αv and α3 integrin subunits are associated with myofibrils during myofibrillogenesis

K. A. McDonald*, M. Lakonishok and A. F. Horwitz

Department of Cell and Structural Biology, University of Illinois at Urbana-Champaign, 511 Morrill Hall, 506 S. Goodwin, Urbana, IL 61801, USA

*Author for correspondence

SUMMARY

The development of the myofibrillar apparatus in skeletal muscle is a process in which transmembrane linkages with adhesion molecules are implicated. Integrins are one class of transmembrane adhesion receptors which appear to mediate these interactions. Two prominent linkages are at the myotendinous junction (MTJ), which resides at the ends of the cell and connects myofibrils to the tendon, and the costameres, which encircle the girth of the cell and connect the Z-discs to the sarcolemma. In this study we report that the αv integrin subunit is a prominent component of the costamere. The αv subunit is present initially on developing myotubes in a diffuse staining pattern with some concentration along nascent myofibrils. However, it appears in a striated pattern at the costamere and inconsistently at the M-line following the striation of α-actinin and titin but before that of desmin. Its recruitment to preformed striation suggests that it is incorporated into a pre-existing structure. The presence of αv in the costamere points to a role in lateral myofibrillar anchorage. In addition, we find that the α3 subunit is transiently associated with myofibrils along portions of their lengths and at their ends during myofibrillogenesis. The α3 subunit staining shows a novel localization and junctional structure. As myofibrils become striated the α3 integrin dissociates from the localized pattern and becomes diffuse. This suggests a possible role in the stabilization of nascent myofibrils prior to striation. Antibody-induced perturbation of adhesion mediated by the integrin β1 subunit in developing myotubes inhibits assembly of the sarcomeric architecture. The observations presented here also contribute to an emerging theme, where different integrins occupy unique junctional regions on muscle.

Key words: costamere, integrin, muscle development, cytoskeleton, myofibril

INTRODUCTION

Skeletal muscle cells have highly organized cytoskeletal-sarcolemmal associations which function to transfer force from the contractile apparatus to the extracellular environment. The contractile cytoskeleton is composed of myofibrils built in a precise hierarchical fashion. The basic cytoskeletal unit of a muscle cell is the sarcomere, anchored at each end by a Z-disc. Sarcomeres are multiplied end to end along the length of the cell to form a myofibril (Craig, 1994). In skeletal muscle, myofibrils terminate at the myotendinous junction (MTJ), a prominent, highly organized region of transmembrane association between the myofibrils and the collagen-rich tendon (Trotter et al., 1985). The Z-discs of the outer-most myofibrils in the muscle cell contact the sarcolemma in another structure, which is called the costamere: the site of lateral cytoskeletal-extracellular matrix attachment (Pardo et al., 1983). The costamere is thought to transmit force laterally to the surrounding connective tissue.

Integrins, a superfamil of heterodimeric cell surface glycoproteins that participate in cytoskeletal-cell surface-extracellular matrix linkages, are likely candidates to mediate myofibrillar-sarcolemmal associations. They are members of a large family of heterodimeric transmembrane molecules that function in a dual binding capacity for both extracellular molecules and the cytoskeleton (Hemler, 1990; Hynes, 1992; Sastry and Horwitz, 1993). Integrins often localize in highly organized structures at sites of transmembrane linkage. The best characterized of these linkages is the focal adhesion present on fibroblasts and some other cells (Burridge and Fath, 1989; Turner and Burridge, 1991). It is the site at which actin stress fibers terminate at the cell surface and connect through integrin with the fibronectin-rich extracellular matrix (ECM).

Vertebrate skeletal muscle expresses a large variety of different integrins (Bao et al., 1993; Lakonishok et al., 1992; Terracio et al., 1989). These show differing ligand specificities for ECM molecules and possibly for cytoplasmic molecules as well (Hynes, 1992). However, the functions of these different integrins in myofibril assembly and organization are only beginning to be characterized. The β1 subunit is found in all major myofibril-sarcolemma junctional areas, including the costamere and the myotendinous junction, and along the sarcolemma of vertebrate skeletal muscle (Bozyczko et al., 1989; Swasdison and Mayne, 1989). The αvβ1 integrin localizes in...
the MTJ where it likely anchors the myofibrils to the tendon. The α3β1 integrin may also associate with myofibrils as it transiently resides in the region of the nascent MTJ prior to membrane infolding and expression of the αvβ1 integrin (Bao et al., 1993).

Other integrins, in addition to the αvβ1 and possibly the α6β1 integrins, appear to mediate associations between myofibrils and the sarcolema. In Drosophila, the β6 integrins localize in regions of the Z-bands and muscle insertions (Volk et al., 1990). Drosophila skeletal muscles that lack PS2 integrins form myotubes without Z-bands and display weakened muscle attachments to surrounding tissue. These adhesions detach following the initial contractions, producing a spherical morphology in the developing embryo (MacKrell et al., 1988; Volk et al., 1990). Although the ligands for these integrins have not been reported, vertebrate vitronectin functions as one of its ligands (Bunch and Brower, 1992), suggesting therefore that a vitronectin receptor may function similarly in vertebrates.

In the present study, we investigate the potential roles of the α3 and α6 integrins in linking myofibrils with the sarcolemma. We find that the α6 subunit initially localizes diffusely along nascent myofibrils on early myotubes. On older myotubes, it is highly enriched at the costamere. α3 is also found associated with early myofibrils; however, it is concentrated along portions of myofibrils and in plaque-like structures at their ends. During myofibrillar development the regionalized concentrations of the α3 integrin disappear as the myofibrils come into register and become striated. Addition of adhesion-perturbing antibody directed against the integrin β1 subunit to developing myotubes inhibits assembly of the sarcomeric architecture. These observations point to both of these integrins as participants in the organization and stabilization of myofibrils. They also show a novel localization and junctional structure for the α3 integrin.

MATERIALS AND METHODS

Antibodies

Two rabbit antisera to the integrin α6 subunit were a gift from Louis Reichardt. The polyclonals employed, α3C and α6L, are against synthetic peptides from the chicken α6 sequence (Bossy and Reichardt, 1990). α3C is against the cytoplasmic domain 27 amino acid carboxyl terminus, KRVRPQQEQQERQLPHEHGETSEA. α6L is against a 27 amino acid peptide, KIKISAPKEDEKNETF-SREDNRHRIS, from the extracellular domain near the membrane spanning region. These antibodies are specific for the peptides against which they were made. The α3C specificity was reconfirmed by peptide blocking in the muscle culture system we used. The monoclonal antibody CHAV-1 is specific for α6, and was also a gift from Lou Reichardt (Neugebauer et al., 1991). Clayton Buck supplied the polyclonal rabbit serum, BLDG06982, against the α3A cytoplasmic domain peptide RIQPSELTLDY (Hynes et al., 1989). The antibody reactivity was blocked in our system by the peptide antigen. Antibodies against the α6 and α7 integrin subunits were described previously (Bao et al., 1993; Muschler and Horwitz, 1991). Antibody against the α3 integrin subunit was a gift from J. Syfrig and M. Paulsson (Syfrig et al., 1991). The W1B10 mAb is an adhesion-perturbing antibody directed against the chicken β1 integrin subunit (Hayashi et al., 1990). Muscle-specific α-actinin monoclonal antibody (9A2B8) was a gift from Donald Fishman (Schultheiss et al., 1990).

Rabbit polyclonal antiserum against desmin (D8281) was obtained from Sigma Chemicals. Monoclonal antibodies 9D10 against titin and MF20 against light meromyosin were obtained from the Developmental Studies Hybridoma Bank, maintained by the Department of Biological Sciences, University of Iowa, Iowa City, IA, under contract N01-HD-6-2915 from the NICHD. Fluorescently labeled secondary goat antibodies against rabbit and mouse whole IgG were purchased from Cappel, Organon Teknika Corp., West Chester, PA. Specificity for the appropriate primary antibody was verified by addition of anti-rabbit and anti-mouse secondary to muscle cultures incubated with either a rabbit or a mouse primary antibody. No cross-reacting activity was observed.

Cell culture

Myocytes were obtained from the breast muscle of 10- to 11-day-old white leghorn chick embryos. Tissue was removed from the embryos and minced in PBS, then enzymatically digested in Dispase for 10 minutes at 37°C. Digestion was ended by addition of excess culture medium (Dulbecco’s modified Eagle’s medium, 10% horse serum, 5% chick embryo extract, penicillin-streptomycin 100 i.u./ml) and centrifugation of the suspension at 800 g for 5 minutes. Pelleted material was resuspended in culture medium prior to filtration through a Swinex syringe filter containing a triple layer of lens paper to remove connective tissue. The resulting cell suspension was plated at 2×10^6 cells per square centimeter on 0.1% gelatin-coated glass coverslips. Medium was changed every 3-4 days.

Immunofluorescence

Cells were grown on glass coverslips. Cell cultures were washed with phosphate buffered saline (PBS) three times, then fixed in 3% formaldehyde-PBS for 15 minutes. Fixative was removed with three washes of PBS, then blocked with 0.1 M glycine-PBS for 10 minutes. Cells were then permeabilized with 0.4% Triton X-100 in PBS for 10 minutes. Detergent was removed by three washes with PBS. Primary antibodies were used simultaneously in double staining, diluted with 5% goat serum-PBS, and allowed to incubate on the cultures for one hour at room temperature. After three PBS washes, the secondary antibody mixture, goat anti-mouse rhodamine and goat anti-rabbit fluorescein, both at 1:200, was added to the cells for 45 minutes. Three washes with PBS preceded mounting of the coverslips on slides with Elvanol mounting medium containing para-phenylene diamine. Fluorescence was viewed on a Zeiss Axioskop microscope with a 63× PlanApo objective. Images were photographed with T-max 400 ASA film. Alternatively, the specimens were observed on a Bio-Rad 600 confocal microscope and image files processed using Photoshop software (Adobe systems).

Antibody perturbation

Myocytes were isolated and cultured as mentioned above. Either CHAV-1, W1B10 or P3 (a control IgG) mAbs purified on Protein A were added to cultures at 50 mg/ml. Medium was changed every third day with antibody-containing medium. The cultures were processed for immunofluorescence as discussed above.

RESULTS

The integrin α6 subunit localizes along Z-bands on skeletal muscle

The integrin β1 subunit has been localized previously to sites of putative association between myofibrils and the sarcolema: the costamere and myotendinous junction (Bozyczko et al., 1989; Hilenski et al., 1992; Terracio et al., 1989; Volk et al., 1990). With the exception of the α6 subunit, which localizes in the myotendinous junction, the specificity of α subunits for the different junctions in vertebrate muscle has not been characterized. Previous studies on Drosophila...
localize the αPS2 subunit at the junction of the Z-band and the sarcolemma. While the ligand specificity of the αPS2 integrin is unclear, it does promote adhesion to vertebrate vitronectin, suggesting that it functions as a vitronectin receptor (Bunch and Brower, 1992; Volk et al., 1990).

We have used three different antibodies to the αv subunit to address the possibility that a vertebrate vitronectin receptor is present at the costamere. Two are rabbit polyclonal antibodies. One, αv C, was raised against the carboxyl-terminal 27 amino acids in the chicken αv cytoplasmic domain. The other polyclonal rabbit antisera, αv L, was raised against a 27 amino acid sequence in the extracellular portion of the αv subunit near the membrane spanning region (Bossy and Reichardt, 1990). Both antisera recognize specifically the αv integrin subunit in chick embryo fibroblasts (Bossy and Reichardt, 1990). Peptides corresponding to those used for immunization blocked the antisera staining. The third antibody is the monoclonal CHAV-1, which is specific for the αv subunit. CHAV-1 inhibits adhesion moderately, and therefore likely interacts with an epitope on the extracellular domain of the αv subunit (Neugebauer et al., 1991). All three antibodies produced similar results except where so noted.

These antibodies reveal the presence of αv in a striated pattern in sarcomemmal regions adjacent to the Z-band of mature myotubes. The striated staining pattern is shown in Fig. 1B. This staining is similar in position to that of muscle α-actinin, which is a component of the Z-disc in striated muscle (Stromer and Goll, 1972) (Fig. 1A). The correspondence between αv and α-actinin is seen most clearly when images from a double-labelled preparation are superimosed (Fig. 1C). The green represents αv staining, and the red represents muscle α-actinin. The yellow areas show regions where the two proteins co-localize. The regions of superimposed localization show that the αv subunit overlies the Z-disc. However the staining for the αv subunit extends slightly beyond the width of the muscle α-actinin band. Such a localization pattern, which was not seen with antisera pre-incubated with the peptide against which it was raised, points to the presence of αv in the costamere.

The staining pattern seen using the CHAV-1 mAb often resolved into a doublet directly adjacent to and partially overlying the Z-disc. This was more prevalent and striking when immunostaining cardiac cultures (not shown). The doublet staining was seen only occasionally with the polyclonal antibodies. Some of the staining variability may arise from differences in the contractile state of the individual myotubes. Doublet staining at the costamere is clearest and most reproducible in stretched muscle fibers, in which the sarcomeres are lengthened and hence the distance between the doublets greater (Pardo et al., 1983). The tendency of CHAV-1 to stain doublets more readily than the other antibodies may be explained by the different epitope that it recognizes. The CHAV-1 mAb is adhesion perturbing. Therefore its epitope could be masked in regions where there is a high density of extracellular ligand. This may occur at the center of the costamere where extracellular matrix fibrils contact the sarcolemma (Craig and Pardo, 1983; Terracio et al., 1989). In addition to the staining at the costamere, the αv C antibody occasionally stained less prominently in the M-line, a band midway between the costameres (Fig. 2). Doublet and M-line staining have been reported previously for other proteins in the costamere including vinculin, clathrin, β-spectrin and dystrophin (Kaufman et al., 1990; Pardo et al., 1983; Porter et al., 1992).

**Fig. 1.** The αv integrin subunit of localizes in the vicinity of the Z-line. Chicken myotubes were cultured for 11 days and then immunostained with the monoclonal antibody 9A2B8 directed against muscle-specific α-actinin (A) and the polyclonal antibody αv C directed against the cytoplasmic domain of the αv subunit of integrin (B). The α-actinin is a marker for the Z-disc. Superimposition of the two images shows a yellow color where the αv and α-actinin are coincident (C). Bar, 10 µm.
The role of the αv subunit in the organization of myofibrils was studied by comparing the time at which it localizes in myofibrils with that of other myofibrillar proteins. During myofibrillogenesis, titin and α-actinin are among the earliest proteins to exhibit periodic localization (Franzini-Armstrong and Fischman, 1994; Wang et al., 1992). Myosin appears in striations at about the same time as titin or shortly thereafter (Isaacs et al., 1992). The intermediate filament protein desmin localizes in Z-bands several days after α-actinin and titin (Vanderloop et al., 1992; Vanderven et al., 1993).

Myotubes were examined from the time of fusion (about 3 days in culture) through the establishment of well developed striations (about 2 weeks). Early in myotube development, prior to titin striation, the distribution of the αv subunit is diffuse, with some concentration along myofibrils that are not yet organized into striated structures, i.e. non-striated fibrils (NSFs) (Fig. 3A,B). The αv subunit appears in a striated pattern only after muscle α-actinin and titin incorporate into developing sarcomeres at about 6 days in culture. This is seen most clearly in myotubes in the process of myofibrillar organization, where regions of α-actinin or titin striations but no αv striations, are seen (arrows in Fig. 3C,D). Desmin has been reported to striate after the titin and α-actinin in other systems (Vanderloop et al., 1992; Vanderven et al., 1993). In our system we confirmed that the desmin filaments striate a couple of days after the α-actinin, i.e. at 8 to 9 days of culture. On the basis of these observations, it appears that the αv subunit is recruited into an already established cytoplasmic sarcomere template at the costamere; but before establishment of the intermediate filament-sarcolemmal linkage (Fig. 3E,F). The recruitment of the β1 subunit, which is also seen in costameres, is a very late event as it is seen only in very old cultures.

### The αv subunit associates transiently with myofibrils prior to striation

Bao et al. (1993) reported that the α3 integrin subunit is present transiently in the vicinity of the myotendinous junction just prior to the appearance of the αv subunit on developing muscle in vivo, suggesting a possible transient association with developing myofibrils. Therefore, we compared the localization of the α3 subunit with that of myofibrillar proteins during the course of myofibrillogenesis. We used a rabbit polyclonal antiserum raised against a synthetic peptide corresponding to the terminal 14 amino acids in the cytoplasmic domain of the chicken α3 subunit (Hynes et al., 1989). This antibody immunoblots a single band at the Mₜ of α3 integrin, and the blotting is inhibited by the synthetic peptide against which the antiserum was raised (not shown).

The integrin α3 subunit has a unique and changing localization on developing myotubes. In contrast to αv, which is present ubiquitously, α3 is expressed in a subpopulation of developing myotubes, i.e. typically less than 50% of the cells at any one time. Initially, α3 tends to concentrate at the ends of nascent non-striated fibrils and along portions of their length (Fig. 4A,B). Subsequently, as the myofibrillar apparatus matures, it localizes in extensive, prominent longitudinal streaks, that occupy an area much broader than the underlying fibrils (Fig. 4C,D). This localization persists until α-actinin shows clear periodic striations. These striated areas tend to exclude the streaks of α3 concentration. This temporal change in α3 localization is seen best in myotubes which are in the

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**Fig. 2.** The αv subunit of integrin localizes to the M-line. The mAb 9A2B8 directed against α-actinin (A) and the polyclonal antibody αvC (B) were used to stain a 17 day culture of chicken myotubes. αv co-localizes with the α-actinin at the Z-line and in this particular example strongly stains midway between the Z-lines. The arrows show the positions of two adjacent Z-discs, arrowheads show the αv staining in the region of the M-line (B). Bar, 10 μm.

The αv subunit is unique among the integrins tested, for its localization at the costamere or M-line. Antibodies against the α3, α5, α6, and α7 integrin subunits do not reveal concentrated staining in these locations (not shown). However, αv is faintly and occasionally present at the Z-band but only in very old cultures (25 day).

The particular β subunit(s) with which αv associates in the costamere is unclear at present. An antibody which recognizes the human αvβ3 heterodimer (LM609) localizes only weakly in the costamere, pointing to the presence of the β3 subunit. Since the β1 subunit appears at the costamere much later in muscle development than αv, it is unlikely that β1 is the major subunit associated with αv in the costamere. The αv subunit also associates with a large number of other β subunits, but a panel of reagents to characterize them is not available.

**The integrin αv subunit is recruited to a preformed cytoskeletal structure during myofibrillogenesis**

The αv in the organization of myofibrils was studied by...
αv and α3 during myofibrillogenesis

process of striating and display both sarcomeres and NSFs. A co-stain using antibodies against both the αv subunit, a marker for striated regions, and the α3 subunit shows α3 predominantly in regions which still have NSFs. However, α3 is absent in regions where the αv has been incorporated into striations (Fig. 4C,D). Older muscle cultures, with well developed sarcomeres, generally show only a diffuse distribution of the α3 subunit (not shown). This interesting transient organization is not shared by α-actinin, talin or any other known integrin-associated molecule (not shown). This points to a novel linkage structure for this integrin on muscle.

β1 Integrins mediate sarcomere assembly

To demonstrate that myofibrillar-associated localization patterns reflect the participation of the α3 and αv integrins in myofibrillogenesis requires a panel of a subunit-specific mAb or cDNA) reagents. While such a library of avian adhesion-perturbing antibodies is not available, the CHAV-1 mAb is
reported to have a modest 20% adhesion-perturbing activity (Neugebauer et al., 1991). The W1B10 mAb directed against the β₁ subunit, while not subunit specific, is also adhesion-perturbing. These antibodies were used to assess the roles of the αv integrin and the β₁ integrins in myofibrillar assembly.

W1B10 or CHAV-1 were added to cultures of myotubes at a concentration of 50 mg/ml. The cultures remained in the antibody until processed for immunofluorescence. Adhesion-perturbing effects of CHAV-1 were not apparent in any of our assays. Myoblast adhesion to a vitronectin substratum by CHAV-1, myotube gross morphology, muscle α-actinin organization, and the timing of developmental events were not inhibited detectably even with concentrations of CHAV-1 as high as 200 mg/ml (data not shown).

In contrast, all cultures exposed to W1B10 showed clear alterations. W1B10, added on the third or fifth day of culture and observed on the sixth day of culture, inhibited myofibrillar organization as assayed by α-actinin localization. In controls a significant fraction of the myotubes showed α-actinin striations, whereas the W1B10-treated cultures did not show well organized muscle α-actinin (not shown). In addition, some cells had detached from the substratum and others showed morphological alterations. Treatment of 7 day cultures, a time at which most cells already showed α-actinin striations, with the W1B10 showed a clear regression in organization of muscle α-actinin when observed on day 8 (Fig. 5). This short treatment of older cultures avoided problems due to detachment and gross morphological alterations. Interestingly, the cultures treated on day 7, but observed on day 10, showed muscle α-actinin striation indistinguishable from that of the controls. Treatment of day 9 cultures with W1B10 showed no apparent alterations in the muscle α-actinin striations when observed on day 10. These latter observations suggest that there are redundant mechanisms contributing to myofibrillar assembly.

**DISCUSSION**

Previous studies of integrin localization on muscle suggest a
The presence of αv in costameres points to its function as a lateral, transmembrane sarcolemmal anchor for sarcomeres. Integrins are generally thought to interact with the actin cytoskeleton via direct associations with proteins like talin and αv-actinin, which interact with actin either directly or through putative linkage proteins like vinculin (Horwitz et al., 1986; Otey et al., 1990; Pavalko and Burridge, 1992). Analogous linkages appear to participate in the organization and stabilization of the actin-rich myofibrils. Caenorhabditis elegans mutants that lack vinculin and Drosophila mutants lacking β integrin show altered myofibrillar structure (Barstead and Waterston, 1991; Volk et al., 1990).

In mature muscle cells the filamentous material that spans the exosarcomeric space between the peripheral Z-discs and the costameres has traditionally been thought to be desmin intermediate filaments and not to contain actin filaments. However γ-actin is reported to be in a striated pattern in close proximity to the costamere (Craig and Pardo, 1983; Wang and Ramirez-Mitchell, 1983). A recent report shows actin filaments emanating peripherally from the Z-line of vertebrate muscle (Bard and Franzini-Armstrong, 1991). Assuming that these actin filaments contact the costamere, the αv integrins may link to them. The actin isoform present in these filaments is not known; they may contain the γ-actin, which is present at the costamere and the periphery of the Z-disks.

In contrast to the association of αv, integrins with mature myofibrils, there is a direct temporal and spatial correlation between the loss of structured αv staining and the presence of striated myofibrils. This agrees with an earlier in vivo report describing its transient presence along myofibrils near the nascent MTJ (Bao et al., 1993). The αv integrin in the chicken embryo hind limb is concentrated in the vicinity of the nascent MTJ prior to the insertion of myofibrils and the presence of the αvβ3 integrin. Bao et al. (1993) speculate on a role for the αv3 integrin in establishing the nascent myofibrillar-sarcolemmal interaction. In addition, αv may participate in a transient early linkage that serves to organize or stabilize non-striated myofibrils prior to registration. In this view, as the nascent non-striated fibrils progress to a mature striated structure with a variety of other associations to the sarcolemma, the αv3 is replaced by other integrins which are unique to each junction. This type of linkage appears novel as the αv3 localizes in a pattern which is different from that described for any of the other integrins or integrin-associated cytoskeletal components found in muscle. However, such a longitudinal association is reminiscent of the myofibrillar-sarcolemmal associations reported in smooth muscle (Small, 1985).

The observations reported here as well as those in previous publications demonstrate that the different types of cytoskeletal-sarcolemmal linkages segregate integrins in muscle (Fig. 6). αv is restricted to the costamere where the myofibrils are laterally linked to the sarcolemma. αv is concentrated in the MTJ, which is the site of terminal anchorage for the contractile apparatus. αv is in the adhesion plaque-like junction where microfilament bundles terminate at substratum adhesions on cultured myotubes. Each junction has a unique function and location within the cell. Such junctional specificity may underlie unique molecular associations that determine the particular function and structure of each junction. The unique

significant role for this class of molecules in myofibrillar development and organization. Vertebrates express a number of integrin heterodimers with different ligand specificities and tissue distribution. Skeletal muscle expresses β1 integrin in a number of structures involved in cytoskeletal-sarcolemmal linkages (Bozyczko et al., 1989). On smooth muscle, β1 integrins are confined to the dense plaques which anchor actin to the sarcolemma (North et al., 1993). On Drosophila muscle, the PS2 integrin is at the costamere, the lateral myofibrillar anchor, and is necessary for normal sarcomere assembly and muscle function (Volk et al., 1990). In vertebrates, the α7 subunit integrin is enriched at the myotendinous junction (MTJ) (Bao et al., 1993). The restriction of αv to the MTJ, where the myofibrils terminate, suggested that the αv subunits are functionally segregated to different types of myofibrillar anchors. In this report, we show that the αv integrins associate with myofibrils at the costamere, that the α3 integrin associates with myofibrils early and transiently during their formation, and that adhesion-perturbing anti-

Fig. 5. An adhesion-perturbing antibody directed against the integrin β1 subunit inhibits sarcomeric assembly of muscle α-actinin. Myotubes were cultured for 7 days and then incubated with the adhesion-perturbing mAb W1B10 at a concentration of 50 mg/ml (A) or a non-specific control mAb P3 at a concentration of 50 mg/ml (B). The cultures were fixed and stained on day 8. Both cultures were then incubated with a mAb directed against muscle α-actinin, mAb 9A2B8, that is directly conjugated with FITC. Few striations are apparent in the W1B10-perturbed cultures (A), in contrast to their presence in the control cultures (B). Bar, 30 µm.
cytoskeletal linkage components at the various junctions is not yet known. Recently, αv-associated molecules have been isolated in our laboratory. Antibodies against these molecules immunostain at the Z-line, supporting the existence of junction-specific linkage mechanisms (work in progress). Two other integrins found on muscle, α4 and α6, do not show any apparent junctional specificity. α4 does not localize in any myofibrillar linkage structure, is transiently expressed on primary myotubes, and is implicated in the formation of secondary myotubes (Rosen et al., 1992). The α6 integrin appears diffusely along myotubes, suggesting possible association with the laminin-rich basal lamina that surrounds skeletal muscle.

Whether integrin is necessary or sufficient for the establishment of diverse muscle junctions is not yet known. The antibody-perturbation experiments demonstrate a clear role for β1 integrins in the assembly of α-actinin into myofibrils; however, even in the continued presence of perturbing antibody, α-actinin-containing sarcomeres do eventually form. This points to the presence of other mechanisms compensating for integrin. The kinetics of α4 incorporation into the costamere, which is subsequent to that of titin and α-actinin but prior to that of the desmin intermediate filaments, suggests that the initial assembly of the sarcomere is independent of the periodic linkage of α6 integrins to the sarcolemma. However, the next stage of sarcomere assembly, in which desmin participates, may involve αv integrins. The results from studies on Drosophila do suggest that the lateral transmission of force in mature muscle requires the lateral linkage provided by integrins. In vertebrates, dystrophin and its associated linkage complex have recently been shown to bind both F-actin and laminin (Ervasti and Campbell, 1993). These dystrophin-associated proteins, like α6, are costameric and involved in linkage with the extracellular matrix. Muscle cells lacking dystrophin protein have numerous pathologies associated with them. It is possible that α6 integrin plays a similar role in maintenance of the mature muscle architecture.

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