Coiled Perimysial Fibers of Papillary Muscle in Rat Heart: Morphology, Distribution, and Changes in Configuration

Thomas F. Robinson, Maria A. Geraci, Edmund H. Sonnenblick, and Stephen M. Factor

The morphology, distribution, and configuration of coiled perimysial fibers of rat heart papillary muscle were studied. Methods included bright-field light microscopy of silver-stained sections, scanning and transmission electron microscopy, and differential interference contrast light microscopy of unfixed and unstained specimens. Coiled fibers, elliptical in cross section, are arranged in a branched network that diverges from the muscle-tendon junction and is continuous throughout the length of the muscle and into the ventricle wall. Most fibers range in diameter from less than 1 μm to 10 μm and are parallel with the long axis of the muscle, although branching is common and oblique orientations are seen. Several myocytes are associated with each coiled perimysial fiber. Constituent fibrils (diameter, 40–50 nm) occur in bundles twisted within the fiber. Small satellite elastic fibers are parallel to the collagen fiber axes. Stereo analysis of the coiled perimysial fibers reveals helical configurations, as opposed to planar waviness, that become less convoluted or even straighten as the resting muscle is stretched. Calculations based on cross-sectional areas of fibers, changes in fiber configurations, and tensile moduli reported for collagen fibers of other tissues show that the potential tensile strength of the network of coiled perimysial fibers is sufficient to contribute significantly to the mechanical properties of papillary muscle. Detailed evaluations of possible roles of the coiled perimysial collagen fiber system as a function of passive stretch and contraction in ventricular wall, as well as in papillary muscle, warrant further study. (Circulation Research 1988;63:577–592)
also investigated for connective tissue fibers in the chorda tendinea. The numerous CPFs of papillary muscle are large relative to the size of myocytes. The functional implications of the structural characteristics of this impressive, branched, continuous array of collagen fibers are discussed for both in vivo and in vitro function in view of the extensive use of the isolated papillary muscle as a model of myocardial contraction.  

**Materials and Methods**

**Tissue Preparation**

Twenty-nine rat hearts were used specifically for these experiments (photographs from previously studied hearts were also reexamined). Sixteen young adult (15 male and one female) Wistar rats (4–6 months old, 250–300 g) were used, and 10 male Fisher rats (strain 344, barrier reared at the National Institute on Aging; either 3.5 or 23 months old) were used. Three Sprague-Dawley rats (heart weights, 250–300 g) were used.

The rats were anesthetized with ether, and their hearts were quickly removed and placed in oxygenated Tyrode’s solution (270–275 mosm) with the following composition (mM): Na⁺ 151.3, Ca²⁺ 2.4, K⁺ 4.0, Mg²⁺ 0.5, Cl⁻ 147.3, H₂PO₄⁻ 12.0, and glucose 5.5. After the hearts in oxygenated Tyrode’s solution were clear of blood, they were placed in high potassium (30 mM) Tyrode’s solution until beating ceased. This solution was maintained at 30°C and bubbled with 95% O₂-5% CO₂.

Both left and right ventricular papillary muscles were used. Papillary muscles examined with stereomicrophotography were from the right ventricle and were exposed by cutting the anterior border of free right wall and folding it back. Papillary muscles ranged in configuration from fan shaped to cylindrical, and the muscle-tendon junctions varied in shape, as did the occurrence of collagen cords (third-order chordae tendineae) attached to the sides of muscles. The appearance of the CPF network depended on the overall appearance of the muscles. The present study describes only CPF networks in cylindrical muscles (Figure 1).

Some isolated papillary muscles were fixed at slack length, whereas others were stretched before fixation. The muscles were continuously superfused with Tyrode’s solution (pH, 7.2; temperature, 30°C) and bubbled with 95% O₂-5% CO₂. The nontendinous ends of the papillary muscles were inserted
MODEL CONSIDERATIONS FOR A SIMPLE COILED FIBER (helix)

Example. Fiber length stays constant, only coiled configuration changes

\[ \text{FL} = \left(3.14 \times D\right)^2 + P^2 \text{m} \]

<table>
<thead>
<tr>
<th>Compression</th>
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<td>1.4</td>
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Figure 2. Diagrams that show method for calculation of convolution index (CI) and that show changes in pitch (P), configuration, and CI for a uniformly coiled thin fiber of constant length as it is stretched or compressed from rest. P, distance between two corresponding points along the coil; FL, fiber length; D, diameter of coil. CI = FL/P; FL = \[ (3.14 \times D)^2 + P^2 \]. (These diagrams demonstrate the geometric changes for helical fibers that are very thin relative to D. Most coiled perimysial fibers, however, are actually thick relative to D. The range and analysis of configurational changes of CPFs is thus similar but more complex than that shown.)

Fixation
Muscles fixed at either slack or stretched lengths were fixed by substitution of Tyrode’s buffer with Tyrode’s buffer that contained either 3.7% formaldehyde (for subsequent silver staining) or 6% glutaraldehyde (for subsequent ultrastructural studies). Hearts filled with barium-gelatin were sectioned in a cryostat microtome at 100-120-µm thickness.

Fixation for silver staining was performed by immersion of papillary muscles in 3.7% buffered formaldehyde for a minimum of 2 weeks.

Fixation for ultrastructural studies was performed by immersion of papillary muscles in 6% glutaraldehyde in Tyrode’s for 3 hours at room temperature or overnight at 4° C, rinsing several times in buffer, postfixation in 1% osmium tetroxide for 1 hour, and rinsing in buffer. Some muscles were fixed with 0.5% tannic acid in the primary fixative. For muscles that were analyzed by stereomicrophotography, fixation was performed in situ with minimal delay after photography.

Silver Staining
The formaldehyde-fixed muscles were prepared as described previously; longitudinal cryosections were cut at a thickness of 80 µm, and the floating sections were stained with a modification of the silver-staining technique of Hasagawa and Ravens, which in turn was a modification of the silver carbonate method of Del Rio Hortega. Stereomicrophotography
Stereomicrophotography was performed with a Zeiss SV-8 stereomicroscope (Zeiss, Thornwood, New York) fitted with an MC-63 camera and Kodak Tri-X, Panatomic X, or Ektachrome 400 film.

Light Microscopy
Light microscopy of silver-stained sections was performed on a Zeiss Photomicroscope III, and...
FIGURE 3. Light micrograph of silver-stained, 100-μm-thick, longitudinal section of left ventricle at low magnification. Branched network of coiled perimysial fibers is continuous through the papillary muscle (P) from muscle-tendon junction (J; see Figure 4) to ventricle wall (W). Individual branched coiled perimysial fibers can be traced over distances equivalent to many myocyte lengths (arrowheads). (Average myocyte length, ~100 μm). Field represented in micrograph is 5,600×4,000 μm.

Micrographs were taken with Kodachrome 25 film. Differential interference contrast light microscopy was performed with a Zeiss WL microscope, and micrographs were taken with Kodak Technical Pan film. Calibration micrographs were taken with a slide graticule (Graticules Ltd, Tonbridge, UK) or hemocytometer. Optical sectioning was performed to trace the branched network of CPFs through the papillary muscle and into the ventricle wall (on cryosections of fixed, barium-gelatin–infused hearts).

**Electron Microscopy**

Fixed and rinsed muscles were dehydrated through a graded ethanol series.

For scanning electron microscopy, dehydrated samples were critical point–dried in carbon dioxide in a Samdri Model 790 dryer (Tousimis, Rockville, Maryland), equilibrated in liquid nitrogen, fractured with a precooled razor blade, and dried by evaporation of the nitrogen under vacuum. Samples were then mounted on stubs, coated with gold-palladium, and viewed in a JSM 25.

For transmission electron microscopy, dehydrated samples were infiltrated with rotation in Spurr’s resin or Embed 812 (EMS, Fort Washington, Pennsylvania), embedded, and cured in an oven at 70°C. Sections were cut with a diamond knife (Diatome USA, Fort Washington, Pennsylvania) on an LKB Ulrotome III (Rockville, Maryland) or a Reichert Ultracut E (Warner Lambert, Edison, New Jersey), collected on copper grids, stained with 1% uranyl acetate in 40% ethanol–60% water and in Reynold’s aqueous lead citrate, and viewed in a JEOL 100CX or JEOL 1200EX electron microscope. Calibration micrographs were taken with a carbon grating replica (54,864 lines/in.; Ladd Research, Burlington, Vermont).

**Measurements**

Diameters and cross-sectional areas of CPFs and myocytes were estimated from electron micrographs of sections cut from blocks oriented for cross sections of the papillary muscles; perpendicularity of the sections was affirmed by the appear-
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FIGURE 4. Light micrograph of silver-stained section of muscle-tendon junction of left ventricular papillary muscle. Convoluted collagen fibers of perimysium and epimysium are stained with silver. Coiled perimysial fibers (CPF) are large relative to myocyte size, and the CPF network extends from muscle-tendon junction through entire muscle. CPFs are highly coiled at slack length of muscle, and are interconnected by struts to other CPFs. Branches of CPFs (arrowheads) vary from parallel to oblique to muscle axis and have been followed over many myocyte lengths by optical sectioning. EC, epimysial collagen fibers; S, struts; bar, 100 μm. Original magnification, ×190.

ance of the myofilaments. The apparent diameters of fibers in muscle cross sections are larger than their intrinsic diameters because CPFs are helical and the coiled fiber is always cut oblique to its own axis. To minimize overestimation of fiber diameters because of their coiled configurations, many of the measurements were made from stretched muscles, whose CPFs have convolution indexes lower than those at slack length. The straightened fibers are, however, still twisted like a stretched, coiled telephone cord. In addition, measurements were made of fibers in which most fibrils were perpendicular to the plane of section.

Sarcomere lengths were averaged from total lengths of eight to 15 sarcomeres. Measurements were made from calibrated prints of Kodachrome micrographs of midmuscle in regions immediately adjacent to CPFs. No correction factor was applied for possible shrinkage during sample preparation.

Convolution indexes, diameters, and pitches of helices. The actual CPF thickness (d) is as much as approximately one third to one half of the diameter of the coil (D). The precision of a convolution index (CI) estimate is thus more difficult to define than in the simple case of a very thin fiber wound around a relatively large hollow core, as shown in Figure 2. Therefore, CIs were estimated from one half the diameters of coils (D/2), where D is measured as the peak-to-peak amplitude of the coil and the pitch (P) is the coil length divided by the turn, according to the Pythagorean theorem. This can be visualized as the opening and flattening of a cylinder constructed around a regular coil to become a rectangle, which is divided into two right triangles by their shared hypotenuse. Therefore, in accordance with the definitions in Figure 2,

\[ FL = \sqrt{(3.14D)^2 + P^2} \]

and

\[ CI = \frac{FL}{P} \]

Results

A branched, continuous network of perimysial and epimysial fibers extends from the muscle-
tendon junction, through the length of papillary muscle, and into the ventricle wall, as seen most effectively by light microscopy of silver-impregnated sections (Figure 3). The most striking features of these perimysial fibers are their highly coiled configurations at slack length, their large size relative to myocytes, and the extent to which their array appears to form an orderly framework, described here for cylindrical papillary muscles (Figure 1). In single, low-magnification micrographs (Figure 3), the continuity of branched CPFs can be followed over distances equal to many myocyte lengths. At higher magnifications, the CPF network is seen to originate in the chorda tendinea, from which CPFs diverge and branch (Figure 4). The branching and continuity of the CPFs have been followed over distances of many cell lengths by means of optical sectioning within the 100-μm-thick sections.

The overall appearance of CPFs is intrinsic and not the result of fixation or staining artifact. Figure 5 is a differential interference contrast light micrograph of a wedge of wet, unfixed, unstained myocardium. CPFs can be studied with differential interference contrast microscopy or with polarization microscopy because of the birefringent nature of collagen fibrils. The images reveal the same highly convoluted configurations seen with light microscopy of silver-stained tissue as well as with ultrastructural techniques.

CPFs range in diameter from less than 1 μm to several microns and are distributed across the muscle in a ratio of one CPF: several myocytes (Figure 6). The cross-sectional profiles of the CPFs are varied and range from slightly elliptical to very elliptical; in some cases they are even ribbonlike. CPFs are oriented in a primarily axial manner. The ribbonlike CPFs are distinguished from other ribbonlike, collagenous fibers that are perpendicular to the long axis of the muscle and are not helical but are wavy and often follow the contours of underlying cells. The degree to which the apparent eccentricity of elliptical cross-sectional profiles is a function of the coiled nature of the CPF rather than the fiber's intrinsic shape depends on the thickness of the fibers and the pitch of the coil. Higher power transmission electron micrographs of cross sections reveal constituent fibrils on the order of 40–50 nm in diameter, often grouped in bundles of two or

Figure 5. Differential interference contrast light micrograph of wet, unfixed, unstained, birefringent coiled perimysial fiber (CPF) in situ in thin end of cut wedge of left ventricular papillary muscle. Convoluted path of fiber is apparent from alternate light and dark regions (arrow). Pitch of coiled perimysial fiber is same order of magnitude as that observed in fixed and stained samples. Bar, 40 μm. Original magnification, ×533.
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Figure 6. Low-magnification transmission electron micrograph of cross-section of central region of right ventricular papillary muscle. Coiled perimysial fibers (CPF) are several microns in diameter and are interspersed among and closely apposed to myocytes. M, myocyte; *, CPF; bar, 10 μm. Original magnification, ×1,900.

three subfibers (Figure 7). The orientation of fibrils varies across the fiber: some fibrils are in perfect cross-sectional alignment, whereas neighboring fibrils are oblique or even longitudinal.

Elastic fibers are associated with each CPF (Figure 7). They run parallel with the axis of the CPF and are composed of amorphous and microfibrillar components. Fibroblast processes are visible in most of the random and serial photographs of CPFs and are often rich in rough endoplasmic reticulum, Golgi apparatus, and secretory vesicles. The cell processes wrap around the CPF, as might be expected from recent studies on synthesis of collagen types I and III by myocardial fibroblasts.25 The extracellular space enclosed by the processes is less electron-dense than that outside the processes. This observation was made in muscles that had undergone primary fixation in solutions containing only glutaraldehyde as well as in solutions that contained tannic acid or the cationic dyes ruthenium red, safranin-O, or Alcian blue.3

The CPFs are highly convoluted, but the issue of whether the fibrils are true coils (helices) as opposed to wavy in only two dimensions (as in a sine wave) was approached in two ways. The first and more direct approach was to view scanning electron microscopic images of the CPFs in stereo. In fortuitous fractures of dehydrated or critical point-dried samples, some of the CPFs remain intact and isolated from surrounding tissue. Fibers, like that in Figure 8, have a pitch (convolution repeat distance) within the range observed in embedded samples and in unfixed, unstained wedge samples and are thus suitable for stereo analysis. The convoluted configuration of the CPF is seen in stereo imaging to be truly coiled (Figure 8B).

The second approach to the issue of helicity depends on the observation that most convolutions are minimal in stretched muscles; that is, most fibers appear straight or much less convoluted at 15% muscle stretch (Figure 9) than at slack lengths, where most fibers appear highly coiled. We can predict that a true coil, when stretched, will have crossover points. An analogy can be made with a coiled telephone cord that is highly stretched; it becomes almost straight but the crossover points between inside and outside surfaces of the coil leave thickened, regularly spaced bulges. Bulges, spaced either in regular or irregular patterns, are seen along the lengths of stretched CPFs (Figure 9).
FIGURE 7. Transmission electron micrograph of cross-section of central region of right ventricular papillary muscle. Coiled perimysial fiber has intrinsically elliptical cross-section, but eccentricity is exaggerated because plane of section cut normal to muscle is oblique to coiled fiber. Differential alignment of apposed constituent fibrils, in true cross-section (arrow) and in oblique section (double arrowheads), are due to both the plane of section relative to the coil of the fiber and the substructural twists of fibrils within the fiber. Fibrils are arranged in bundles within the fiber (arrowheads). Small elastic fibers, associated with the coiled perimysial fiber, have amorphous and microfibrillar components. Coiled perimysial fiber is enveloped by fibroblast process; the extracellular space between the fiber and fibroblast is less electron-dense than that between the fibroblast and myocyte. cf, collagen fibrils; E, elastic fiber; Fb, fibroblast; rER, rough endoplasmic reticulum; ×14,700 bar, 1 μm. Original magnification, ×14,700.

Furthermore, although considerable variation in regularity of coiling exists among fibers or regions of fibers, most of the CPFs are coiled with a pitch that corresponds to several sarcomere lengths. In a sample calculation, for example, a convolution index of about 1.4 (see “Materials and Methods” and Figure 2) at slack length would yield a convolution index of 1.2 as a consequence of a 15% stretch. The convolution indexes of many of the CPFs in muscles stretched by 15% are in the range of 1.1–1.2, although some regions actually seem to have a convolution index of 1.0 (Figure 9).

Some of the variations in pitch of a given CPF or between different CPFs within a single muscle in a given state are so large that the CPFs must be visualized as a population with distributions of diameters, pitches, regularities of pitch, and starting points of pitch at any given muscle length.

CPF convolutions were studied at two selected, extreme mechanical states of papillary muscles, slack length, and 15% stretch beyond slack length. The concept of continuous change in convolution of each CPF throughout the entire range of muscle lengths is not, however, only deduced from different CPFs in different muscles fixed at predetermined lengths. Wedges of living myocardium were stretched and compressed, in a qualitative manner. The CPFs stretched and compressed reversibly, like coiled springs.

The CPFs are laterally connected to myocytes (Figure 10) and to each other (Figure 4) by struts that are on the order of 0.1 μm in diameter. The struts have much smaller diameters than those of the CPFs. They also are quite numerous and can be seen draped along the sides of CPFs isolated for scanning electron microscopy.
FIGURE 8. Scanning electron micrograph of right ventricular papillary muscle fractured while frozen. Muscle was fixed and processed at slack length. Panel a: Muscle axis (double-ended arrow) and coiled perimysial fiber (CPF). Sample calculation for one selected turn of coil (see Figure 2 and “Materials and Methods”): coil diameter (D)/2, 2.2 μm; pitch (P), 7.3 μm; convolution index (CI), <1.4. Increase in P with stretch to a perfectly straight configuration: percent change in P=(1.4–1.0)/1.4×100=29%. Increase to more likely CI values of 1.1 or 1.2 give percent changes in P=(1.4–1.1)/1.4×100=21%, or 14%, respectively. Bar, 20 μm. Original magnification, ×1,200. Panel b: Stereo pair of same coiled perimysial fiber in different orientation. Note “corkscrew” appearance and surface fibrils; that is, fiber has helical configuration, as opposed to planar convolutions. Angular difference between micrographs, 3° (stereo viewer recommended). Bar, 25 μm. Original magnification, ×700.
The configurations of collagen fibrils in chordae tendineae differ in muscle–chordae tendineae preparations that have been fixed at slack length (Figure 11A) from muscle–chordae tendineae preparations that have been stretched before fixation (Figure 11B). Quantitation of the relative changes in segment length of the muscle itself versus that of the chorda tendinea as a function of total stretch of the muscle–chordae tendineae preparations remains to be studied, as do the accompanying relative changes in CPF convolutions and waviness of collagen fibrils in chordae. Cross sections reveal that the abundant elastic fibers in chordae tendineae contain a large proportion of microfibrils and that the collagen fibrils vary noticeably in diameter (Figure 11C).

**Discussion**

**Configurations and Orientation of Coiled Perimysial Fibers**

CPFbs comprise a branched network that is continuous throughout the length of the papillary muscle of rat heart (Figure 12). They are longitudinal or oblique to the long axis of the muscle. Although CPFbs do not surround groups of myocytes in transverse section, they are classified as perimysial because each fiber is associated with more than two myocytes in longitudinal directions and often in transverse directions.

Several types of imaging have revealed the configurations of the CPFbs. Stereo viewing in the scanning electron microscope was performed on fibers from muscles that were processed in the critical point dryer, frozen, and then fractured to reveal fibers free of surrounding tissue. The truly helical fibers whose surfaces are thus analyzed have the same projected two-dimensional conformation as fibers studied by transillumination in the light microscope, in both silver-stained sections and unfixed, unstained wedges of myocardium viewed with differential interference contrast optics. The convoluted, embedded fibers studied in the light microscope were not subject to forces of fracture, and the wet wedges of tissue were not subject to fixation, dehydration, or staining protocols but were imaged by virtue of the birefringent nature of the constituent collagen fibers. CPFbs studied in serial sections by transmission electron micros-
copy had tortuous paths relative to neighboring myocytes and different alignment of constituent fibrils across the fiber and along its length. These observations are consistent with the concept that CPFs are helically configured as opposed to convoluted in two dimensions.

Many of the bundles of collagen fibrils and rodlike collagen fibers referred to in other studies of papillary muscles are probably part of the axial, branched network of CPFs. The CPF network is distinct, however, from other components of the myocardial perimysium in papillary muscle in the weaves and septa of collagen fibers that surround or separate myocytes or groups of myocytes, the transverse collagenous ribbons that follow cell contours, the axial tendonlike fibers, and the collagenous strands that interconnect the weaves.

The network of CPFs is also different from the highly regular, three-dimensional crisscross array of perimysial fibers in bovine and sheep skeletal muscle. The less-regular array of CPFs in rat papillary muscle could reflect the differences in sizes, configurations, and orientations of myocytes in cardiac and skeletal muscle, as well as differences in degree of elaboration of perimysium as a function of muscle size (e.g., the perimysium is more ordered and elaborate in skeletal muscles of large mammals than in those of small mammals. The structural and configurational differences between perimysial arrays in skeletal and cardiac muscles presumably reflect a difference in their functions. The dependence of the pitch of CPFs on the degree of stretch of the muscle supports this idea. The convolution indexes of CPFs decrease as the papillary muscle is stretched (Figures 2 and 12); this is expected from the continuity of the branched network of CPFs between anchor points in the muscle-tendon junction and the ventricle wall. In contrast, the perimysial "neutral connecting fibers" of skeletal muscle are transverse to the direction of muscle shortening, remain slack as myocytes change length, and apparently serve only as scaffolds for attachments of myocytes.

Estimates of Tensile Strength of Coiled Perimysial Fibers

In the absence of any direct measurements of the mechanical properties of CPFs, tensile strengths can be estimated as the product of cross-sectional areas of the CPFs and the modulus of tensile strength of collagen fibers from similar tissue. For example, if the modulus of skeletal muscle fascia is...
used $^{39} (15 \mu N/(\mu m)^2)$, the ultimate tensile strengths of CPFs with range in cross-sectional area of 0.19–68 (\mu m)^2 would be 2.9–1,020 \mu N. These calculated values correspond to elliptical CPFs whose cross-sectional areas can be approximated as ellipses with minor and major axes of 0.2 x 0.3 \mu m and 3 x 7 \mu m. If the calculation is made for only the smaller axes (0.2 and 3 \mu m) the ultimate tensile strengths are calculated as 1.9–424 \mu N. The best estimates are probably intermediate between these two sets of values because at least some of the eccentricity of the elliptical cross-sectional profile arises from the transverse sectioning of a coil, which will not yield an intrinsically circular cross section. In addition, to relate stress to strain in an axially loaded helical spring, corrections must be made for small values of $D/d$ (see “Materials and Methods”) and for large pitch angles ($\alpha>10^\circ$). For the CPF in Figure 8, $D/d=2.7$ and $\alpha=47^\circ$; in this case, the corrections balance and yield a correction factor $\sim 1$ according to the formula of Rover. $^{30}$

These calculations are intended to serve only as preliminary estimates in the absence of direct measurements of isolated CPFs. The values may represent an overestimate of the tensile strengths actually invoked during the cardiac cycle because not all the fibers fully straighten, even in vitro at 15% stretch beyond slack length, and because the moduli of rat cardiac CPFs relative to skeletal muscle fascia depend on an incompletely understood set of variables that includes the amounts and ratio of type I and type III collagen, degree of cross linking, diameters of constituent fibrils, and glycosaminoglycan and glycoprotein contents. Differences in these parameters can result in altered mechanical function; for example, Viidik $^{31}$ has shown that human chordae tendineae are more extensible than skeletal muscle tendon and that chordae from the left ventricle can be stretched 21% compared with 14% in the lower-pressure right ventricle.

A preliminary comparison of estimates of tensile strengths can be made between individual CPFs and published values of measurements made from individual myocytes freshly isolated from rat heart. $^{32,33}$ This type of analysis is complex because stiffness depends on myocyte length, and myocytes display

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**Figure 11.** Transmission electron micrographs of chordae tendineae of papillary muscles. Panel a: Longitudinal section, slack length. Collagen fibrils (cf) are wavy. Bar, 3 \mu m. Original magnification, x6,700. Panel b: Longitudinal section, muscle–chorda tendinea preparation stretched 15% beyond slack length. Collagen fibrils are relatively straight. Bar, 3 \mu m. Original magnification, x6,200. Panel c: Transverse section. Collagen fibrils and microfibril-rich elastic fibers are interspersed. Fibroblasts are more abundant in periphery than in central regions of chorda tendinea. Bar, 3 \mu m. Original magnification, x6,800.
FIGURE 12. Diagrams that summarize multiple observations of configurations of the branched network of coiled perimysial fibers (CPF) and epimysial fibers at slack length (top panel) and 15% stretch beyond slack length (bottom panel) in cylindrical papillary muscle. The branched CPF network is continuous from muscle-tendon junction to ventricle wall. CPFs diverge from the muscle-chorda tendinea junction and are highly convoluted at slack length and less convoluted or even straight at 15% stretch. Obliquely oriented CPFs are more aligned with the muscle axis at 15% stretch. The biaxially arranged epimysial collagen fibers are also less convoluted and more highly aligned at 15% stretch (“cargo net model”). Collagen fibrils of chordae tendineae are crimped at slack length and straight when stretched. These various changes in configuration are correlated with a striking increase in resistance to passive stretch in papillary muscles at 15% stretch (near “L_upper”) and with a sarcomere length at the upper limit of maximal cross-bridge overlap.33

The questions then become what fraction of the population of CPFs exert their full tensile strengths when muscles are at 15% stretch and what increases in myocyte stiffness occur at corresponding sarcomere lengths? The effective values of CPF tension are the magnitudes of the axial vectors of tension for branches of a CPF network that are not colinear with the muscle axis. Although a quantitative estimate of vector components would be premature and is not attempted, the oblique angles of some of the CPFs would suggest that the tensile strengths actually manifest are somewhat less than the calculated ultimate tensile strengths. If this were true, then the actual tensile strengths of the CPF and myocyte systems could be fairly closely matched. Caulfield and Borg3 point out that the presence of more tendonlike collagen fibers in papillary muscle than in other muscle of the ventricle could account for its higher stiffness constant.

Possibility of Energy Storage and Energy Dissipation in Elastic and Collagenous Components of Coiled Perimysial Fibers

The presence of satellite elastic fibers parallel to the CPF axes warrants further investigation. Elastic fibers adjoin essentially all of the CPFs, although their cross-sectional area is far smaller than that of the bundles of collagen fibrils. The elastic fibers could serve as elastic forces of resistance to stretch. A possibility (not a conclusion) is that stored energy from both sources could then help promote subsequent shortening or could preload the muscle for the subsequent contraction. This type of model has been invoked to explain biphasic stress-strain curves in elastic fiber–collagen systems in bovine neck ligament.34 Additional or alternative functions have been ascribed to tendons subject to rapid loading. Energy dissipation might act as shock absorption to prevent structural damage to the muscle during sudden stretch. The elastin might act against the creep phenomenon to restore collagen configuration.35 Another possibility is that the elastic fibers might serve as part of a scaffold in the domain of remodeling close to the fibroblast.25 A model for extracellular compartments of fibroblasts of cornea and tendon has been proposed, although a role for elastin was not proposed in those studies.36,37

A role of energy storage by the collagen of CPFs should not be ruled out without direct experimental tests. Tendons in jumping animals such as Alsatian dogs and kangaroos have been shown to store large amounts of elastic energy (on the order of 5 J/g)38,39 over defined ranges of stretch. CPFs look like springs, but whether the coiled configurations of the CPFs contribute significant elastic storage of energy with gradual onset over the physiological range of lengths remains to be determined. The possibility of springlike behavior of the CPFs is consistent with but not proven by the observation that CPFs are coiled, even when isolated from the muscle, and change convolution continuously with stretch and compression (authors’ published observations). This CPF configuration in the absence of external forces implies a prestressed system with reversibly distensible constraints built into the coil itself. An energy storage system comprised of CPFs acting in concert with the myocytes themselves could provide the large stress relaxation.33 However, if we consider a cell stiffness (measured force of an isolated cell relative to length-normalized length perturbation) of 10 μN,33 it seems reasonable to suggest that the stiffness of the CPF array, with its higher modulus but smaller total cross-sectional area, is significant relative to the total stiffness of the muscle at long lengths.
source of the elastic recoil and suction exhibited during the cardiac cycle. 40–42

*Functional Implications of Configurational Changes in Coiled Perimysial Fibers and Epimysial Fibers*

To describe changes in muscle length, CPF configuration, and sarcomere lengths on a quantitative basis, a starting point needs to be selected. Slack length has been used as this arbitrary point where resting tension approaches zero and the muscle appears straight (Figure 12). However, the accuracy of estimates of the percent changes in convolution indexes and sarcomere lengths does not permit an assessment of whether the CPFs or the myocytes pull up the load first during stretch or if the two systems act in concert. Uncertainty attributable to normal variations in size and pitch of CPFs, extent of differential preset stretch, possible differences in myocyte stimulation and shrinkage during fixation and preparation, and uncertainty in measurements of coil pitch limit the assessment of a differential onset of stretch in CPFs and myocytes in intact muscle.

CPF and myocytes are interconnected (Figure 10). The cardiac endomysial connective tissue is mechanically linked to myocytes and their contractile lattices and cytoskeletons, 15 which have significant tensile properties, 32 but the slack and compliance in their interconnections is not yet known. A current approach to further investigate this question involves our use of living muscles monitored for CPFs, sarcomere lengths, and muscle force simultaneously as a function of muscle length.

CPF is abundant in the ventricular walls, and their changes in configuration with ventricular distension in isolated, arrested rat hearts (authors’ unpublished observations) suggest a possible role in passive compliance that currently is under investigation.

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**References**


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