction.

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FIG. 5. Costameres in stretched muscle. The chicken anterior latissimus dorsi muscle was frozen while it was under tension. (A) Cryostat section stained for vinculin. The costameres overlie the I bands and appear to split into doublet bands, the discontinuity (arrow) occurring directly over the Z line. (×688; bar represents 20 µm.) (B) Phase-contrast micrograph of A, arrow at Z line. Similar patterns were observed in chicken pectoralis and posterior latissimus dorsi (not shown). (C) Immunoelectron microscopy of vinculin in chicken pectoralis. Routine 4-µm cryostat sections were stained with affinity-purified anti-vinculin followed by protein A-coated colloidal gold. The sections were then processed for electron microscopy. The contracted chicken pectoralis muscle shows dense clusters of gold particles near the cell margin to either side of the Z line. (×40,000.) Control sections treated with an irrelevant antibody (to keyhole limpet hemocyanin) have minimal labeling (not shown).