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Abstract

We analyzed the structural features of the perimysium collagen network in bovine *Flexor carpi radialis* muscle using various sample preparation methods and microscopy techniques. We first observed by scanning electron microscopy that perimysium formed a regular network of collagen fibers with three hierarchical levels including (i) a loose lattice of large interwoven fibers ramified in (ii) numerous collagen plexi attaching together adjacent myofibers at the level of (iii) specific structures that we call perimysial junctional plates. Second, we looked more closely at the intracellular organization underneath each plate using transmission electron microscopy, immunohistochemistry, and a three-dimensional reconstruction from serial sections. We observed the accumulation of myonuclei arranged in clusters surrounded by a high density of subsarcolemmal mitochondria and the proximity of capillary branches. Third, we analyzed the distribution of these perimysial junctional plates, subsarcolemmal mitochondria, and myonuclei clusters along the myofibers using a statistical analysis of the distances between these structures. This revealed a global colocalization and the existence of adhesion domains between endomysium and perimysium. Taken together, our observations give a better description of the perimysium organization in skeletal muscle, and provide evidence that perimysial junctional plates with associated intracellular subdomains may participate in the lateral transmission of contractile forces as well as mechanosensing.

Keywords: Perimysium; Myonuclear domains; Mitochondria; Muscle contraction; Skeletal muscle

1. Introduction

The extracellular matrix (ECM) plays a fundamental role, both in structural and functional aspects of skeletal muscle. Its two components are the endomysium and the perimysium. The endomysium is made of a continuous sheath covering the full length of myofibers until the myotendinous junction (Borg and Caulfield, 1980; Swasdison and Mayne, 1989; Trotter and Purslow, 1992). It is organized as a regular network of thin fibrillar collagen fibers composed mostly of type IV, as well as types III, VI (Listrat et al., 1999), and XII (Listrat et al., 2000) to a minor extent. This network attaches myofibers sarcolemma to specific transmembrane proteins (Kovanen, 2002), thereby forming a regular mosaic pattern (Borg and Caulfield, 1980). These proteins, such as integrins or dystrophin–glycoprotein complexes (Rando, 2001), interact directly with the cytoskeleton (Berthier and Blaineau, 1997) and transmit the contractile forces between adjacent myofibers (Monti et al., 1999; Sheard et al., 2002) as far as the tendon (Purslow, 2002). Taking into account this particular anatomy and
associated roles, the endomysium can be considered the main ECM component involved in muscle flexibility.

In contrast, the second component of ECM, i.e., the perimysium, is formed by an areolar network of crimped collagen fibers varying in diameter and composition, including essentially type I collagen in conjunction with types III, VI (Listrat et al., 1999), and XII (Listrat et al., 2000). However, few studies have analyzed the perimysium’s fine organization so perimysium continues to be considered a simple packing tissue, even though some early observations have suggested a connection with endomysium (Borg and Caulfield, 1980; Moore, 1983) and a possible role in the lateral transmission of contractile forces (Huijting et al., 1998). In particular, there is a lack of detail concerning contact sites between perimysium and endomysium, their organization, distribution along myofibers, and underlying intracellular environment. We analyzed these features of the perimysium collagen network with a particular emphasis on endomysium contact sites, as well as the associated intracellular subdomains in bovine Flexor carpi radialis muscle using scanning electron microscopy (SEM), transmission electron microscopy (TEM), three-dimensional reconstruction from serial sections, and immunohistochemistry techniques.

Our results show that the perimysium forms a network of collagen fibers with three hierarchical levels including (i) a regular lattice of interwoven fibers with (ii) collagen plexi at each angle that attach adjacent myofibers at (iii) particular domains that we call perimysial junctional plates (PJPs). A three-dimensional reconstruction from serial sections showed that, underneath each plate, there was an accumulation of myonuclei arranged in clusters near capillary branches. Surrounding these clusters, we observed a high density of subsarcolemmal mitochondria by immunohistochemistry and TEM. Comparison of the data obtained by these various techniques enabled us to propose a model of perimysium organization that may underline its role in lateral transmission of contractile forces and thus in various aspects of muscle flexibility.

2. Materials and methods

All procedures were performed in accordance with institutional guidelines for animal care. The Flexor carpi radialis muscles of the foreleg were taken from ten different cows, just after slaughter, and immediately fixed for electron microscopy analyses or frozen for immunohistochemistry, as detailed below.

2.1. Scanning electron microscopy

The Flexor carpi radialis was immersion-fixed in 10% paraformaldehyde for 3 days before being sectioned in samples of 20 cm³. To study the perimysium collagen network’s fine structure, we conducted an scanning electron microscopy (SEM) analysis on muscle samples submitted to either (i) a digestion technique (NaOH) that enabled to free and detach partially the endomysium so we could more easily visualize the perimysium between adjacent myofibers, or (ii) a fracture technique that does not alter the chemical structure of connective tissue, and makes it possible to remove the endomysium and visualize perimysium connections at the surface of myofibers. The digestion technique of NaOH cell maceration was adapted from Ohtani et al. (1991). For this, muscle samples were immersed in NaOH 6N and maintained at a temperature of 18 °C for 5 days, before being rinsed for 3 days in water at 18 °C. Afterward, the samples were fixed in 2% tannin, freeze-dried, and fractured. The sections were coated with gold and examined on a Philips 515 scanning electron microscope. The fracture technique consisted in the fixation of muscle samples in 2% osmic acid, followed by freeze-drying and fracture. For each analysis, a series of at least five different samples were taken from three different muscles.

2.2. Transmission electron microscopy

The Flexor carpi radialis was stretched by a 300-g weight to preserve muscle fiber straightness and immediately fixed by the injection of solution A (0.5% glutaraldehyde, 2% paraformaldehyde, 7% saccharose, and 4% polyvinylpyrrolidone in 0.1 M cacodylate buffer). This made it possible to increase intramuscular pressure and separate muscle fibers for better visualization of the connective tissue. The muscle was sectioned in 40 μm thick blocks, immersed for 1 h in fixative solution A, and rinsed with water. These blocks were recut into smaller samples of about 12 mm² and immersed in a second fixative solution B (2% osmic acid in 0.1 M cacodylate buffer) for 1 h. After dehydration, these blocks were embedded in epoxy (epon) resin and cut into longitudinal or transversal sections 0.1 μm thick. These sections were stained with a solution of uranyl acetate and lead citrate. They were observed on a Philips CM10 microscope to analyze the contact sites between perimysium and myofibers as well as the intracellular subdomains.

2.3. Three-dimensional reconstruction

Samples of embedded material (see above) were cut by ultramicrotome parallel to myofiber direction to obtain a series of 55 successive sections (2 μm thick). Sections were stained with toluidine blue and photographed on an light microscope (Zeiss). These photographs were then analyzed with Photoshop 7.0 (Adobe) to isolate the surroundings of six adjacent myofibers, and to determine the respective positions of myonuclei and capillaries. Images of the myonucleus and capillary positions along the myofibers from each section were submitted to a three-dimensional reconstruction (stereo pairs) using Image J software (http://rsb.info.nih.gov).

2.4. Immunohistochemistry

Samples of Flexor carpi radialis muscle were rapidly frozen in liquid nitrogen-cooled isopentane and stored at.
−80 °C. Longitudinal and transversal sections (10 μm thick) were obtained using a cryostat (Frigocut 2800, Reichert Jung) at −22 °C. The sections were fixed with a solution of 4% paraformaldehyde (Sigma–Aldrich) for 30 min at 25 °C. After two washes with PBS (5 min each), the sections were dehydrated by successive baths of 70, 90, and 100 ethanol (2 min each), followed by rehydration with successive baths of 90 and 70% ethanol, followed by two washes in PBS (2 min each). Sections were then incubated in a blocking solution [10% foetal calf serum (Hyclidean) diluted in PBS] for 2 h at 25 °C. Next, they were incubated overnight with two primary antibodies (rabbit polyclonal anti-collagen III, Rockland and mouse monoclonal antitoxine cytochrome c oxidase subunit IV, Mitosciences). After two washes in PBS (10 min each), the sections were incubated in 10% normal goat serum (Sigma) in PBS for 1 h at 25 °C. Subsequently, sections were incubated with the secondary antibodies (Alexa Fluor 546 goat anti-rabbit IgG and Alexa Fluor 488 goat anti-mouse IgG, Molecular Probes) for 90 min at 25 °C before being washed three times with PBS (15 min each). Finally, sections were incubated with 300 nM 4',6-diamidino-2-phenylindole, dihydrochloride (DAPI, Molecular Probes) for 10 min at 25 °C, washed three times in PBS (10 min each), and mounted with Mowiol 4–88 (Calbiochem). The sections were observed on an epifluorescence microscope (Nikon Eclipse E600), photographed by a digital camera (COHU High Performance CCD Camera), and acquired using Visiolab software (Biocom).

2.5. Morphometric and colocalization analysis

To determine the distribution of PJPs, images obtained by SEM and immunohistochemistry (fluorescence microscopy) were analyzed using Image J software. The contours of each PJP were traced on electron micrographs, and the distances between their respective centers were measured and expressed in μm. On images acquired by fluorescence microscopy, the PJPs were visualized using an antibody raised against type III collagen (red alexa fluorescent staining). This type of collagen is contained in both the endomysium and perimysium so that their focal colocalization appears in the form of deep red domains. Successive dense regions of collagen were considered to belong to the same PJP when there was a series of dots (≥2) interspaced at a distance of ≤50 μm. These criteria were obtained from observing PJPs on an electron micrograph. For this analysis, we did not consider the regions corresponding to capillary walls that were identified by comparing with phase contrast images. The distances between the centers of successive PJPs were once again measured and expressed in μm. The myonuclei were stained using 4',6-diamidino-2-phenylindole (DAPI) to visualize DNA as deep blue dots. Successive nuclei were considered to belong to the same cluster when they were interspaced by a distance of ≤50 μm as determined on the three-dimensional reconstruction from serial sections. The presence of capillaries was identified on each section using phase contrast microscopy, and the corresponding nuclei were excluded from the analysis. The distances between the centers of myonuclei clusters were measured and expressed in μm. For three-dimensional reconstruction, distances between the centers of myonuclei clusters were measured on a series of 5 sections (2 μm thick) to compare with the results of immunohistochemistry on 10 μm sections. Mitochondria were stained green using an antibody directed against one subunit of the cytochrome c oxidase (COX) complex of the respiratory chain. The distances between the centers of subsarcolemmal mitochondria sections were measured and expressed in μm. This entire analysis was performed on 10 images of SEM, 20 immunohistochemistry photographs, and 55 optic microscopy photographs (three-dimensional reconstruction) using Image J software. We also measured the distances between the centers of the various structures by pairs for an interobject distribution analysis.

2.6. Statistics

An initial statistical analysis was performed to individually analyse the intra-distribution of various structures (PJPs, myonuclei clusters, and subsarcolemmal mitochondria). Another statistical analysis was also performed to ascertain their inter-distribution and potential colocalization. We represented histograms of the distribution of distances between the centers of these structures as performed in Kassab et al. (1997) to study the intra-distribution of one type of structure along individual fibers. We used two different methods to study the inter-distribution and potential colocalization between different types of structures. First, we compared histograms obtained from the intra-distribution analysis using the Kolmogorov–Smirnov test (Mehrotra et al., 2005) (KS test) and Statview software 5.0 (SAS Institute). This test determines whether or not two datasets differ significantly, and has the advantage of making no assumptions about data distribution (non-parametric and distribution-free). The threshold of significance for colocalization between the various structures was defined at p ≥ 0.05. Second, we performed a statistical analysis of the distances between the centers of the different structures by pairs. We did this by calculating the frequency of the peak value as determined on the distribution histograms.

3. Results

3.1. Perimysium collagen network and junctional plates

Muscle digestion with NaOH 6 N at 18 °C resulted in the loosening and partial removal of the endomysium from embedded adjacent myofibers (Figs. 1A–D). This enabled us to directly observe the perimysium network and its connections with the myofiber surface. On lateral (Fig. 1A) and front (Fig. 1B) views, the perimysium network appeared to consist of a combination of several collagen elements including numerous large interwoven fibers at an angle of about 60° with regard to myofiber
length. These fibers formed a plexus of thin branches (noted p on Figs. 1C and D) at each angle bound to the surface of adjacent myofibers. This resulted in a patchwork of plexi at the surface of myofibers where large portions of membrane are devoid of any contact with the perimysium for distances of up to 300 μm (Fig. 1C). To study the adhesion between perimysium and myofiber endomysium in more detail, we performed a muscle fracture procedure (Figs. 1E and F). We again observed the branching lattice of the perimysium collagen network, and more particularly the plexus that bound myofiber endomysium for approximately 150–200 μm. This formed...
an adhesive structure (Fig. 1E) that we called perimysial junctional plate (PJP) consisting of about 10 principal branches interspaced by a distance of \( \leq 50 \mu m \). Higher magnification revealed the attachment of branches at the myofiber surface, forming adhesive regions both on the top and between costameric structures (Fig. 1F). Moreover, we observed that, despite the fracture procedure, fibrils of the endomysium (e) remained present only in those regions where perimysium plexi were attached to myofibers. At this particular level, the perimysium (which could not be distinguished from the endomysium), formed an important area for adhering to the plasma membrane that may constitute focal points for attachment between connective tissue and muscle.

3.2. Existence of intracellular subdomains associated with the perimysial junctional plates

To study PJP's and associated intracellular subcomposition, we performed a TEM analysis on muscle samples injected with a fixative solution. On longitudinal sections (Fig. 2A), we observed the association of myonuclei (noted mn) and subsarcolemmal mitochondria (noted sm), in proximity to bundles of collagen (noted p) and capillary branches (noted Ca). Then, we looked more closely at the junction between connective tissue and these intracellular domains using a higher magnification (Figs. 2B–D). The cross-striation of collagen fibers indicated a type I origin that is the major component of the perimysium, but absent

![Fig. 2. Intracellular subdomains associated with PJP's.](image-url)
from the endomysium (Listrat et al., 1999). Thus, the regions of connective tissue observed between adjacent myofibers (Fig. 2B) must belong to the perimysium in contrast with the endomysium (noted e) that solely envelopes myofibers. Moreover, the presence of type I collagen fibers infiltrated between adjacent myofibers indicated that the perimysium network not only covered and connected longitudinal muscle surface (as seen in Fig. 1), but also strongly attached individual myofibers within the interstitial space. In each instance, attachment occurred at the level of PJPs that formed strong adhesive structures on myofibers as seen on Figs. 1F, 2B–D. At these junctions, the sarcolemma showed darker regions that corresponded to membrane densifications (noted d on Figs. 2B and C) with an accumulation of caveolae (noted cv) in unusual places that did not correspond to the costameric structures of cytoskeleton attachment on sarcolemma. We frequently observed myonuclei and subsarcolemmal mitochondria underneath these membrane densifications.

To better identify and characterize the organization of intracellular subdomains with relation to extracellular environment, we realized a three-dimensional reconstruction of capillary bed position and the position of myonuclei in a muscle volume containing six adjacent myofibers (Fig. 3A). Capillary bed reconstruction (Fig. 3B) from the adjoining faces of six adjacent myofibers (noted A–B–C–D–E–F) revealed a longitudinal and linear network with the typical branched organization described by Schraufnagel et al. (1983) and Reina-De La Torre et al. (1998). The three-dimensional view of the capillary bed showed short transversal ramifications between adjacent myofibers that often formed branches every 150 μm as well as often colocalizing with myonuclei. This indicates a possible topological association between PJPs, myonuclei, and capillary branches. The position of myonuclei on the adjoining faces of four adjacent myofibers (A–B–C–D) was examined (Fig. 3C) to identify the possible association between PJPs and myonuclei. We observed that myonuclei belonging to different individual fibers formed clusters of approximately 15 U where myonuclei were interspaced by a distance of ≤ 50 μm. Each cluster was approximately 150–200 μm in length, and separated from subsequent clusters by a distance of approximately 150 μm. This topological position of myonuclei is very much in accordance with the SEM micrograph (Fig. 1D), with perimysium plexi binding adjacent myofibers.

We repeated this structural analysis of PJPs, myonuclei, and subsarcolemmal mitochondria by fluorescence microscopy on both longitudinal and transversal muscle sections. The PJPs corresponded to the deep red fluorescent domains (please see criteria in the methods section) and appeared to be variable in length and number according to the myofiber (Figs. 4A, B, and F). The myonuclei were visualized as blue dots (Figs. 4A–D and F) and showed the cluster organization previously determined by phase contrast microscopy (see Fig. 3C). The mitochondria corresponded to green fluorescence (Figs. 4A–D and F). We observed differences in COX expression between intermyofibrillar (low) and subsarcolemmal (high) mitochondria. We frequently observed myonuclei clusters surrounded by subsarcolemmal mitochondria (Figs. 4C and D) under regions of deep red fluorescence identified as PJPs. The presence of capillaries in interstitial space was also observed both in transversal and longitudinal sections (noted Ca on Figs. 4A and B), and confirmed by phase contrast microscopy (Figs. 4E and F). However, the existence of capillary branches was only revealed by the 3D reconstruction described in Fig. 3.

3.3. Colocalization of perimysial junctional plates and associated intracellular subdomains

We conducted a morphometric analysis on images obtained by SEM and immunohistochemistry to study the intra-distribution of PJPs and associated intracellular components along myofibers. The respective histograms of the mean distances between the centers of successive PJPs are given in Figs. 5A and B. These both show a wide distribution of PJPs with the same most frequent value of 50–60 μm. Likewise, the distribution of distances between
the centers of successive myonuclei clusters is indicated in Fig. 5C. This also shows a wide distribution, with a most frequent value of 50–60 μm. A similar result was also observed for subsarcolemmal mitochondria (Fig. 5D). Hence, we observed a strong similarity between the histograms obtained for PJPs, myonuclei clusters, and subsarcolemmal mitochondria. Moreover, the comparative statistical analysis of these histograms by pairs using the Kolmogorov–Smirnov test further indicated a significant correspondence, with a p value of 0.88 between PJPs and myonuclei clusters, 0.99 between PJPs and subsarcolemmal mitochondria, and 0.61 between myonuclei clusters and subsarcolemmal mitochondria. All these values are superior to 0.05, which implies a similar organization. To ascertain potential colocalization, we studied the inter-distribution between PJPs, myonuclei clusters, and subsarcolemmal mitochondria. To do so, we once again conducted a morphometric analysis of the distances between the centers of the different structures by pairs. The corresponding histograms are shown in Figs. 5E–G. They reveal a major colocalization of these three structures, as peak values were obtained at a distance of <5 μm, with a frequency ranging from 0.63 to 0.8. This indicates that the distribution of PJPs in muscle closely corresponds to the distribution of myonuclei clusters and subsarcolemmal accumulations of mitochondria.

4. Discussion

We analysed the fine organization of the perimysium network in bovine Flexor carpi radialis muscle using various sample preparation methods and microscopy techniques. We looked more into detail than previous studies at the contact sites between the perimysium and the endomysium, their distribution along myofibers, and the underlying intracellular environment using SEM, TEM, three-dimensional reconstruction from serial sections, and immunohistochemistry techniques.

Our observations of the perimysium collagen network showed three hierarchical levels of organization that include a loose lattice of interwoven fibers with collagen plexi at each angle attaching adjacent myofibers at the level of specific domains that we call perimysial junctional plates (PJPs). Prior studies on the organization of the perimysium

Fig. 4. Distribution of PJPs and associated intracellular components. Images of muscle sections from fluorescence microscopy with specific labeling of type III collagen (red), nuclei (blue), and mitochondria (green). (A) Observations at low magnification on longitudinal sections show the existence of deep red domains corresponding to the PJPs associated with clusters of myonuclei. Images of (B) transversal or (C and D) longitudinal sections at higher magnification show the close association between PJPs, myonuclei, and subsarcolemmal mitochondria. Note the difference between subsarcolemmal mitochondria (deep green) and intermyofibrillar mitochondria (paler green). (E) Phase contrast observation of a transversal section shows that the capillary (Ca) is also associated (F) with deep red domains, myonuclei clusters, and subsarcolemmal mitochondria.
in skeletal muscle essentially described this tissue as a loose lattice surrounding myofiber bundles (Borg and Caulfield, 1980; Moore, 1983; Rowe, 1981). All these works were performed on various rat, bovine, and ovine skeletal muscles including the diaphragm, adductor major, gastrocnemius, pectoralis, biceps, psoas major, soleus, semitendinosus, semimembranosus, and longissimus dorsi. They all gave the same description of the perimysium that seemed to be similar between species, which may underly a possible functionality of this tissue. We obtained more details on perimysial structural features in bovine skeletal muscle, and specified its organization while observing a predominant pattern of interwoven fibers at the surface of myofibers. At high magnification, we observed that each perimysium collagen fiber formed a plexus in close proximity to muscle surface and attached adjacent myofibers at a level we call “perimysial junctional plates” (PJP). These can be defined as contact regions between the endomysium and the perimysium. Micrographs taken by SEM clearly demonstrated the nature of the strong attachment between the two components of the extracellular matrix, i.e., the endomysium and perimysium which could indicate a focal region for delivery tension during muscle contraction. Moreover, we observed a sarcolemmal membrane densification in these regions in addition to those observed at the costameric junction of the endomysium (Young et al., 2000), and which also resembled those of the myotendinous junction (Swasdison and Mayne, 1989). These two systems, i.e., tendon and endomysium, were previously described as playing a fundamental role in the transmission of contractile forces (Trotter and Purslow, 1992; Tidball, 1991), which further suggests that PJP could take part in this process. We also observed the presence of numerous caveolae under each PJP, possibly indicating a role for these domains in muscle plasticity.

Fig. 5. Colocalization of PJP, myonuclei clusters, and subsarcolemmal mitochondria. Histograms of the intra-distribution of (A, by SEM and B, by fluorescence microscopy) PJP, (C) myonuclei clusters, and (D) subsarcolemmal mitochondria. Histograms of the inter-distribution between (E) PJP and subsarcolemmal mitochondria, (F) PJP and myonuclei, and (G) PJP and subsarcolemmal mitochondria. Each bar corresponds to the mean distance between the centers of one type of structure (intra-distribution) or different types of structures by pairs (inter-distribution). This is expressed in μm and was determined from measurements of 20 various longitudinal muscle sections observed in immunohistochemistry.
and physiological adaptation (Minetti et al., 2002; van Deurs et al., 2003; Woodman et al., 2004).

We thus looked more closely at PJPs to show their potential role in the functioning of muscle physiology. In particular, we investigated the composition, position, and arrangement of the intracellular environment underneath each plate using TEM and immunohistochemistry techniques. Interestingly, we observed that there was an accumulation of myonuclei arranged in clusters, surrounded by a higher density of subsarcolemmal mitochondria and nearby capillary branches, below each PJP. This cluster arrangement of myonuclei is in agreement with previous studies (Roy et al., 1999; Smith et al., 2000), but is also in slight contrast with the concept of “myonuclear domains,” which considers individual myonuclei at the center of cytoplasmic domains (Bruusgaard et al., 2003; Hall and Ralston, 1989). Our statistical analysis of myonuclei cluster distribution showed that these domains exist, but instead of one they contain 10–15 nuclei. One might also expect the Golgi apparatus and sarcoplasmic reticulum to be present in these regions seeing as an association with myonuclear domains was previously shown in rat gastrocnemius (Ralston et al., 1999) or soleus muscle (Nori et al., 2003). In addition, the association of mitochondria and capillary branching was also reported in other studies (Mathieu-Costello et al., 2002; Suarez et al., 1991). Therefore, our structural study showed that the perimysium network was regularly associated with the distribution of myonuclei and subsarcolemmal mitochondria, but also with the capillary bed that was previously described as being included in a part of the perimysium network (Bosman and Stamenkovic, 2003). We conducted a statistical analysis on the distribution of PJPs, myonuclei clusters, and subsarcolemmal mitochondria along fibers to quantify our findings. To do so, we measured the distances between the centers of each structure and looked at their distribution in different individual myofibers, as well as potential colocalization. Histograms of these distances showed a disperse value ranging between 60 and 300 μm, with the most frequent distance of 50–60 μm for PJPs, myonuclei clusters, and subsarcolemmal mitochondria. In addition, the distribution of distances between the centers of each PJP was similar when performed on either electron micrographs or on immunohistochemistry images as verified using the Kolmogorov–Smirnov test (p > 0.05). This also validated the use of type III collagen dense regions as a means to identify PJPs in muscle sections. The histograms for these three different structures were similar, as verified using the KS test (with p > 0.05). This suggested the idea of a succession of domains where PJP, myonuclei clusters, and subsarcolemmal mitochondria are located in close proximity to one another. This view was supported by an inter-distribution analysis that also revealed a frequent colocalization: ≈70% of cases with a distance of <5 µm between PJPs, subsarcolemmal mitochondria, and myonuclei clusters by pairs. These domains are 200 µm long and regularly repeated along fibers. This particular repeated motif along muscle fibers recalls the notion of myonuclear domains (Hall and Ralston, 1989), and could suggest that perimysium organization is associated with myofiber activity. This hypothesis is supported by the existence of a functional collaboration between these myonuclei, subsarcolemmal mitochondria, and perimysium. In addition, a functional connection between collagen VI, a component of perimysium (Listrat et al., 1999), and each of these structures was recently demonstrated for calcium transport and muscle contraction (Irwin et al., 2003). Moreover, our study suggests that the association between mitochondria and O2 supply (Mathieu-Costello et al., 2002; Suarez et al., 1991) may be completed by the presence of adhesive plates (PJPs) and myonuclei clusters, possibly to form a functional domain involved in muscle contraction and mechanosensing.

Thus, our study reports the existence of particular 200 µm long domains where junctional structures and associated intracellular components are colocalized with each other that may participate in muscle integrity through the adhesion of adjacent myofibers. Such a colocalization also supports previous observations (Huijing et al., 1998) that demonstrate the perimysium’s role in the lateral transmission of contractile force. In this study, the contractile forces of rat Extensor digitorum longus were maintained even when the distal tendon was cut, thereby proving a “non-myotendinous force transmission.” Our structural observations suggest that such forces could originate from contact sites between the perimysium and myofibers attached at the level of PJPs, and be

![Fig. 6. Model of perimysium organization in skeletal bovine muscle.](image-url)
transmitted within muscles through the well-ordered lattice of perimysium collagen also observed in our study. Furthermore, we noticed that each PJp connected with the edges of costameric structures lying at the surface of myofibers. These transmembranous proteins are likely to correspond to the integrins involved in signal transduction (Anastasi et al., 2003; Samson et al., 2004; van der Flier and Sonnenberg, 2001). Interestingly, the presence of integrins at myotendinous junction with a possible implication in mechano-transduction has been previously observed (Tidball, 1991). We believe that the PJPs observed in our study may also participate in a similar mechanosensing system in skeletal muscle.

Taken together, our observations give a better description of the organization of the perimysium in skeletal muscle. On the basis of numerous data provided by SEM and TEM micrographs, as well as fluorescence microscopy and subsequent statistical analysis, we proposed a model represented in Fig. 6. It highlights specific junctional structures (PJPs) with associated intracellular subdomains.

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