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Feet, Bridges, and Pillars in Triad Junctions of Mammalian Skeletal Muscle: Their Possible Relationship to Calcium Buffers in Terminal Cisternae and T-Tubules and to Excitation-Contraction Coupling

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Summary. The structure of the triad junction was examined in thin sections of mammalian fast-twitch skeletal muscle. The aims of the experiments were twofold: first, to examine relationships between the contents of the junctional gap and the terminal cisternae that could be significant in excitation-contraction coupling and, second, to look for structures in the transverse tubules that could support a calcium buffer system. Procedures known to stabilize cytoskeletal elements were used in an attempt to retain the original structure. "Feet," "pillars" and "bridges" were often seen side by side in the same junction. In one such junction, the average center-to-center spacing between four bridges was 30.9 ± 1.7 nm and between five foot-like structures was 29.2 ± 1.4 nm. The subunit structure of the feet could be seen in many sections. The lumen of the terminal cisternae was filled with a tetragonal network of calsequestrin which formed parallel strands near the junctional membrane, in register with the feet. The strands overlay the area occupied by "rods" seen in freeze-fracture replicas of terminal cisterna membrane. The contents of the transverse tubules were aggregated into bands, or "tethers," which extended across the short axis of the tubule at regular intervals of about 30 nm. The tethers consisted of flattened discs, stacked across the long axis of the tubule, aligned with the junctional feet. Lanthanum staining of the tethers indicated cationic binding sites that could buffer luminal calcium ion concentration in the vicinity of the voltage sensor for contraction. It is suggested (i) that the control of calcium concentration near the voltage sensor is necessary for normal activation, (ii) that feet, pillars and bridges are different images of a spanning structure, and (iii) that the regular alignment of tethers, feet and calsequestrin is functionally significant in excitation-contraction coupling.

Key Words T-tubule calcium buffer · triad junction · mammalian skeletal muscle · excitation-contraction coupling

Introduction

The first aim of this study was to examine structures in the triad junction that might play a role in excitation-contraction coupling in skeletal muscle. The mechanism by which depolarization of the transverse (T-) tubule membrane initiates calcium release from the terminal cisternae is unknown. One hypothesis is that there is mechanical coupling between voltage-sensitive molecules in the T-tubule and the calcium release apparatus (Chandler, Rakowski & Schneider, 1976). Recent evidence supports this hypothesis: high molecular weight (350 to 450 kD) ryanodine receptor proteins from triads (Lai et al., 1988) form calcium channels in lipid bilayers, which could be involved in calcium release since the pharmcological properties of the channels and calcium release from terminal cisternae vesicles are the same (Liu et al., 1988; Smith et al., 1988). It is not clear which of the several structures seen in the triad might be associated with the high molecular weight protein complexes. Junctional "feet" (Franzini-Armstrong, 1970) are probably involved: each of the four subunits of a foot (Ferguson, Schwartz & Franzini-Armstrong, 1984) contains a 600-kD spanning protein with two 300-kD subunits (Kawamoto et al., 1986). Negative staining of isolated ryanodine receptor/calcium channels reveals four subunit structures with dimensions similar to feet (Lai et al., 1988; Saito, Inui & Fleischer, 1988). These observations suggest that the calcium release channel is an integral part of the foot structure and is physically linked to the T-tubule membrane. Consequently, the simplest model for activation is a mechanical model in which depolarization of the T-tubule membrane produces a conformational change in the foot protein, which opens calcium channels in the terminal cisternae.

Other structures identified in the triad junction include "pillars" (Eisenberg & Eisenberg, 1982) and "bridges" (Somlyo, 1979). Analysis of fish muscle suggests that pillars are feet in which a pore between the subunits forms a central electron-lucent area (Franzini-Armstrong & Nunzi, 1983). It is not clear whether bridges (Somlyo, 1979) are also a

different image of the foot. Further, the relationship between "rods" (Dulhunty, 1988) and the junctional processes has not been established. The results of this study suggest that feet, bridges and pillars in mammals are different images of a spanning process and are structurally related to rods, to calcium-binding protein in the terminal cisternae, and to electron-dense T-tubular inclusions.

The second aim was to look for structures, analogous to calsequestrin in the terminal cisternae, that might be associated with a calcium buffer in the T-tubule lumen. A calcium buffer in the T system would explain two anomalous observations. First, contraction is strongly depressed in low calcium solutions containing 20 to 80 mM EGTA (Barrett & Barrett, 1978; Spiecker, Melzer & Luttgau, 1979; Dulhunty & Gage, 1988), but much less depressed in 1 to 2 mM EGTA (Armstrong, Bezanilla & Horowicz, 1972) or unbuffered calcium-free solutions (Edman & Grieve, 1964; Caputo & Giminez, 1967; Dulhunty & Gage, 1988); a strong external calcium buffer may be necessary to overcome the influence of an intrinsic buffer. Second, isolated T-tubule vesicles have a total calcium concentration of 28 mM (C. Hidalgo, *personal communication*), indicating that there is a store of bound calcium.

This report provides the first direct evidence for a cationic binding substance in the T-tubule lumen. Structures are described that span the tubules at the triad junction and appear to tether the tubule walls into the characteristic elliptical shape. The "tethers" (i) have a high affinity for lanthanum ions and (ii) are apparently continuous with structures in the junctional gap and terminal cisternae. It is suggested that tethers play a role in excitation-contraction coupling by buffering calcium ions in the vicinity of the voltage sensor for contraction.

Materials and Methods

Preservation of the Cytoskeleton

One aim of the experiments was to preserve the cytoskeleton. This was achieved by (i) blocking calcium-activated proteases with Ep-475 (Blest, Stowe & Eddey, 1982; de Couet, Stowe & Blest, 1984), (ii) by light tannic acid fixation (Stowe, Fukudome & Tanaka, 1986), and (iii) by reducing osmium concentration and exposure time during osmication (Stowe et al., 1986).

Biological Material and Solutions

Sternomastoid muscles were isolated from male wistar rats, pinned out at rest length in a Sylgard (Dow Corning) lined petri dish and bathed in a Krebs solution containing (in mM): NaCl, 142; KCl, 3.5; $CaCl_2$, 2.5; $MgCl_2$, 1.0; glucose, 11; TES (N-tris (hyroxymethyl)methyl-2-aminoethanesulphonic acid) buffer, 10 (ph = 7.4). Ca^{2+} was omitted from the Krebs solution in some experiments. The muscles were fine dissected into small bundles of 20 to 30 fibers. The fibers were preincubated for 2 hr in 2 mM EP-475, or 2 mM $LaCl_3$, added to the Krebs solution. EP-475 was dissolved with equimolar $Ca(HCO_3)_2$.

Fixation and Processing for Electron Microscopy

Following preincubation, the fibers were fixed for 2 hr in 2.5% glutaraldehyde and 3.5% paraformaldehyde in either Krebs solution or 0.1 M cacodylate buffer, washed in Krebs solution or cacodylate buffer, postfixed in 0.1% tannic acid for 3 hr, washed overnight, osmicated (0.5% osmium) for 45 min, washed in H_2O, dehydrated through ethanol and propylene oxide and embedded in araldite. Some preparations were stained for 2 hr in 1% uranyl acetate (in 70% alcohol) at the beginning of dehydration.

The following list summarizes the several different procedures used. Methods for fixation through to dehydration were the same in each case. The vehicles used for fixatives, and for washing the tissue, are given in parentheses:

Method 1—preincubation in EP-475 in a calcium-containing Krebs solution, standard fixation (cacodylate buffer), dehydration and embedding.

Method 2—preincubation in EP-475 in a calcium-free Krebs solution, standard fixation (cacodylate buffer), dehydration and embedding.

Method 3—no preincubation, standard fixation (calcium-free Krebs solution), dehydration and embedding.

Method 4—preincubation in 2 mM $LaCl_3$ in a calcium-containing Krebs solution, standard fixation (cacodylate buffer), *en bloc* uranyl acetate, dehydration and embedding.

Method 5—preincubation in EP-475 in a calcium-containing Krebs solution, standard fixation (cacodylate buffer), *en bloc* uranyl acetate, dehydration and embedding. Sections were stained with lead citrate.

No major differences were noted between the first two methods. The tetragonal structure of the contents of the terminal cisternae was better preserved in fibers preincubated in EP-475 (Methods 1 and 2) than in fibers fixed without preincubation (Method 3). No sections of material fixed using Method 3 are shown. Thin longitudinal sections of the fibers were examined in a Phillips 301S electron microscope.

Analysis of Micrographs

The orientation of structures in the triad is described relative to the axes of the T-tubule, which are defined in Fig. 1*A*. The long and short axes describe the cross-section of the T-tubule, and a third axis, *X*, is parallel to the length of the tubule. Transverse sections of the triad are in the plane of the long and short axis and reveal both axes of the T-tubule (Fig. 1*C*). Longitudinal sections of the triad, in the plane of the short axis and the *X* axis, reveal only the short axis of the tubule (Fig. 1*B*). Both longitudinal and transverse sections of triads are seen in longitudinal sections of muscle fibers.

Results

In these experiments the membranes of the triad and the contents of the T-tubule lumen, junctional gap and terminal cisternae demonstrated a similar electron density after light tannic acid and osmium

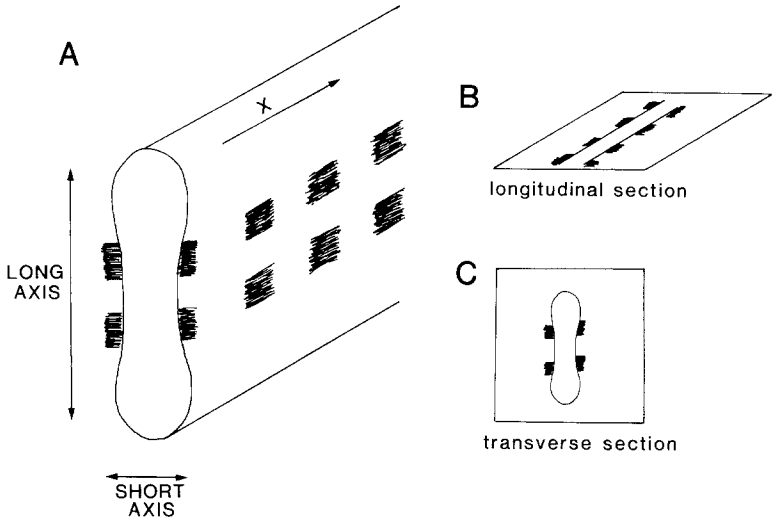

Fig. 1. A schematic illustration of the axes of the T-tubule (*A*) and the orientation of the T-tubule in longitudinal (*B*) and transverse (*C*) sections of the triad. The shaded areas represent junctional feet severed between the T-tubule and terminal cisternae. The terminal cisternae membranes are not shown

fixation. Consequently, the sections had an overall low contrast appearance. The advantage of the technique was that more fine structure was visible in the cytosol, particularly in the terminal cisterna lumen and in the junctional gap, as well as in the T-tubule lumen.

Contents of T-Tubule Lumen

Organization of the intraluminal contents of the T system was apparent in most sections (*see*, e.g., Fig. 2). Bands of electron-dense material occurred at regular intervals along the length of the tubule and were oriented perpendicular to the tubule membrane (large arrows in Fig. 2). In transverse sections of the triad (Fig. 3) similar, although narrower, bands of material were stacked across the long axis of the tubule. The bands were named "tethers" because they appeared to pull the T-tubule into its characteristic elliptical cross-section.

Two micrographs of the same area are shown in Fig. 3. The first (Fig. 3*a*) is printed on low contrast paper and the second (Fig. 3b) on high contrast paper, and the features referred to in Fig. 3*a*, i.e., tethers, junctional structures and calsequestrin, are outlined with pen and ink for clarity. Figures 6 to 9 have been arranged in the same way.

Although the edges of the tethers were not clearly defined, a rough estimate of their dimensions could be obtained. The average width of 26 tethers in longitudinal sections of T-tubules (Fig. 2) was 18.7 ± 1.2 nm (mean ± SEM). In contrast, the tethers in transverse sections were narrower (Fig. 3), with an average width of 7.5 ± 0.4 nm (25 examples). The longitudinal space between tethers was 13.4 ± 1.1 nm in 26 observations, giving a periodicity 32 nm, similar to the periodicity of feet (Franzini-Armstrong, 1970) and bridges (Somlyo, 1979). Indeed, a regular coincidence between the tethers and junctional structures can be seen in Figs. 2 and 3. The space between 23 tethers in transverse sections was 9.0 ± 0.8 nm; two tethers were usually associated with each foot (Fig. 3). The different longitudinal and transverse profiles suggest that the tethers are flattened disks of material aligned across the T-tubule lumen at regular intervals, in register with the junctional feet.

An unusually clear image of the tethers is shown in Fig. 4. Tannic acid is known to produce variable staining of membranes and intracellular inclusions (Somlyo, 1979). The T-tubule membrane and tethers in the "freak" image in Fig. 4 were stained more densely than the surrounding tissue. The tethers appear to be continuous with the T-tubule membrane, and the membrane is slightly scalloped at the junction with the structures.

Some preparations were preincubated in 2 mM $LaCl_3$ before fixation. Lanthanum did not penetrate well and only T-tubules near the fiber surface were stained. Lanthanum produced a high contrast stain that was easily recognized against the background of low contrast tannic acid/osmium stained material. Both the membrane and the repeating tethering structures are outlined in the T-tubule shown in Fig. 5, showing that lanthanum was bound to both structures. The apparent affinity of the tethers for lanthanum suggests that the material contains cationic binding sites and could, therefore, function as a calcium buffer system.

Contents of the Junctional Gap between the T-System and Terminal Cisternae

The appearance of junctional "feet" in thin sections can vary, depending on the orientation and thick-

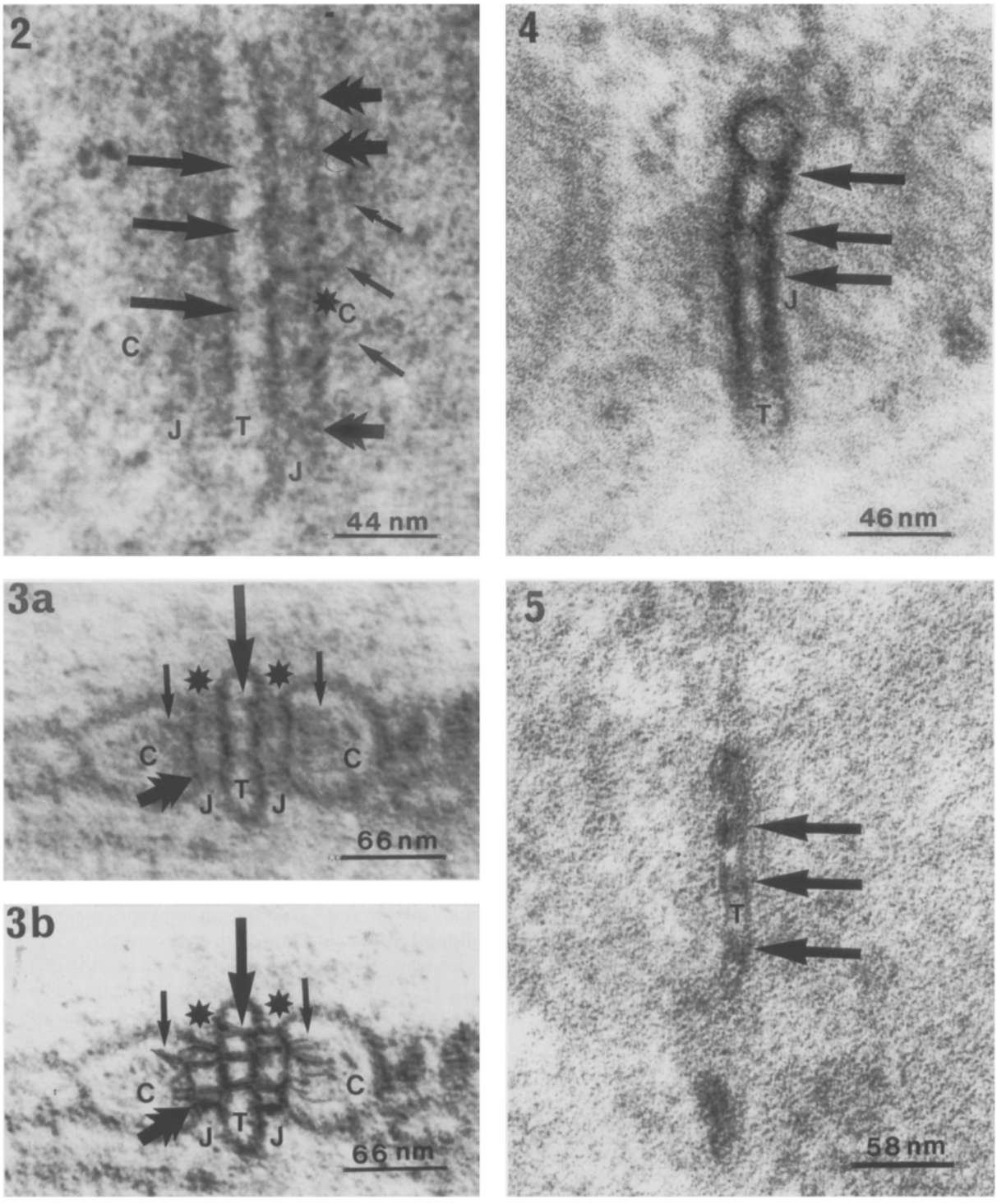

Fig. 2. A longitudinal section through a triad, showing a regular pattern of tethers spanning the short axis of the tubule (large, single arrows). The material was processed using Method 1 (*see* Materials and Methods). *—columned junctional foot (*see text*); large double arrows—amorphous junctional feet (*see text*); small single arrows—parallel strands of calsequestrin terminating on the junctional membrane; *C*—terminal cisternae membrane; *J*—junctional gap; *T*—T-tubule. The calibration bar is 44 nm

Fig. 3. A transverse section through a triad, showing tethers spanning the short axis of the T-tubule and stacked along the long axis. The material was processed using Method 1 (*see* Materials and Methods). The symbols are the same as in Fig. 2. *3a*—an original micrograph; *3b*—a high contrast copy of *3a* in which the relevent structures have been outlined with pen and ink for clarity. The calibration bar is 66 nm

ness of the section. Three types of image have been defined by Franzini-Armstrong and Nunzi (1983):

Type 1 feet—partially cross the junctional gap and are mostly associated with the terminal cisterna. Type 1 feet are referred to as "partial feet."

Type 2 feet—form one amorphous electron dense column which contacts both the T-tubule and terminal cisterna membranes. Type 2 feet are referred to as "amorphous feet."

Type 3 feet—form two dense columns separated by a less dense core and contact both the T-tubule and terminal cisterna membranes. Type 3 feet are referred to as "columned feet" and have also been described as "pillars" (Eisenberg & Gilai, 1979; Eisenberg & Eisenberg, 1982).

The most common image was of amorphous feet (Type 2 feet, double arrow Fig. 2). The center-to-center spacing between 26 densities in longitudinal sections of tubules was 31.0 ± 0.5 nm, and the spacing in transverse sections was 34.4 ± 0.8 nm (25 densities). The spacing was similar to that of junctional feet in frog skeletal muscle (Franzini-Armstrong, 1970).

Columned feet (type 3 feet) revealed the subunit structure of the foot (Ferguson et al., 1984) and were seen in longitudinal (star, Fig. 2) and transverse sections (stars, Fig.3) of the junction. Although an electron-lucent area between the columns in the light tannic acid-stained material is not as clear as that seen with other fixation techniques (Eisenberg & Eisenberg, 1982; Franzini-Armstrong & Nunzi, 1983), the feet indicated by the stars in Figs. 2 and 3 are clearly not amorphous and show a central discontinuity, either more lucent (Fig. 2) or more opaque (Fig. 3) than the columns on either side, which are presumably two subunits seen in a side-on view of a four-subunit foot. Ideally, all sections through a four-subunit foot should have a columned appearance. The more general amorphous image can be attributed to the alignment of sections and the superimposition of feet in thicker sections.

Both amorphous and columned feet were continuous across the junction, making contact with opposing T-tubule and terminal cisternae membranes (Figs. 2 and 3). Partial feet (type 1 feet) were discontinuous and mostly associated with the terminal cisternae (tripple arrow head, Figs. 6 and 8). Franzini-Armstrong and Nunzi (1983) attribute the structure of partial feet to very thin sections (about 30 nm) cutting transversely through the junction, so as to miss the part of the foot attached to the T-tubule. Since partial feet are preferentially associated with the terminal cisterna side of the junction (Fig. 8), it seems likely that the segment of the foot attached to the T-tubule is thinner, or less electron dense, than the segment attached to the terminal cisterna.

Partial, amorphous and columned feet were often seen in the same junction. The five feet in the left-hand junctional gap in Fig. 6 are (listing up from 1 to 5): amorphous, columned, partial, amorphous, columned. Thin parallel densities, probably corresponding to bridges (Somlyo, 1979), were commonly seen in the same junctions as the feet. The bridges (long thin arrows, Fig. 6) extended from the tip of scallops in the terminal cisternae membrane, coinciding with the expected central area of a foot and the central edges of the electron-dense regions of columned feet. The regular periodicity of the five feet in Fig. 6 extends to the bridges, and the average center-to-center spacing between the nine structures in the junction was 29.6 ± 1.0 nm. There was no significant difference between the average spacing between the five feet, 29.2 ± 1.4 nm, or four bridges, 30.9 ± 1.7 nm. It is likely that the four types of structure in the junction are different images of identical spanning structures.

Contents of the Terminal Cisternae

The lumen of the terminal cisternae was filled with strands of calsequestrin, which had an ordered network structure that was most obvious in longitudinal sections of triads (Figs. 6 and 8). The tetragonal network extended across the lumen from the junction to the opposing nonjunctional membrane. The calsequestrin formed parallel strands near the junction and terminated on the membrane, in register with the junctional feet (short arrows, Figs. 2 to 9). The calsequestrin strands appeared to terminate near the edges of the junctional processes, in register with the outer edges of the junctional processes,

Fig. 4. A tangential section through a T-tubule which has been stained with tannic acid. The micrograph provides a particularly clear image of the tethers. The symbols are the same as in Fig. 2. The tissue was processed using Method 2 (*see* Materials and Methods). The calibration bar is 46 nm

Fig. 5. A longitudinal section through a triad in which the T-tubule has been stained with lanthanum. Note the staining of the tethers as well as the T-tubule membrane. The tissue was processed using Method 4 (*see* Materials and Methods). The symbols are the same as in Fig. 2. The calibration bar is 58 nm

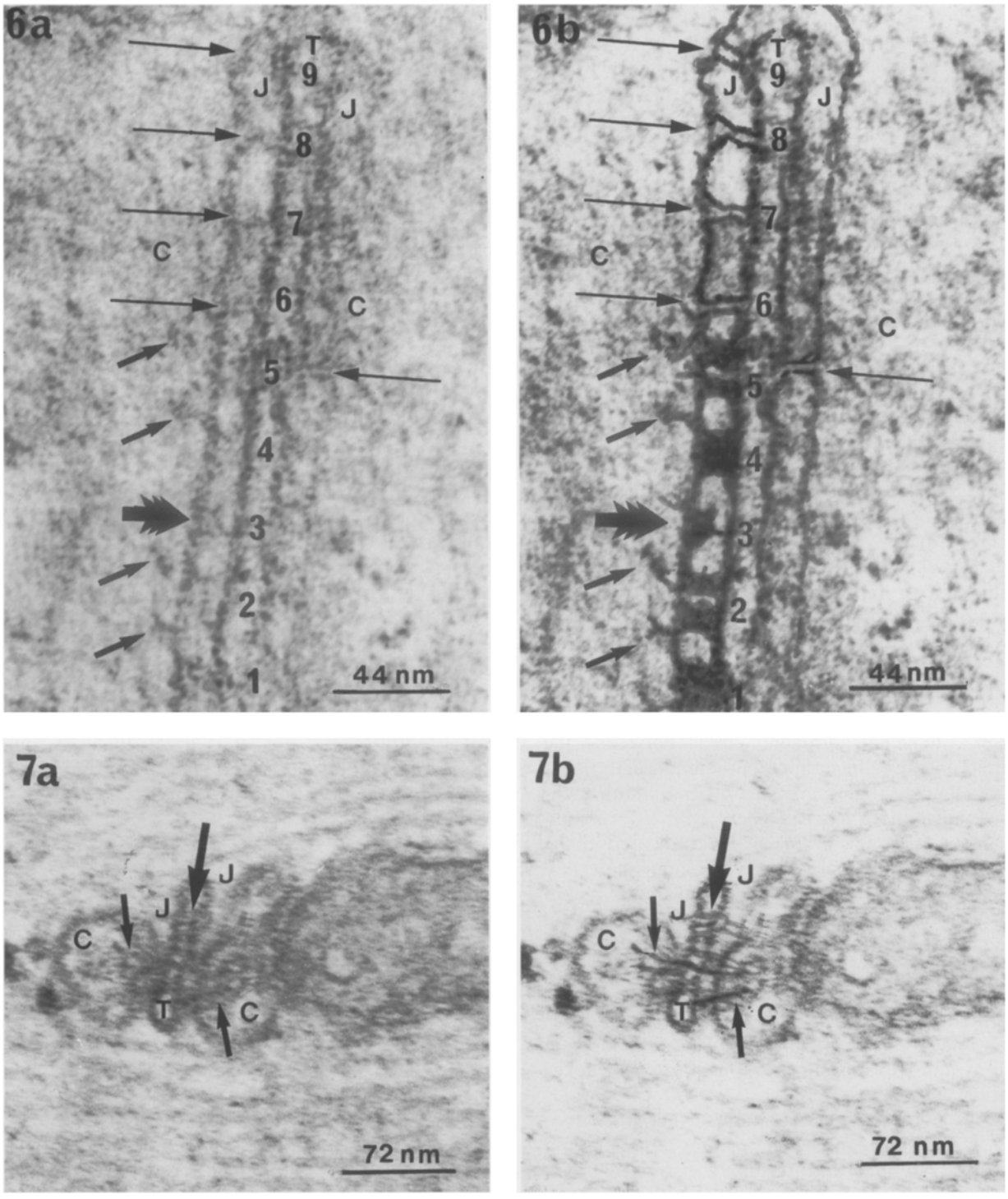

Fig. 6. A tangential (mostly longitudinal) section through a triad. The tissue was processed using Method 1 (*see* Materials and Methods). The numbers in the T-tubule lumen are placed beside the eight processes in the adjacent junctional gap. The micrograph shows (i) partial (tripple arrow), amorphous and columned junctional feet (*see text*) and bridges (long thin arrows) in the same junction, (ii) parallel bands of calsequestrin terminating on the junctional membrane of the terminal cisternae (shown more clearly in Fig. 8), and (iii) the tetragonal network of calsequestrin in the body of the terminal cisternase. *6a*—the original micrograph; *6b*—a high contrast copy of 6a in which the relevant structures have been outlined with pen and ink for clarity. Other symbols are the same as in Fig. 2. The calibration bar is 44 nm

Fig. 7. A tangential (mostly transverse) section through a triad showing continuity between tethers, feet and calsequestrin. The tissue was processed using Method 2 (*see* Materials and Methods). *7a*—the original micrograph; *7b*—a high contrast copy of *7a* in which the relevant structures have been outlined with pen and ink for clarity. The symbols are the same as in Fig. 2. The calibration bar is 72 nm

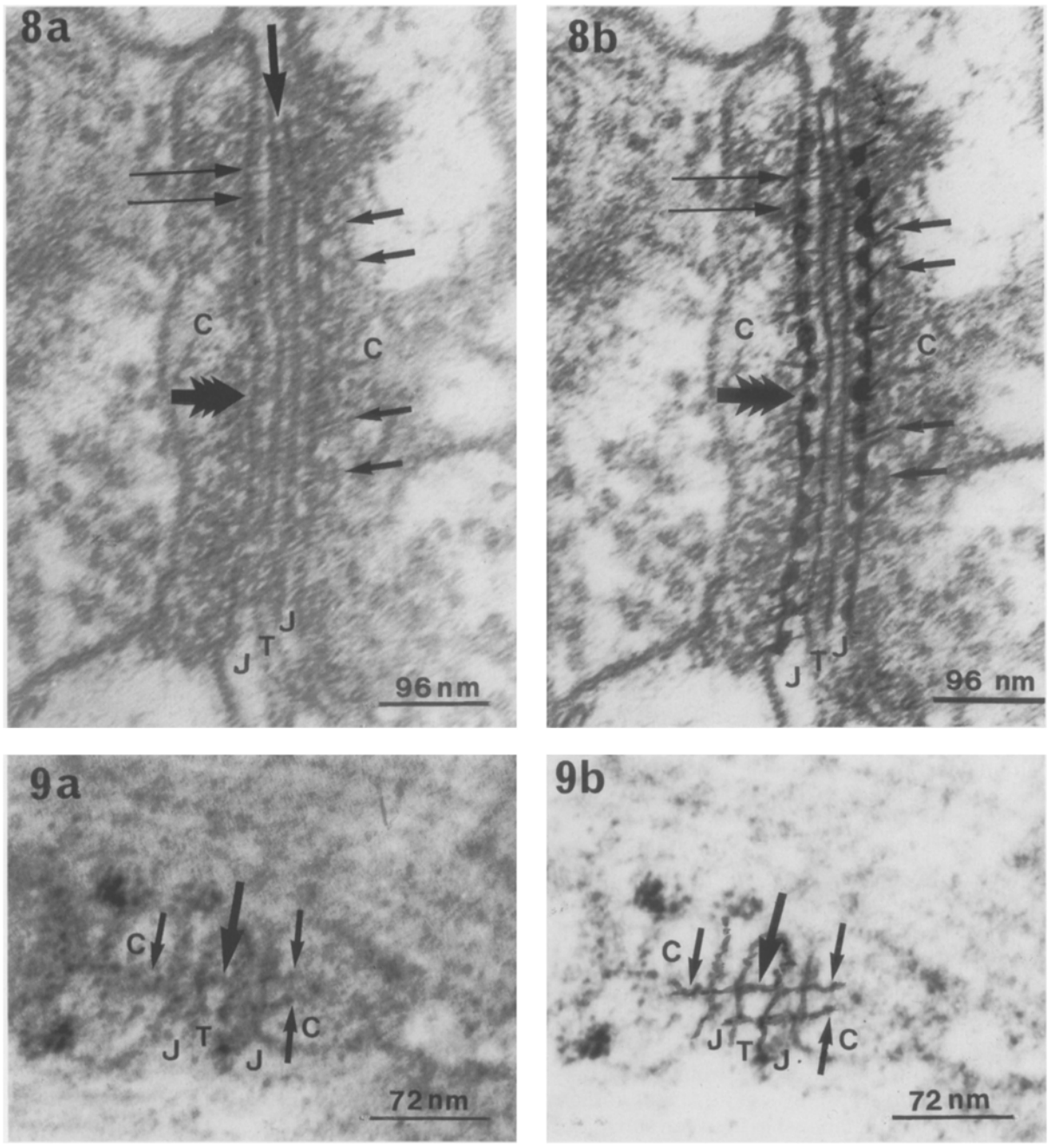

Fig. 8. A tangential (mostly longitudinal) section through a triad showing partial feet (tripple arrow) and bridges (long thin arrows). The micrograph also shows the tetragonal structure of calsequestrin in the body of the terminal cisternae. The tissue was processed using Method 5 (*see* Materials and Methods). *8a*—the original micrograph; *8b*—a high contrast copy of *8a* in which the relevant structures have been outlined with pen and ink for clarity. Other symbols are the same as in Fig. 2. The calibration bar is 96 nm

Fig. 9. A transverse section through a triad showing the alignment of tethers, feet and calsequestrin. The tissue was processed using Method 1 (*see* Materials and Methods). *9a*—the original micrograph; *9b*—a high contrast copy of *9a* in which the relevant structures have been outlined with pen and ink for clarity. The symbols are the same as in Fig. 2. The calibration bar is 72 nm

in register with the outer edges of the subunits, rather than the centers of the feet. The network structure was not seen as clearly in transverse sections of triads (Figs. 3, 7 and 9). The parallel strands near the junctional membrane were closer together, with two to three strands approaching one foot (Fig. 2).

Thinner filaments appeared to anchor the strands of calsequestrin to the parajunctional membrane (Fig. 3). The filaments terminated on the flat

surface of the terminal cisternae membrane in the position occupied by rods in freeze-fracture replicas (Dulhunty, 1987). It is likely that the rods delineate anchor points for calsequestrin which extend from the junctional gap to the parajunctional membrane.

Continuity between Contents of the T-System, Junctional Gap and Terminal Cisternae

One of the most notable aspects of the micrographs in Figs. 2 to 9 is the alignment of structures across the triad. Tethers in the T-tubule appear to be aligned with feet in the junctional gap on either side and the feet aligned with calsequestrin strands in the terminal cisternae (Figs. 3, 7 and 9). The overall impression in many triads, particularly in transverse sections (Fig. 3 and Fig. 7), is that the calsequestrin strands in the opposing terminal cisternae of the triad are connected, through the feet in each junctional gap and tethers in the T-tubule. The alignment was most clearly seen when the plane of the section was exactly longitudinal (Fig. 2) or transverse (Fig. 3). The less regular alignment in tangential sections (e.g., Figs. 6 and 8) may be attributed to the orientation of the section. The alignment of structures suggests a physical association that may be functionally important in excitation-contraction coupling.

Discussion

Feet, pillars and bridges in mammals were similar to those in fish and amphibian muscle (Franzini-Armstrong, 1970; Somlyo, 1979; Eisenberg & Eisenberg, 1982; Franzini-Armstrong & Nunzi, 1983). The regular periodicity of bridges and feet and their occurrence in the same junctions, support the conclusion that there is only one spanning structure and suggest that different images result from the orientation of the subunits (Ferguson et al., 1984) to the section. The physical relationship between subunits may change during activation so as to increase the probability of visualizing columned feet (i.e., pillars; Eisenberg & Eisenberg, 1982).

Two newly described features are the tethers in the T-tubule lumen and the alignment of calsequestrin, tethers and feet. It is suggested that calcium buffering by the tethers is important since excitation-contraction coupling is sensitive to external calcium concentrations (Dulhunty & Gage, 1988; Brum, Rios & Stefani, 1988). A buffer near the voltage sensor for contraction would dampen fluctuations in calcium concentration arising from activity of the T-tubule calcium pump (Malouf & Meissner, 1979) and from voltage-activated calcium channels (Almers, Fink & Palade, 1981).

A functional role for the tethers is indicated by their alignment with the feet. The feet have a high affinity for T-tubule proteins (Caswell, Brandt & Kawamoto, 1988) and a high molecular weight complex, including ryanodine receptors (in the terminal cisternae membrane; Lai et al., 1988) and dihydropyridine receptors (in the T-tubule membrane), has been isolated (Knudson et al., 1988). Dihydropyridine receptors are thought to be the voltage sensors for excitation-contraction coupling (Lamb, 1986; Lamb & Walsh, 1987; Rios & Brum, 1987; Dulhunty & Gage, 1988; Tanabe et al., 1988). The physical association between the feet and proteins in the T-tubule membrane could extend to structures in the T-tubule lumen, especially if the role of the structures was to regulate calcium concentration at the voltage-sensitive site.

The tethers may be associated with the major intrinsic 72-kD T-tubule protein (Caswell et al., 1988). Alternatively, since no specific calcium-binding proteins have been demonstrated in T-tubule fractions, it is possible that the tethers are a part of the glycocalyx, which is negatively charged and could trap positively charged ions. The a_2 subunit of the dihydropyridine receptor is glycosolated, and it is likely that the glycosolation sites are on the extracellular (i.e., luminal) side of the T-tubule membrane (Takahshi et al., 1987; Tanabe et al., 1987). The glycosolated proteins may well be distributed unevenly in the membrane and, as a part of the dihydropyridine receptor complex, may be specifically associated with the junctional feet (Knudsen et al., 1988).

The lability of the structure of calsequestrin is well recognized (Franzini-Armstrong, Kenney & Varriano-Marston, 1987), and procedures to preserve the cytoskeleton probably helped to maintain the alignment between calsequestrin and the feet, which is not seen in deep-etched freeze-fracture replicas (Franzini-Armstrong et al., 1987). The structural alignment between calsequestrin and the feet was not surprising since the protein is associated with junctional membranes in thin-sectioned and deep-etched intact fibers (Johnson & Sommer, 1967; Walker, Schrodt & Edge, 1971; Somlyo, 1979; Eisenberg & Gilai, 1979; Saito et al., 1988; Franzini-Armstrong et al., 1987) and in isolated terminal cisternae vesicles (Campbell, Franzini-Armstrong & Shamoo, 1980; Brunschwig et al., 1982). A specific association with the foot is indicated by the affinity of calsequestrin for spanning protein (Caswell & Brunschwig, 1984; Costello et al., 1986; Kawamoto et al., 1986) and by its ability to modify calcium release (Ikemoto & Koshita, 1988) through the

ryanodine receptor/foot complex (Lai et al., 1988). It is possible that there is a molecular connection between the dihydropyridine receptor and calsequestrin, through the foot complex, which provides the physical basis for a mechanical coupling mechanism in excitation-contraction coupling.

The structural relationship between the feet and calsequestrin suggests a model for excitation-contraction coupling in which the feet communicate directly with the calcium-binding protein. One such model is that T-tubule depolarization allows voltage-sensitive molecules to undergo a conformational change, which is transmitted through the spanning protein to calsequestrin. As a result, the affinity of calsequestrin for calcium is reduced, calcium ions dissociate and flow into the sarcoplasm through channels which have an open time that is regulated by calcium concentrations, adenine nucleotide levels (Smith, Coranado & Meissner, 1985; Rousseau et al., 1986) and by inositol (1,4,5)-trisphosphate (Suarez-Isla et al., 1988). This model differs from other mechanical models in that the function of the junctional structures is to transduce the depolarization signal to a reduction in calsequestrin affinity for calcium, followed by a secondary extrajunctional calcium release through open channels. In more conventional models the junctional structures are coupled with junctional sarcoplasmic reticulum calcium channels and facilitate the opening of the calcium channels in response to T-tubule membrane depolarization (Ma et al., 1988). In either case, the transduction mechanism depends on a physical relationship between the feet and the terminal cisternae membrane. It has been shown that an intact triad structure is essential for voltage-activated contraction, since both contraction and asymmetric charge movement are depressed when the triad is disrupted by gentle glycerol treatment which preserves T-tubule geometry (Dulhunty & Gage, 1973; Dulhunty, Gage & Barry, 1981).

A model for excitation-contraction coupling in which the junctional processes are coupled with the calcium-release channels implies that the calcium-release channels open directly into the junctional gap. Such a model is currently popular because (i) calcium-activated calcium channels are apparently located in the junctional membrane of the terminal cisternae (Zorzato & Volpe, 1988), (ii) negatively stained ryanodine receptors form foot-like structures when viewed electron microscopically (Lai et al., 1988; Saito, 1988), and (iii) ryanodine receptors form calcium channels with the properties of calcium-activated calcium channels in lipid bilayers (Liu et al., 1988; Smith et al., 1988). The question of whether or not the ryanodine-receptor/calcium-activated-calcium-release channel is the channel activated during excitation-contraction coupling in intact fibers remains unresolved. While T-tubule dependent calcium release in skinned fibers is in fact calcium dependent (Stephenson, 1987), there is other compelling evidence that calcium-activated calcium release is not the mechanism activated during normal depolarization-induced contraction (Endo, 1977; Donaldson, 1985; Brunder & Palade, 1988).

One problem with a model in which calcium is released into the junctional gap is that unstirred layer effects in the small volume would restrict diffusion from the release sites to the myofilaments. Furthermore, free calcium in the gap would have to increase to at least 35 mM to raise the myoplasmic calcium concentration to 10^{-5} (Dulhunty, 1988). It would be more logical if calcium release sites were on the flat surfaces of the terminal cisternae in the vicinity of indentations (Dulhunty & Valois, 1983). Although the ryanodine-receptor/calcium-activated-calcium-release channels have been located in the junctional membrane (*see above*), there is no evidence to suggest that the channels are confined to that area of membrane. The channels may well be distributed over the junctional and flat surfaces of the terminal cisternae to allow calcium release into the myoplasm where it is closer to, and has easier access to, its site of action at the myofilaments.

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