INTRODUCTION

The degeneration of skeletal muscle fibers is caused by defects of the intermediate filament (IF) cytoskeleton. Mice lacking desmin, an IF protein predominantly found in all muscle tissues (Bennett et al., 1979; Lazarides, 1980), have shown severe disruption and disintegration of myofibrils (Milner et al., 1996; Li et al., 1996), whereas mice lacking plectin, an IF-associated protein (Foisner and Wiche, 1991), have exhibited focal disruption of myofibrils and the sarcolemma (Andrä et al., 1997). In humans, the defective expression of plectin has been found to cause an autosomal recessive disease, epidermolysis bullosa simplex with muscular dystrophy (EBS-MD) (Gache et al., 1996; Smith et al., 1996; Mclean et al., 1996). These phenomena suggest that the IF cytoskeleton has an important role in maintaining the structural integrity of skeletal muscle fibers. However, how the IF cytoskeleton maintains the structural integrity remains elusive, because nothing is known of the physical association of desmin IF with other cytoskeletal components, e.g. myofibrils and sarcosomes.

Plectin is widely distributed in a variety of tissues and cells where it codistributes with various types of IF proteins, such as vimentin, cytokeratins, and glial fibrillary acidic protein (Wiche et al., 1983; Hieda et al., 1992; Errante et al., 1994; Yaoita et al., 1996; Svitkina et al., 1996; Eger et al., 1997). Besides its IF-binding property (Foisner et al., 1988, 1991b), plectin possesses binding affinities to multiple cytoskeletal proteins. Plectin contains a highly conserved actin-binding domain at its amino terminus (Mclean et al., 1996; Elliot et al., 1997), and in fact, its association with actin-containing focal contacts and actin filaments has been demonstrated by immunofluorescence and immunoelectron microscopic studies (Seifert et al., 1992; Foisner et al., 1995). Furthermore, α-spectrin (fodrin), high molecular mass microtubule-associated proteins, integrin β4 and myosin II have been biochemically and ultrastructurally identified as plectin-binding partners (Hermann and Wiche, 1987; Svitkina et al., 1996; Rezniczek et al., 1998).

In skeletal muscle fibers, plectin is localized at the level of Z-discs and beneath the sarcolemma (Wiche et al., 1983; Gache et al., 1996; Andrä et al., 1997; Schröder et al., 1997). This localization is reasonable when one considers the IF-binding property of plectin and the previous observations that desmin IFs surround Z-discs and run beneath the sarcolemma (Lazarides 1980; Pierobon-Bormioli, 1981; Tokuyasu et al., 1983; Ishikawa, 1983, 1988; Wang and Ramirez-Mitchell, 1983; Fujimaki et al., 1986). However, it has not yet been determined whether or not plectin has physical interactions with Z-discs and sarcolemma as well as desmin IFs.

Here, we have documented the topological and structural relationships of plectin to IF and Z-discs in rat skeletal muscle fibers. The interaction of plectin with the sarcolemma will be reported elsewhere. The present study has clearly demonstrated...
that plectin fine threads link desmin IFs to Z-discs just to form lateral linkages among individual myofibrils. Based on the results obtained, we have discussed the functional significance of plectin in the force generation of muscle fibers as well as the possible mechanism of degeneration of muscle fibers lacking plectin.

MATERIALS AND METHODS

Antibodies

The primary antibodies (Abs) used in this study were monoclonal plectin Ab, clone 7A8 (Sigma Chemical Company, St Louis, MO, USA), polyclonal α-actinin Ab raised against chicken pectoral muscle α-actinin (Tokue et al., 1991), and polyclonal desmin Ab (Progen Biotechnik GMBH, Heidelberg, Germany). The secondary Abs were horseradish peroxidase (HRP)-conjugated goat IgG to mouse or rabbit IgG (Organon Teknika Corp., Durham, NC, USA), FITC-conjugated goat affinity purified Ab to mouse IgG (H+L) (Kirkegaard & Perry Laboratories Inc., Gaithersburg, MD, USA), Texas red-conjugated goat affinity purified Ab to mouse IgG (Organon Teknika Corp., Durham, NC, USA), 5 and 10 nm gold-conjugated Ab to mouse or rabbit IgG (BioCell Research Laboratories, Cardiff, UK).

Electrophoresis and immunoblot analysis

Diaphragm muscles were dissected out from male Wistar rats (9 weeks), quickly submerged in liquid nitrogen, and ground in a porcelain mortar to yield a fine powder. The powder was suspended in SDS gel-loading buffer (50 mM Tris-Cl, pH 6.8, 100 mM DTT, 2% SDS, 0.1% Bromophenol Blue and 10% glycerol) and boiled for 5 minutes. SDS-PAGE was performed in 3-12% polyacrylamide gradient gels according to the method of Laemmli (1970). Protein bands were stained with Coomassie brilliant blue.

For immunoblot analysis, proteins in gels were transferred to polyvinylidene difluoride membranes (Immobilon-P, Millipore, Bedford, MA, USA). After being blocked with 5% nonfat dry milk in TBS (pH 7.5), the membranes were incubated with primary Ab for 5 hours at room temperature, washed with TBS containing 0.1% Tween-20, and then incubated with secondary HRP-conjugated Ab for 3 hours.

For conventional thin section EM, small pieces of diaphragm muscle were fixed in 1.5% paraformaldehyde, 2.5% glutaraldehyde, 0.2% tannic acid and 0.02% saponin in 0.1 M sodium cacodylate buffer, pH 7.4, overnight. They were post-fixed in 1% OsO4 in 0.1 M sodium cacodylate buffer and then block-stained with 0.5% aqueous uranyl acetate. They were dehydrated in a series of increasing concentrations of ethanol and embedded in Epon 812. Thin sections were cut, stained with uranyl acetate and lead citrate, and then observed with a Hitachi H-800 electron microscope (Hitachi, Japan).

Preparation of skinned muscle fibers

Strips of diaphragm muscles (2x 5 mm) were incubated in 50% (v/v) glycerol containing 0.5 mM NaHCO3, 5 mM EGTA, and 1 mM leupeptin at 0°C overnight and then stored in the fresh solution at −20°C for 1 week. From these glycinated muscles, smaller bundles of muscle fibers (approximately 200 μm x 3 mm) were carefully teased and chemically skinned with 1% Triton X-100 in EGTA rigor solution (0.17 M KCl, 1 mM MgCl2, 10 mM 3-[N-morpholino]propanesulfonic acid (MOPS; pH 7.0), 1 mM EGTA, 2 mM disopropyl fluorophosphates (DFP), 2 mM phenylmethylsulfonyl fluoride (PMSF), and 2 mM leupeptin) for 30 minutes on ice.

Immunoelectron microscopy

For ultrathin cryosection EM, diaphragm muscles perfusion-fixed with 1.5% paraformaldehyde in PBS were cut into small blocks and further fixed in the fresh fixative for 1 hour. The blocks were transferred into 20% polyvinyl pyrrolidone (MW=10,000), 1.84 M sucrose and 0.01 M Na2CO3 in PBS as cryoprotectant for 2 hours. They were mounted on aluminum stubs and plunge-frozen in liquid nitrogen. Ultrathin sections were cut using a Reichert Ultracut S microtome fitted with a FC S cryomicrotome attachment (Leica, Wien, Austria). They were picked up with a drop of 2.3 M sucrose and 0.75% gelatin in PBS and then placed on Formvar/carbon-coated grids. The grids were washed by floating on drops of PBS, blocked with 3% BSA in PBS and then incubated on drops of primary Abs for 1 hour. After being washed on drops of 1% BSA in PBS, they were incubated with gold-conjugated secondary Ab for 1 hour and washed again. The grids were fixed on drops of 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer and postfixed with 1% OsO4 in the buffer. They were stained with 0.5% aqueous uranyl acetate, dehydrated in a series of increasing concentrations of ethanol and then embedded in LR white (London Resin Company Limited, Berkshire, England). Some sections in LR white were stained with aqueous uranyl acetate and lead citrate, but others were not.

For double immunolabeling, we paid special attention. The sections were first labeled with monoclonal plectin Ab and gold-conjugated Ab to mouse. After being washed, they were labeled with polyclonal desmin Ab and a different size of gold-conjugated Ab to rabbit. To check possible crossreactivity or steric hindrance, the labeling was carried out in the reverse sequence and by using different sizes of gold.
Linkage of plectin to Z-disc particles conjugated secondary Ab for each primary (desmin or plectin) Ab.

Skinned muscle fibers were incubated with primary Ab for 5 hours at 2°C and washed in EGTA rigor solution containing 0.1% Triton X-100. Subsequently, they were incubated with gold-conjugated secondary Ab at 2°C overnight. After being washed, they were fixed with 2.5% glutaraldehyde and 0.2% tannic acid in 0.1 M sodium cacodylate buffer, pH 7.4, and processed for EM preparation, as described above.

For controls, ultrathin cryosections and skinned fibers were incubated with only secondary gold-conjugated Ab, which did not stain any specific structures.

Quantification of electron microscopic data
The relative localization of plectin to desmin IF and Z-discs was determined by measuring the distance of plectin- and desmin-specific gold particles (center) from the peripheral margin of Z-discs in electron micrographs of ultrathin cryosections doubly-labeled with desmin and plectin Abs. When gold particles intervened between two Z-discs, we delineated midline between the Z-discs and measured the distance from the nearest Z-disc. To determine the zone of

Fig. 1. Immunoblotting examination of antibodies used for immunocytochemical studies. An equal amount of whole skeletal muscle extract was loaded into each lane. CBB: Coomassie brilliant blue staining.

Fig. 2. The colocalization of plectin with desmin at the periphery of Z-discs. Confocal laser micrographs of longitudinal and cross sections doubly-stained with FITC-labeled anti-plectin (A,C,F,I; green) and Texas red-labeled anti-α-actinin (B,D,G; red) or anti-desmin antibody (J; red) Note in superimposed images (E and H) that plectin is localized around Z-discs. Arrowheads in I and J indicate coincident localization. Bars: 5 μm (A); 1 μm (C and F); 2 μm (I).
sarcoplasmic reticulum (SR), we randomly chose Z-discs, where we first measured the maximum and the minimum distance between the distal margin of SR apposing the Z-disc and the peripheral margin of Z-discs and then regarded their median value as the average of SR zone.

RESULTS

By immunoblotting of skeletal muscle tissue, we checked the reactivity of the antibodies (Abs) to be used for the following immunocytochemistry. The reactivity of the Abs was monospecific and essentially in accord with previously published data (Fig. 1). Monoclonal plectin Ab (clone 7A8) labeled an approximately 500 kDa band (Wiche et al., 1991), which was slightly higher than dystrophin, 427 kDa (Koenig et al. 1988), and lower than the ryanodine receptor, 564 kDa (Zorato et al., 1990). Polyclonal desmin Ab stained a band at 50 kDa (Geisler and Weber, 1982), whereas polyclonal α-actinin Ab labeled a major band at 100 kDa (Endo and Masaki, 1984) with weaker bands of lower molecular mass, possibly degradation products.

Confocal microscopic analysis of longitudinal cryosections of muscle fibers stained with anti-plectin showed a cytoplasmic cross-striation staining pattern (Fig. 2A). Each plectin striation was well aligned with Z-discs, and consisted of intermittent immunofluorescent dots rather than a continuous line (Fig. 2A-E). Double-immunofluorescence staining for plectin and α-actinin demonstrated that fluorescent dots of plectin intervened between adjacent α-actinin-positive Z-discs. In cross sections, a honey-comb plectin staining pattern was observed in which plectin immunoreactivity surrounded individual Z-discs (Figs. 2F-H). On the other hand, double-immunofluorescence staining for plectin and desmin showed coincident localization (Fig. 2I and J). These results indicated that plectin was colocalized with desmin at the periphery of Z-discs.

The localization of plectin at the periphery of Z-discs was constantly retained irrespective of the contracted or extended state of the muscle fibers. Superimposition analysis of plectin striation images and sarcomere images, obtained from contracted and extended muscle fibers, showed constant plectin localization aligned with Z-discs (Fig. 3, left). To verify this plectin localization statistically, we examined the correlation between the width of plectin striations and the sarcomere lengths. This dot plot analysis indicated that the width of plectin striations concomitantly changed with the sarcomere lengths (Fig. 3, right). The Pearson’s correlation showed a very high value of 0.997. These results suggest either direct or indirect association of plectin with Z-discs.

To examine topological relationships of plectin to IF and Z-discs at the ultrastructural level, we carried out immunoelectron microscopic analysis by using ultrathin cryosections. As revealed by thin-section EM with tannic acid enhancement, individual Z-discs were surrounded by or closely apposed fenestrated sarcoplasmic reticulum (SR), outside of which IF bundles were running (Fig. 4A). At closer inspection of such Z-disc areas in ultrathin cryosections labeled with plectin Ab, gold particles indicative of the plectin immune

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**Fig. 3.** Constant alignment of plectin localization with Z-discs. Left: Confocal laser micrographs of sarcomere images in differential interference contrast phase (upper) and plectin striation images in fluorescence phase (lower). In both contracted and extended state of muscle fibers, plectin localization is aligned with Z-discs. Bar, 2 μm. Right: The correlation between the sarcomere length and the width of plectin striation is a linear one, showing a very high value of 0.997 in Pearson’s correlation (n=103).
Fig. 4. Electron micrographs of Z-disc area demonstrated by conventional thin-section EM (A) and immuno-EM using ultrathin cryosections (B-D). (A) Z-discs closely appose fenestrated sarcoplasmic reticulum (SR), outside of which intermediate filaments (IFs) run. (B-C) Plectin-specific gold particles (5 nm) are localized close to IFs, around SR and on the peripheral margin of Z-discs. Arrowheads in C show plectin-specific gold particles preferentially localized within fenestrations of SR (also see Fig. 5, upper). (D) In longitudinal section, plectin-specific gold particles (arrowheads) are found around SR at Z-discs levels. Bars, 0.1 μm.
complex were found close to IFs, around SR, and on the peripheral margin of Z-discs (Fig. 4B). More numerous gold particles were found in SR zones intervening between IFs and Z-discs (Fig. 4C,D). In these zones, plectin-specific gold particles were frequently localized within fenestrations or pores of SR.

Next, we statistically determined the relative localization of plectin to desmin IF, Z-discs and SR by measuring the distance of plectin- and desmin-specific gold particle from the peripheral margin of Z-discs and that of the distal margin of SR from it. The distal margin of SR was at an average distance of 53±8.7 nm (n=156) from the Z-discs. Based on this average distance of the SR zone, more than 70% plectin-specific gold particles were located at a distance of between 0 and 50 nm, namely in the SR zone, whereas more than 70% of the desmin-specific gold particles were localized at >50 nm, outside of the SR zone (Fig. 5). Considering the extraordinary length of individual plectin molecules (mean lengths of 191-193 nm, Foisner and Wiche, 1987; Weitzer and Wiche, 1987) and the antigenic epitope of the present Ab (clone 7A8) on the middle portion of plectin rod (Foisner et al., 1991a), plectin-specific gold particles in the SR zone imply that plectin molecules would span the IFs and Z-discs.

Based on this assumption, we assessed the presence of cytoskeletal elements spanning the IF and Z-discs. In this assessment, SRs intervening between IFs and Z-discs obscure such cytoskeletal elements if present. Thus, we chemically skinned muscle fibers with Triton X-100 and observed them by thin-section EM with tannic acid enhancement. Membranous organelle, such as SR, T-tubules and mitochondria, were almost completely removed, and fine threads associated with IFs were clearly visualized at the periphery of the Z-discs (data not shown). These fine threads were 2-4 nm in diameter, much thinner than actin thin filaments, and varied in length, up to approximately 190 nm long. They often linked IFs to Z-discs and connected IFs to each other.

In addition to their association with IF, fine threads matched plectin molecules in their dimensions (Foisner and Wiche, 1987). Therefore we attempted to identify fine threads with anti-plectin Ab and gold conjugated secondary Ab in skinned muscle fibers. As expected, treatment of skinned fibers with anti-plectin Ab resulted in the association of plectin-specific gold particles with the fine threads spanning the IF and Z-discs and between IFs (Fig. 6A,B). Gold particles appeared preferentially to lie on the mid point of fine threads, consistent with the plectin epitope recognized by the 7A8 Ab. The number of gold particles per fine thread ranged from 1 to 8 in 5 nm-gold labelings, implying that the polymer form of plectin constitutes fine threads. Double-immunolabeling with plectin and desmin Abs clearly demonstrated that desmin IF projected

**Fig. 5.** Preferential localization of plectin in SR zones. Upper: Electron micrograph of ultrathin cryosection doubly labeled with plectin (10 nm gold) and desmin (5 nm gold) antibodies. Bar = 0.1 μm. Lower: Statistical analysis of plectin- and desmin-localization relative to Z-discs and SR. In double-staining with 5 nm gold for plectin and 10 nm gold for desmin, 78% plectin-specific gold particles (453/580) are localized in SR zones, whereas 79% desmin-specific gold particles (1297/1641) are localized outside of SR zones.

This localization pattern holds true for double-staining with 10 nm gold for plectin and 5 nm gold for desmin (plectin: 75% (201/268) in SR zones, desmin: 74% (1090/1473) outside of SR zones).
Fig. 6. Electron micrographs of skinned muscle fibers after immunogold labeling for plectin (A and B) and after double-labeling for plectin and desmin (C). (A and B) Plectin-specific gold particles (5 nm) lie on fine threads linking IF to Z-disc and to each other. Note immunolabels on the midpoint of fine threads. (C) Desmin-specific gold particles (5 nm) lie along IFs from which fine threads labeled with plectin (10 nm gold) antibody project to Z-discs. Bars, 0.1 μm.
plectin assembles into homotetramers composed of two to 8) on a single thread might be consistent with the notion that molecules (Foisner et al., 1991a). Multiple gold particles (up wide and up to 190 nm long) match the size of isolated plectin because: (1) the dimensions of connecting threads (2-4 nm would be morphological counterparts of plectin molecules (Foisner and Wiche, 1987; Weitzer and Wiche, 1988); and (2) antibody (7A8) labeling in the middle of threads molecules (Steinert et al., 1985). Conversely, the assumption that plectin binds to the highly conserved rod domain would plausibly explain the plectin association with various types of IF proteins with distinct head and tail domains, such as vimentin, glial fibrillary acidic proteins, neurofilaments, keratin 10, 11 and lamin B (Foisner et al., 1988, 1991b) and furthermore expects plectin-desmin interaction as well.

A likely candidate for a binding partner for plectin in Z-discs would be actin, based on the following circumstantial evidence: (1) the amino terminus of plectin contains a highly conserved actin-binding site (Mclean et al., 1996; Elliot et al., 1997). This site shares sequence homology with that of BPAG1n, which has been shown to bind actin filaments by transfection experiments and in vitro binding assays (Yang et al., 1996; Fuchs et al., 1997). (2) Plectin localizes not only to IF but also to actin stress fibers and focal contacts in cultured cells (Seifert et al., 1992). Crossbridge-like structures labeled by plectin antibody connect IFs with actin microfilaments (Foisner et al., 1995). (3) In skeletal muscle fibers, Z-discs are peculiar structures in which actin filaments provide binding sites for various kinds of actin-binding proteins, such as α-actinin, cap Z, and filamin-like protein (Blanchard et al., 1989; Casella et al., 1987; Bechtel, 1979). However, the identity of plectin binding partners within Z-discs remains to be established.

The configuration of Z-discs, SR, plectin threads and IFs is worth elaborating on. Z-discs closely appose or are surrounded by SRs, around which IF bundles run. In this configuration, one might pose the question of how plectin threads go through SR and span between Z-discs and IFs. SR is not a simple membranous sac but has many fenestrations or pores, in which plectin-specific gold particles are frequently localized. Based on this observation, plectin threads would go through the fenestrae of SR and link IFs to Z-discs (Fig. 7). This configuration, the penetration of plectin threads through SR, also seems supportive for preserving the specific location of SR membrane relative to the sarcomere during contraction and relaxation cycles, in addition to short filaments connecting the Z-discs to SR (Nunzi and Franzini-Armstrong, 1980).

The tension of a single muscle fiber is represented by the sum of the tensions generated by individual myofibrils. Thus, effective force generation requires synchronous contraction of individual myofibrils with similar sarcomere lengths. If individual myofibrils with different sarcomere lengths contract, they produce different tensions since the length-tension curve for vertebrate sarcomeres shows an inverted-U shape (Gordon et al., 1966; Edman, 1966; Edman and Reggiani, 1987). These

**DISCUSSION**

In the present study we have ultrastructurally demonstrated that plectin fine threads link desmin IFs to Z-discs and connect IFs to each other. These structural couplings of desmin IFs/plectin fine threads/Z-discs link myofibrils laterally at Z-disc levels (Fig. 7). Functionally, these lateral linkages would prevent individual myofibrils from disruptive contraction and ensure effective force generation. Morphogenetically, the lateral linkages would be formed during myogenesis, namely IF translocation, to which plectin molecules could contribute.

Because our structural evidence for plectin association with IFs and Z-discs was obtained from electron microscopy of muscle fibers chemically skinned with Triton 100-X, we must consider the possibility that this procedure may introduce artifacts of plectin redistribution or adventitious superimposition onto Z-discs or IFs. The results obtained by other methods or approaches suggest that the observed plectin distribution or association was not artificial. First, a similar distribution pattern of plectin, e.g. its preferential localization between IFs and Z-discs, was observed in immuno-EM using ultrathin cryosections which are not treated with Triton X-100. Second, morphometric analysis of plectin localization in immunofluorescence-labeled muscle fibers indicated that plectin localization was constantly in register with Z-discs irrespective of contracted or extended muscle fibers, suggesting the association of plectin with Z-discs. Accordingly, we believe that the association of plectin with not only IF but also Z-discs fairly reflects the organization of plectin in situ.

Fine threads connecting IFs to Z-discs and to each other would be morphological counterparts of plectin molecules because: (1) the dimensions of connecting threads (2-4 nm wide and up to 190 nm long) match the size of isolated plectin molecules (Foisner and Wiche, 1987; Weitzer and Wiche, 1987); and (2) antibody (7A8) labeling in the middle of threads is consistent with the location of antibody epitope on plectin molecules (Foisner et al., 1991a). Multiple gold particles (up to 8) on a single thread might be consistent with the notion that plectin assembles into homotetramers composed of two parallel dimers, arranged in an antiparallel fashion and overlapping by their entire length (Wiche et al., 1991). Accordingly, the fine threads could be plectin molecules themselves and thus the linkage of fine threads to Z-discs and IF would represent the association of plectin molecules with desmin IF and components of Z-disc.

Direct evidence for plectin’s binding to desmin has not yet been shown, but the binding is implicated by the present morphological result of plectin association with desmin IF. This binding of plectin to desmin is anticipated, for plectin seems to bind to the rod domain of vimentin (Foisner et al., 1988) and this α-helical domain is highly conserved among IF proteins (Steinert et al., 1985). Conversely, the assumption that plectin binds to the highly conserved rod domain would plausibly explain the plectin association with various types of IF proteins with distinct head and tail domains, such as vimentin, glial fibrillary acidic proteins, neurofilaments, keratin 10, 11 and lamin B (Foisner et al., 1988, 1991b) and furthermore expects plectin-desmin interaction as well.

Fig. 7. Schematic drawing of IF-plectin threads-SR-Z-disc configuration in skeletal muscle fibers. Plectin threads are represented by thin-short red lines spanning between IF (blue) and Z-disc.
uneven tensions within a muscle fiber would result in the reduction of generated tension. To ensure effective force generation, the lateral linkages formed by IFs and plectin would keep the sarcomere length of individual myofibrils almost equal during contraction and relaxation. In support of this notion, the force generated by myotubes lacking the lateral linkages which were dismantled by expression of truncated desmin cDNA was reduced into 1/2 to 1/4 of control myotubes with normal desmin IF (Feng et al., 1994). On the other hand, the lateral linkages or frameworks formed by plectin and IF seem to be not so rigid, but would have some degree of junctional compliance between adjacent Z-discs. The displacement of adjacent Z-discs from one another has been often noted in EM studies (Tokuyasu et al., 1983; also see Fig. 4D).

Skeletal muscle fibers in plectin-knockout mice and in humans with plectin deficiency show disorganization or disruption of myofibrils, particularly of the Z-discs (Gache et al., 1996; Andrä et al., 1997). This disruption most likely occurs as a consequence of disruptive contractions of myofibrils with uneven sarcomeres that result from defective lateral linkages lacking plectin threads. This pathogenic mechanism would hold true for similar pathological alteration of skeletal muscles found in desmin-knockout mice (Milner et al., 1996; Li et al., 1996). As demonstrated in the present study, both plectin and desmin are crucial structural components of the lateral linkages, which would be indispensable for normal synchronized and harmonized contraction and relaxation activity of myofibrils. However, neither plectin nor desmin IF seem to be required for the normal assembly of myofibrils including Z-disks during myofibrillogenesis. Intact Z-discs and myofibrils are also formed in plectin-knockout mice (Andrä et al., 1997), and the dismantlement or the knockout of desmin IFs do not affect the assembly of morphologically normal Z-discs and myofibrils (Schultheiss et al., 1991; Milner et al., 1996; Li et al., 1996).

During myogenesis, IFs oriented longitudinally in the cytoplasm of the myotube achieve their final disposition surrounding the Z-discs (Bennett et al., 1979; Holtzer et al., 1982, 1985). However, nothing is known of the underlying mechanism of this translocation of IFs. The current finding of plectin molecules linking desmin IFs to Z-discs suggests that plectin could be one of the molecules involved in the translocation of IFs. In this context, it is noteworthy that desmin appears delocalized at the periphery of muscle fibers in the EBS-MD skeletal muscle fibers without plectin expression (Gache et al., 1996). For this phenomenon, one interpretation is that plectin deficiency, that is, the lack of linkage between IF and Z-discs, leads to the abnormal localization of desmin during myogenesis, whereas another is that the abnormal localization secondarily results from the degeneration of myofibrils. Inspection of the IF arrangement in skeletal muscles of or muscle cells cultured from plectin null mice would provide some insights into the mechanism of IF translocation.

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REFERENCES


