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Selective modulation of the interaction of $\alpha_7\beta_1$ integrin with fibronectin and laminin by L-14 lectin during skeletal muscle differentiation

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SUMMARY

The $\alpha_7\beta_1$ integrin was originally identified and isolated from differentiating skeletal muscle and shown to be a laminin-binding protein (Song et al. (1992) *J. Cell Biol.* 117, 643-657). Expression of the α_7 gene and protein are developmentally regulated during skeletal muscle differentiation and have been used to identify cells at distinct stages of the myogenic lineage (George-Weinstein et al. (1993) *Dev. Biol.* 156, 209-229). The lactoside-binding protein L-14 exists as a dimer and has been localized on a variety of cells, in association with extracellular matrix. During myogenesis in vitro, L-14 is synthesized within replicating myoblasts but it is not secreted until these cells commence terminal differentiation and fusion into multinucleate fibers (Cooper and Barondes, *J. Cell Biol.* (1990) 110, 1681-1691). Addition of purified L-14 to myogenic cells plated on laminin inhibits myoblast spreading and fusion, suggesting that the L-14 lectin regulates muscle cell interactions with the extracellular matrix that are germane to myogenic development (Cooper et al. (1991) *J. Cell Biol.* 115, 1437-1448). We demonstrate here, using affinity chromatography and immunoblots, that $\alpha_7\beta_1$ also binds to fibronectin and to the L-14 lectin. L-14 binds to both laminin and to the $\alpha_7\beta_1$ integrin, and it can effectively inhibit the association of laminin and this integrin. Modulation of $\alpha_7\beta_1$ interaction with its ligands by L-14 is selective: L-14 does not bind to fibronectin, nor does it interfere with the binding of fibronectin to $\alpha_7\beta_1$. These results are discussed in the context of the potential roles of $\alpha_7\beta_1$ in its interaction with laminin and fibronectin during myogenesis.

Key words: $\alpha_7\beta_1$ integrin, L-14 lectin, fibronectin, laminin, skeletal muscle, myogenesis

INTRODUCTION

During the development of skeletal muscle, myogenic precursor cells migrate from somite myotomes into peripheral mesenchyme, proliferate, differentiate and fuse to form post-mitotic, multinuclear and contractile myofibers. The formation of the limb musculature takes place in two phases as a consequence of the development of two lineages of skeletal myoblasts (Miller and Stockdale, 1986; George-Weinstein et al., 1993). The formation of primary fibers takes place first and secondary fibers subsequently develop around these (Kelly and Zachs, 1969; Harris, 1981; Ontel and Kozeka, 1984; Ross et al., 1987). These developmental processes are believed to be regulated by the interactions of a family of helix-loop-helix proteins with regulatory sites in the genome (for reviews see Olson, 1990; Weintraub et al., 1991; Funk et al., 1991), by growth factors (for review see, Funk et al., 1991), and by extracellular matrix proteins and their receptors (Foster et al., 1987; George-Weinstein et al., 1993).

Laminin is a major component of the extracellular matrix and it is believed to play a prominent role in promoting myoblast adhesion, migration, proliferation and differentiation (Foster et al., 1987; Ocalan et al., 1988; Goodman et al., 1989a,b; von der Mark and Ocalan, 1989). Proliferating myoblasts initially increase their adhesion to laminin, but prior to fusion they lose adhesion and responsiveness to laminin (Goodman et al., 1989a). Upon continued development, mature myofibers become ensheathed in a continuous laminin-rich basement membrane (Mayne and Sanderson, 1985; Sanes et al., 1986; Kuhl et al., 1982, 1984).

The $\alpha_7\beta_1$ integrin is the predominant, if not sole, laminin-binding integrin on differentiating skeletal muscle cells (Song et al., 1992; von der Mark et al., 1991). During the development of primary skeletal fibers, the $\alpha_7\beta_1$ integrin first appears subsequent to differentiation, when these fibers envelop themselves in a matrix rich in laminin. In contrast, during the formation of secondary fibers (which takes place within this same basal lamina), the $\alpha_7\beta_1$ integrin is expressed prior to differentiation on replicating myoblasts (Kaufman and Foster, 1988). Upon differentiation there is an increase in the expresson of the α_7 chain. There also are alternate forms of α_7 mRNA and these encode different cytoplasmic domains (Song et al., 1993; Collo et al., 1993). Expression of the alternate forms of α_7 is developmentally regulated and these likely underlie the diversity in function of the $\alpha_7\beta_1$ integrin at different stages of muscle development.

L-14 is a homodimeric lactose-binding lectin with 14,000 Da subunits (Harrison, 1991). It is expressed in a wide range of vertebrate tissues, but is particularly abundant in developing cardiac, smooth and skeletal muscle (Catt et al., 1987; Levi and Teichberg, 1989; Poirer et al., 1992). In skeletal muscle, L-14 is secreted during differentiation and acccumulates with laminin in the basement membrane surrounding each myofiber (Cooper and Barondes, 1990). Deposition of L-14 in basement membrane is presumed to be due to its interaction with laminin. L-14 has particular affinity for polylactosamine oligosaccharides (Zhou and Cummings, 1990; Cooper et al., 1991; Castronovo et al., 1992), and laminin is one of the few glycoproteins with this type of glycosylation (Arumugham et al., 1986; Fujiwara et al., 1988; Knibbs et al., 1989). Laminin is the major L-14 binding glycoprotein in muscle cell extracts (Cooper et al., 1991).

Previous studies indicate that the interaction of L-14 with laminin may be physiologically significant to the development of skeletal muscle. Purified L-14 was found to inhibit adhesion and spreading of C2C12 myoblasts on laminin. This was shown to depend on L-14 binding to the laminin and not to the cell surface, as predigestion of the laminin with glycosidase blocked the L-14 inhibition of cell adhesion. These results led to the proposal that L-14 binds to laminin oligosaccharides and thereby sterically inhibits laminin recognition by a cell surface receptor (Cooper et al., 1991).

The coincidence of L-14 secretion with the onset of myoblast differentiation and fusion, with the transition in myoblast adhesion and mobility on laminin, and with laminin deposition in muscle basement membrane, suggested that L-14 might be involved in regulating these processes. As $\alpha_7\beta_1$ is the major laminin binding integrin on myoblasts, we have investigated the effects of the L-14 lectin on $\alpha_7\beta_1$ binding to laminin. We demonstrate here that the L-14 lectin can not only bind to laminin, but can also bind to its integrin receptor. As a consequence, L-14 inhibits the association of $\alpha_7\beta_1$ and laminin, and it thereby has the potential to prevent and dissociate, in a dose-dependent fashion, the interaction of cells with laminin. Secondly, we note that the $\alpha_7\beta_1$ integrin can also bind to fibronectin and this interaction is not affected by L-14. Therefore, the onset of L-14 secretion with differentiation may shift myoblast interactions away from laminin to fibronectin, and thereby have important consequences on myogenic development.

MATERIALS AND METHODS

Cell extracts

L8E63 rat myogenic cells were grown on 100 mm tissue culture dishes, in Dulbecco's modified Eagle's medium (DME) containing 10% horse serum, penicillin, streptomycin and kanamycin, as described (Kaufman and Parks, 1977). Under the conditions used these myoblasts commence differentiation between days 3 and 4. Extracts were prepared from cells grown for 7 days, at which time approximately 86% of the nuclei were in fibers. The cells were washed three times with cold Dulbecco's phosphate-buffered saline (DPBS), collected by scrapping with a rubber policeman, pelleted by centrifugation, and extracted twice, for 30 minutes, at 4°C, in 200 mM *n*-octyl-β-D-glucopyranoside, 1 mM phenylmethylsulfonyl fluoride (PMSF), 100 mM Tris-HCl, pH 7.4 (Kramer et al., 1991). A 50 μl sample of extraction buffer was used per 100 mm plate of cells, the extracts were pooled and adjusted to 2 mM $MnSO_4$ and immediately used for chromatography.

Laminin and fibronectin affinity chromatography

Purified Englebreth-Holm-Swarm mouse tumor laminin and human serum fibronectin were obtained from Dr A. F. Horwitz (University of Illinois, Urbana, IL) and from Becton Dickinson (Bedford, MA). Fibronectin was dialysed against 0.1 M MES, pH 4.3, and laminin was dialysed against 0.1 M HEPES, pH 7.0. The proteins were then coupled to Bio-Rad Affigel 10 beads at 1 mg protein/ml beads by incubation with rotation, for 4 hours at 4°C. Unreacted sites on the beads were blocked by addition of 1 M ethanolamine-HCl, pH 8.0, for 1 hour at 4°C. Control columns were not reacted with protein but were blocked with ethanolamine.

The fibronectin, laminin and control columns were equilibrated with buffer A (50 mM *n*-octyl-β-D-glucopyranoside, 2 mM $MnSO_4$, 50 mM Tris-HCl, pH 7.4) (Kramer et al., 1991), and cell extracts were loaded on the columns and circulated at 1 ml/min, in a closed system, for 16 hours at 4°C. Unbound material was collected, the columns were washed with 12 volumes of buffer A and with 4 volumes of buffer A + 0.1 M NaCl. The columns were then eluted with four volumes of 10 mM EDTA in buffer A (without $MnSO_4$). The eluates were immediately adjusted to 20 mM $MgCl_2$, dialysed overnight at 4°C against buffer B (0.1% SDS, 10 mM Tris-HCl, 1 mM EDTA, pH 7.2), passed through Centricon 30 filters (Amicon, Danvers, MA), concentrated to dryness using a Speed Vac, and stored at −76°C.

To demonstrate the specificity of integrin binding to fibronectin, the columns were first eluted with buffer A containing either 1 mg/ml RGDS or 1 mg/ml GRGESP, and finally with 10 mM EDTA in buffer A without $MnSO_4$. Peptides from Immuno-Dynamics (La Jolla, CA) and Peninsula Labs (Belmont, CA) gave the same results. To test the effect of L-14 on integrin binding to laminin or fibronectin, the columns were equilibrated either with buffer A, buffer A containing 100 μg/ml L-14 lectin, or buffer A containing 100 μg/ml L-14 and 20 mM D-galactopyranosyl-β(1→1)-thiogalactopyranoside (thiodigalactoside, TDG), and L-14 and TDG were added to the extracts used on the respective columns.

L-14 affinity chromatography

Recombinant L-14 was synthesized in *Escherichia coli* using a pET expression vector, purified by affinity chromatography, alkylated for protection against oxidation, and used directly, or coupled to cyanogen bromide-activated Sepharose, as described by Cooper et al. (1991). A 1.2 mg sample of L-14 was reacted per ml of activated Sepharose. The columns were equilibrated with buffer C (0.15 M NaCl, 3.4 mM *n*-octyl-β-D-glucopyranoside, 50 mM Tris-HCl, pH 7.2), loaded with cell extract and incubated overnight in the continuous flow system described above. In some cases, 10 mM EDTA was added to the extracts to dissociate any laminin-$\alpha_7\beta_1$ complexes. The columns were washed with several volumes of buffer C followed by buffer B containing 0.1 M cellobiose and then eluted with buffer C containing 0.1 M lactose. The fractions were collected and prepared for electrophoresis as above.

Immunoblots

Column eluates and cell extracts were electrophoresed in 8% (w/v), 1 mm polyacrylamide gels at 20 mA for 50 minutes. The gels were equilibrated in Tris-glycine buffer containing 20% methanol and transferred onto nitrocellulose (Song et al., 1992). The blots were rinsed and blocked at room temperature for 2 hours in TSTB (5 mM Tris, 75 mM NaCl, and 0.5% Tween-20, pH 7.5) 2% gelatin, then reacted with the appropriate primary and secondary alkaline phosphatase-conjugated goat anti-rabbit or goat anti-mouse immunoglobulins (Jackson Immunoresearch), and developed with nitroblue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate as reported (Song et at., 1992). Prestained molecular mass markers were included in each gel. The primary antibodies used were: 05-α_7, a monoclonal

antibody reactive with the extracellular domain of the α_7 chain (Song et al., 1992); 014, a monoclonal antibody reactive with β_1 chains (Song et al., 1992); and anti-β_1, a rabbit antiserum reactive with the cytoplasmic domain of the β_1 chain (provided by A. F. Horwitz, University of Illinois, Urbana, IL).

RESULTS

$\alpha_7\beta_1$ Integrin binds to both laminin and fibronectin

L8E63 myotube extracts in octylglucoside and 2 mM $MnSO_4$ were passed through EHS laminin and human plasma fibronectin affinity columns as indicated in Materials and Methods. Bound integrin was eluted from both columns by cation chelation with EDTA, and from the fibronectin columns by competition with the fibronectin peptide RGDS. Protein eluted from the laminin column and in the original myotube lysate immunoblotted identically with monoclonal 05-α_7 and 014-1 anti-β_1 antibodies, and with a rabbit antiserum raised against the cytoplasmic domain of integrin β_1 chain (Fig. 1). In this experiment, the fibronectin columns were first eluted with buffer containing either 1 mg/ml RGDS or 1 mg/ml GRGESP, and subsequently with 10 mM EDTA. The results demonstrate that $\alpha_7\beta_1$ can bind fibronectin in a cation-dependent fashion and that the peptide RGDS, but not GRGESP, can elute the bound $\alpha_7\beta_1$ integrin from fibronectin (Fig. 2A). In an analogous experiment, RGDS or GRGESP peptides were added to the extracts to a final concentration of 1 mg/ml. As shown in Fig. 2B, RGDS, but not GRGESP, also inhibited $\alpha_7\beta_1$ binding to fibronectin. At the same concentration, inhibition of binding of the $\alpha_7\beta_1$ integrin to fibronectin by RGDS was more complete than dissociation of bound integrin. This may be due to additional stability between fibronectin and $\alpha_7\beta_1$ upon binding, or to differences in on- and off-rates.

L-14 lectin inhibits binding of $\alpha_7\beta_1$ integrin to laminin but not to fibronectin

Since the $\alpha_7\beta_1$ integrin can bind both laminin and fibronectin, we wanted to test the influence of L-14 on these ligand-receptor interactions. Myotube extracts were mixed either with purified L-14 lectin, or with L-14 and 20 mM thiodigalactoside, the most potent simple saccharide inhibitor of this lectin. The mixtures were then passed over laminin or fibronectin columns and eluted with 10 mM EDTA. The eluted fractions were electrophoresed and immunoblotted with 05-α_7 antibody. As seen in Fig. 3, L-14 lectin effectively and selectively inhibited the association of $\alpha_7\beta_1$ integrin with laminin, but not with fibronectin. These results are consistent with our earlier report that L-14 inhibits myoblast attachment to laminin, but has little effect on attachment to fibronectin (Cooper et al., 1991). Competitive inhibition with thiodigalactoside blocked L-14 inhibition of $\alpha_7\beta_1$ binding to laminin. Immunoblot analysis of the proteins that did not bind to the laminin columns confirms these results (Fig. 3A).

L-14 can dissociate $\alpha_7\beta_1$ integrin bound to laminin

As the L-14 lectin can alter the association of myogenic cells with their extracellular matrix (Cooper et al., 1991), it was of interest to determine whether L-14 could also dissociate $\alpha_7\beta_1$ integrin bound to laminin. Extracts prepared from myotubes were passed over a laminin affinity column. The column was then sequentially eluted, first with one column volume of purified L-14 (0.2 mg/ml), then with 0.5 mg/ml L-14, and finally with 10 mM EDTA. The eluted fractions were immunoblotted with 05-α_7 antibody. Most of the $\alpha_7\beta_1$ integrin was eluted with 0.5 mg/ml L-14 (Fig. 4), indicating that the lectin can modulate the interaction of the $\alpha_7\beta_1$ integrin with laminin.

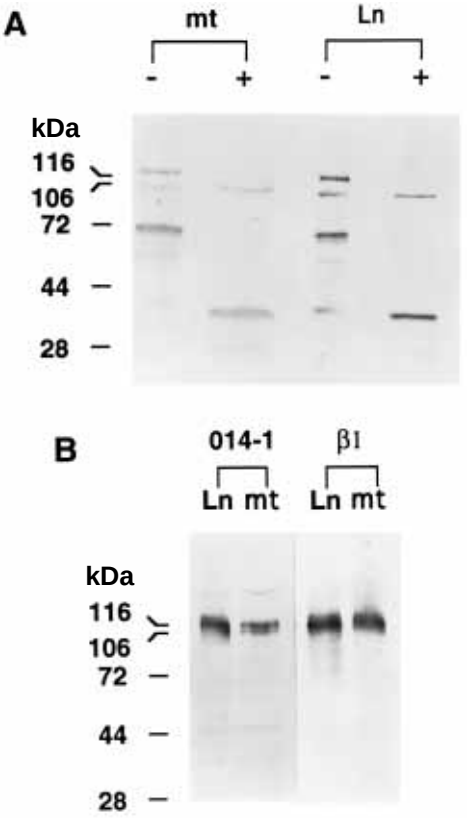

Fig. 1. $\alpha_7\beta_1$ Integrin is a laminin-binding protein. Myotube (mt) extract and integrin purified using a laminin affinity column (Ln) were electrophoresed under nonreducing (–) and reducing conditions (+) in 8% polyacrylamide-SDS gels. (A) Immunoblot using 05-α_7 monoclonal antibody. Protein eluted from the laminin column (Ln) and in the original myotube lysate (mt) immunoblot identically. (B) Immunoblot using mouse monoclonal 014-1 anti-β_1 antibody and rabbit antiserum raised against the cytoplasmic domain of integrin β_1 chain. The intact 121,000 Da α_7 chain and 70,000 Da fragment detected with 05-α_7 antibody predominate in unreduced samples. The relative amounts of each varied between experiments and is believed to reflect in situ proteolysis of the α_7 chain at one or both of its protease cleavage sites (Song et al., 1992). Two-dimensional gel electrophoresis demonstrates that the 100,000 Da and 35,000 Da fragments are detected upon reduction and dissociation of a disulfide-linked fragment from the intact α_7 chain and 70,000 Da fragment, respectively (Song et al., 1992). The apparent molecular masses of prestained β-galactosidase (116,000 Da), phosphorylase *b* (106,000 Da), bovine serum albumin (72,000 Da), ovalbumin (44,000 Da), and carbonic anhydrase (28,000 Da) are indicated.

$\alpha_7\beta_1$ Integrin binds to L-14 lectin

Because integrin oligosaccharides have been implicated in integrin function (Chammas et al., 1991, 1993), we tested whether the L-14 lectin recognizes oligosaccharides on the

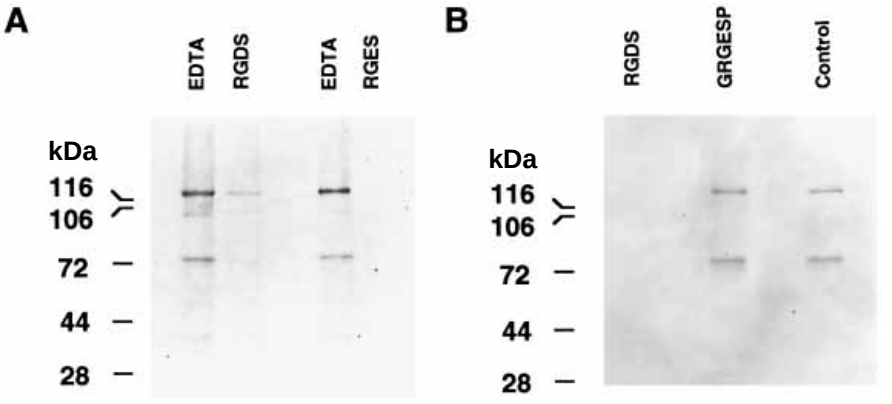

Fig. 2. $\alpha_7\beta_1$ Integrin binds to fibronectin in an RGD-dependent fashion. L8E63 myotube cultures were extracted with octylglucoside and passed through 1-ml columns of Affigel-10 (Bio-Rad) conjugated with 1 mg human plasma fibronectin. (A) The columns were washed as indicated in Materials and Methods, then first eluted with buffer containing either 1 mg/ml RGDS or 1 mg/ml GRGESP, and subsequently with buffer containing 10 mM EDTA. The eluates were concentrated, electrophoresed and analyzed by immunoblotting with 05-α_7 monoclonal antibody. $\alpha_7\beta_1$ binds to fibronectin in a cation-dependent fashion and RGDS, but not GRGESP, can elute the bound integrin from fibronectin. (B) Addition of 1 mg/ml RGDS, but not GRGESP, to extracts prior to chromatography inhibits binding of $\alpha_7\beta_1$ to fibronectin, as detected by EDTA elution, electrophoresis and immunoblotting with 05-α_7 antibody. Control, no peptide added to extract.

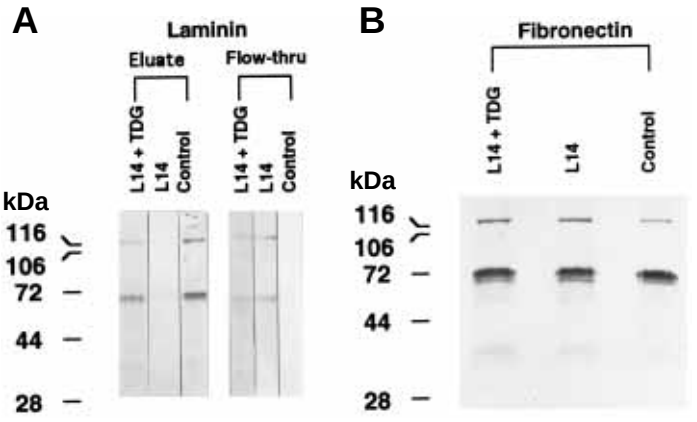

Fig. 3. L-14 lectin inhibits binding of $\alpha_7\beta_1$ integrin to laminin but not to fibronectin. Myotube extracts (control) were mixed with purified L-14 lectin, or with L-14 and 20 mM thiodigalactoside (TDG). The mixtures were then passed over individual (A) laminin, or (B) fibronectin columns, and eluted with 10 mM EDTA. The eluted fractions were immunoblotted with 05-α_7 antibody. L-14 lectin effectively inhibited the association of $\alpha_7\beta_1$ integrin with laminin, but not with fibronectin. In the presence of L-14, the $\alpha_7\beta_1$, which did not bind to laminin, was detected in the column flow-through. TDG largely, but not completely, prevented the inhibitory effect of L-14 on $\alpha_7\beta_1$ binding to laminin.

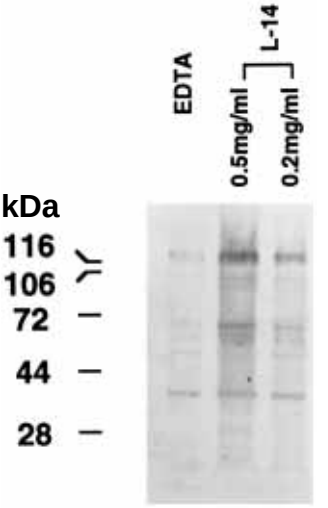

Fig. 4. L-14 can dissociate $\alpha_7\beta_1$ integrin bound to laminin. Myotube extracts were passed over a laminin affinity column. The column was then sequentially eluted, first with one column volume of purified L-14 (0.2 mg/ml), then with 0.5 mg/ml L-14, and finally with 10 mM EDTA. The eluted fractions were immunoblotted with 05-α_7 antibody. Most of the α_7 integrin was eluted by 0.5 mg/ml L-14.

$\alpha_7\beta_1$ integrin. Extracts prepared from L8E63 myotubes were passed over an L-14 Sepharose column, washed with 0.1 M cellobiose to elute non-specifically bound glycoprotein, and finally with 0.1 M lactose to elute glycoprotein specifically bound by the L-14 lectin. The fractions eluted with cellobiose and with lactose were then immunoblotted using 05-α_7 antibody. The results demonstrate that the $\alpha_7\beta_1$ integrin binds to L-14 lectin (Fig. 5A).

In the above experiment it is possible that a complex of $\alpha_7\beta_1$ and laminin bound to the L-14 column as a consequence of the interaction of L-14 and laminin. To demonstrate that the $\alpha_7\beta_1$ integrin can directly bind to L-14, 10 mM EDTA was added to the extract to dissociate any complex of $\alpha_7\beta_1$ and laminin. The extract was then passed over an L-14 column and eluted with lactose. Immunoblot analysis demonstrates that the L-14 lectin can directly bind to the $\alpha_7\beta_1$ integrin and in a cation-independent fashion (Fig. 5B).

DISCUSSION

The interactions of cells with their extracellular environment, in particular the proteins that comprise the extracellular matrix, has profound effects on morphogenesis and development, and stabilization and function of the differentiated state. The proliferation (Foster et al., 1987; Ocalan et al., 1988), migration (Jaffredo et al., 1988) and differentiation of skeletal myogenic cells (Foster et al., 1987; Menko and Boettiger, 1987) are markedly influenced by receptor recognition of extracellular matrix proteins. Integrin receptors also appear to be involved in myoblast fusion (Rosen et al., 1992), the organization of myofibrils and dystrophin (Lakonishok et al., 1992; Enomoto et al., 1992), the transmembrane association of the cytoskeleton and basement membrane (Lowrey and Kaufman, 1988; Song et al., 1993), and the organization of myotendinous and neuromuscular junctions. Laminin and fibronectin and their

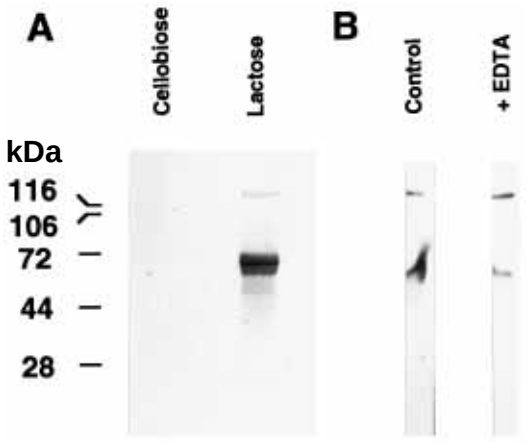

Fig. 5. $\alpha_7\beta_1$ Integrin binds to L-14 lectin. (A) Myotube extracts were passed over an L-14 Sepharose column, washed with buffer, then with 0.1 M cellobiose to elute non-specifically bound glycoprotein, and finally with 0.1 M lactose to elute glycoconjugates specifically bound by L-14. (B) EDTA was added to the extract to dissociate any complexes of integrin and laminin that may have been present. Control extract (no EDTA) and extract containing 10 mM EDTA were then passed through the L-14 Sepharose columns and eluted with 0.1 M lactose. Immunoblot analysis of eluted fractions with 05-α_7 antibody demonstrates that $\alpha_7\beta_1$ integrin (A) specifically and (B) directly binds to L-14 lectin in a cation-independent fashion. No α_7 bound to an unconjugated Sepharose column.

receptors are believed to be critical to many of these interactions.

The $\alpha_7\beta_1$ complex is the major functional laminin binding integrin expressed by differentiating skeletal (Song et al., 1992; von der Mark et al., 1991). It is expressed at selective stages during development of the primary and secondary myogenic lineages and is believed to function in the localization and expansion of the population of presursor cells that give rise to secondary fibers (Kaufman et al., 1991; George-Weinstein et al., 1993). $\alpha_7\beta_1$ is also localized around adult fibers (Song et al., 1992) and at the myotendinous junction (George-Weinstein and Kaufman, unpublished results; Bao et al., 1993). This diversity in the temporal expression and localization of the $\alpha_7\beta_1$ integrin suggests that it serves multiple and distinct functions at different stages of development. At least two mechanisms appear to underlie the diversity in $\alpha_7\beta_1$ function. First, the α_7 chain has at least three different cytoplasmic domains that arise by alternate RNA splicing and the expression of these alternate forms is developmentally regulated (Song et al., 1993; Collo et al., 1993). Second, as reported here, diversity in function is likely to arise from $\alpha_7\beta_1$ integrin interactions with multiple ligands, including laminin, fibronectin and L-14 lectin.

Fibronectin also seems to play a key role in myogenesis. Myoblasts express multiple fibronectin receptors including $\alpha_5\beta_1$, $\alpha_4\beta_1$ and $\alpha_3\beta_1$, and as shown in these experiments $\alpha_7\beta_1$ also can bind to fibronectin. The occupancy of fibronectin receptors appears to modulate skeletal muscle development in both positive and negative fashions. For example, soluble fibronectin (Podleski et al., 1979), and antibodies against β_1 integrin chain (Menko and Boettiger, 1987), against fibronectin, and against the α_5 integrin chain (Menko et al., unpublished data), inhibit myogenic differentiation.

Immunolocalization of the different alpha chains suggests that they have distinct functions. The $\alpha_4\beta_1$ complex appears to function in the fusion of secondary myoblasts rather than as a fibronectin receptor (Rosen et al., 1992), and any role for the $\alpha_3\beta_1$ integrin in myogenesis remains to be determined (von der Mark et al., 1991). It is of interest to note that the cytoplasmic domain of the α_7B isoform undergoes a change in conformation upon crosslinking the α_7 extracellular domain with antibodies or upon $\alpha_7\beta_1$ binding laminin, and, these same conditions promote the association of the integrin with the cell cytoskeleton (Lowrey and Kaufman, 1989; Song et al., 1993). Fibronectin too will promote these changes in conformation and cytoskeletal association and therefore fibronectin may also initiate signal transduction from the extracellular environment to within the cell via the $\alpha_7\beta_1$ receptor. The α_7B cytoplasmic domain contains a rich potential for participating in signal transductions, including those mediated by receptor-like protein phosphatases and serine/threonine kinases, and interactions with the cell cytoskeleton (Song et al., 1993).

The selective inhibition by L-14 lectin of $\alpha_7\beta_1$ binding to laminin but not fibronectin may have several important consequences on myoblast development. Although L-14 is synthesized in myoblasts, it is not secreted until terminal differentiation. This suggests that the association of secreted L-14 with laminin and $\alpha_7\beta_1$ may be functionally significant at this time in myogenic development. At least four potential roles for the L-14 lectin are consistent with our present findings and earlier reports. (1) As myoblasts terminally differentiate, they cease proliferation. Since laminin promotes myoblasts proliferation (Foster et al., 1987; Ocalan et al., 1988), the asscoiation of L-14 with either laminin or the $\alpha_7\beta_1$ receptor, could effect an end to the proliferative phase of development that is coincident with terminal differentiation. (2) The mobility of myoblasts seen upon growth on laminin also ceases upon differentiation (Goodman et al., 1989a) and the L-14 lectin could also promote this transition by binding to laminin or $\alpha_7\beta_1$. In vivo, L-14 could halt myoblast migration and promote their localization at the sites of secondary fiber formation. (3) Topological considerations indicate that in order for myoblasts to fuse they must alter their attachment to extracellular matrix. Secretion of the L-14 lectin could promote this by disrupting the association of $\alpha_7\beta_1$ and laminin, thereby facilitating cell interactions and fusion. (4) Lastly, since L-14 does not inhibit association of $\alpha_7\beta_1$ with fibronectin, secreted L-14 could dissociate $\alpha_7\beta_1$ receptors associated with laminin, freeing them to bind fibronectin. This new or enhanced interaction of $\alpha_7\beta_1$ with fibronectin (or the absence of association of myoblasts with both laminin and fibronectin) could stimulate further myogenic differentiation. This suggestion is consistent with previous results that indicate that the interaction of fibronectin with myoblasts may be essential for further differention (Menko and Boettiger, 1987; Boettiger et al., 1989; Menko et al., unpublished data) and infers a role for the $\alpha_7\beta_1$ integrin in this process. At this same time in myogenesis there is a switch in expression in the α_7 cytoplasmic domains (Song et al., 1993; Collo et al., 1993) and a commensurate change in the potential for signal transduction into the cell.

During later stages of myogenesis, laminin is deposited along muscle fibers and forms a major component of the

basement membrane. Immunofluorescence demonstrates that $\alpha_7\beta_1$ is expressed on adult muscle fibers (Song et al., 1992) and L-14 is part of the basement membrane (Cooper and Barondes, 1990). Since L-14 is a homodimer, in addition to its capacity to dissociate or inhibit association between $\alpha_7\beta_1$ and laminin, it may also form bridges between laminin molecules, between laminin and $\alpha_7\beta_1$, or between adjacent $\alpha_7\beta_1$ molecules, and thereby stabilize the association of muscle fibers with the extracellular matrix.

In summary, it is clear that the development of skeletal muscle involves complex changes in the interactions of multiple integrins and their respective ligands. We propose that the developmentally regulated interaction of L-14 with laminin and $\alpha_7\beta_1$ regulates myogenesis by inhibiting the association of laminin with $\alpha_7\beta_1$ and perhaps also by stabilizing cell-matrix intermolecular interactions. The concentration of L-14 relative to $\alpha_7\beta_1$ and laminin, and the state of glycosylation of $\alpha_7\beta_1$, laminin and fibronectin, would determine which effect it promotes.

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