Abstract. PDZ motifs are protein–protein interaction domains that often bind to COOH-terminal peptide sequences. The two PDZ proteins characterized in skeletal muscle, syntrophin and neuronal nitric oxide synthase, occur in the dystrophin complex, suggesting a role for PDZ proteins in muscular dystrophy. Here, we identify actinin-associated LIM protein (ALP), a novel protein in skeletal muscle that contains an NH₂-terminal PDZ domain and a COOH-terminal LIM motif. ALP is expressed at high levels only in differentiated skeletal muscle, while an alternatively spliced form occ-
urs at low levels in the heart. ALP is not a component of the dystrophin complex, but occurs in association with α-actinin-2 at the Z lines of myofibers. Biochemical and yeast two-hybrid analyses demonstrate that the PDZ domain of ALP binds to the spectrin-like motifs of α-actinin-2, defining a new mode for PDZ domain interactions. Fine genetic mapping studies demonstrate that ALP occurs on chromosome 4q35, near the hetero-
chromatic locus that is mutated in fascioscapulohumeral muscular dystrophy.

The cytoskeleton is a complex protein network that provides cellular structure. By partitioning the cell, the cytoskeleton can also provide microdomains that allow specific responses to localized stimuli. The assembly and maintenance of the cytoskeleton is mediated, in large part, by high affinity interactions between modular consensus protein-binding motifs. These sites for protein–protein interaction are often multifunctional, and the specific binding partners are determined by the variations in amino acid sequences between the individual domains. A recently identified motif, the PDZ domain, is an 80–120-amino acid domain that was first identified in the postsynaptic protein, PSD-95, which contains three PDZ domains in tandem (Cho et al., 1992). Sequence analysis has subsequently demonstrated that PDZ domains are common protein motifs that occur in a variety of dissimilar proteins that interact with the cytoskeleton (Ponting and Phillips, 1995). Individual PDZ domains occur in neuronal nitric oxide synthase (nNOS), 1 syntrophins, p55, dishev-
elled and CASK, while multiple PDZ domains occur in PSD-95, dlg, and zona occludens (ZO)-1 and -2 proteins; and PTP-BAS. Recent work indicates that PDZ domains are multifunctional protein–protein interaction motifs (Brenman and Bredt, 1997; Kornau et al., 1997; Sheng, 1996). One mode for interaction of PDZ domains involves association with the COOH terminus of target proteins. Thus, the COOH terminus of Fas binds to the third PDZ domain of PTP-BAS, and this interaction participates in Fas-mediated apoptosis of T cells (Sato et al., 1995). Similarly, the first and second PDZ domains of PSD-95 bind to the COOH termini of certain ion channels in the brain, and they anchor these channels to synaptic sites at the plasma membrane (Kim et al., 1995; Kornau et al., 1995). PDZ–PDZ interactions have also been identified. The PDZ domain of nNOS binds to the second PDZ domain of PSD-95 (Brenman et al., 1996). These multifunctional interactions of PDZ domains assemble a complex of nNOS / PSD-95/ the N-methyl-D-aspartate receptor calcium channel at the postsynaptic density.

Functional roles for PDZ domains have been demonstrated in diverse tissues. Mutations in the PDZ domain of Drosophila inactivation no afterpotential D (INAD) alter transduction of visual signals (Shieh and Zhu, 1996), while mutations in the Caenorhabditis elegans PDZ proteins Lin-2 and Lin-7 result in abnormal vulval development (Hoskins et al., 1996). In all these cases, the PDZ domains are implicated in targeting intracellular proteins to appropriate multiprotein complexes at the plasma membrane.

1. Abbreviations used in this paper: ALP, actinin-associated LIM protein; EST, expressed sequence tag; FISH, fluorescence in situ hybridization; FSHD, fascioscapulohumeral muscular dystrophy; GST, glutathione S-transferase; INAD, inactivation no afterpotential D; nNOS, neuronal nitric oxide synthase; ORF, open reading frame; RT-PCR, reverse transcription; ZO, zona occludens.
To understand and further define the role of PDZ domains in cytoskeletal assembly, we have focused on skeletal muscle as a model system. The regular and defined structure of skeletal muscle makes it an ideal tissue for study. Previous studies have demonstrated that the two known PDZ domain proteins in skeletal muscle, the family of syntrophins and nNOS, are both components of the dystrophin complex (Adams et al., 1993; Brennan et al., 1995). nNOS isoforms lacking the PDZ domain do not interact with the dystrophin complex and occur in the skeletal muscle cytosol. The PDZ domains of nNOS and syntrophin directly interact with each other, and this linkage anchors nNOS to the dystrophin complex (Brennan et al., 1996). The absence of dystrophin in Duchenne muscular dystrophy results in a loss of syntrophins and nNOS from the sarcolemma, and these abnormalities may contribute to the disease process.

We hypothesized that other PDZ proteins in muscle may also occur in the cytoskeleton. Characterization of these proteins will help better understand the function of PDZ domains and may identify candidate genes for inherited muscular dystrophies. Here, we report the cloning of a novel cDNA encoding a protein of 39 kD that consists of an NH2-terminal PDZ domain and a COOH-terminal LIM domain. The protein is expressed at high levels only in skeletal muscle, where it occurs at the Z lines in association with α-actinin-2. We therefore name this protein alpha-actinin-associated LIM protein (ALP). Biochemical and two-hybrid analyses indicate that the PDZ domain of ALP binds to the spectrin-like repeats of α-actinin-2, establishing a novel mode of interaction for PDZ domains. Chromosomal mapping indicates that the ALP gene occurs at 4q35 within 7–10 megabase (Mb) of the heterochromatic region that is deleted in fascioscapulohumeral muscular dystrophy (FSHD; Altherr et al., 1995).

Materials and Methods

**mRNA Isolation and cDNA Analysis**

mRNA was isolated using the guanidine isothiocyanate/CSCl method, and mRNA was selected with oligo dT sepharose. For degenerate reverse transcriptase-PCR (RT-PCR), rat skeletal muscle mRNA was reverse transcribed with RTⅠ polymerase using random hexamer primers. PCR, using a 54°C annealing temperature, was then performed using the following primer pair: GGGGGATCCGGXGGXCTXGGXHT and GCGGAA-TTCCCCARXATRTCXCC. Amplified products were resolved on agarose gels. Bands of 120–160 bp were isolated and subcloned into pBluescript IKS. PCR and confirmed by sequencing.

**Myogenic Cell Culture**

C2 myogenic cell line was grown on gelatin-coated dishes in Ham’s F-10 nutrient mixture plus 0.8 mM extra CaCl₂, supplemented with 15% horse serum (Life Technologies, Inc., Bethesda, MD) and 2.5 ng/ml basic fibroblast growth factor (Promega, Madison, WI). Myoblasts were fused in media containing DME (GIBCO BRL, Gaithersburg, MD) supplemented with 2% horse serum. Protein was extracted from cultured cells in buffer containing 25 mM Tris-HCl, pH 7.4, containing 1% Triton X-100, 1 mM EDTA, and 100 µM PMSF. RNA was purified from cultured cells as described above.

**GST Fusion Protein Affinity Chromatography and Immunoprecipitation**

Skeletal muscle tissue was homogenized in 10 vol of cold water containing 1 mM PMSF and centrifuged at 15,000 g for 10 min. To extract cytoskeletal proteins, the pellet was treated with 10 vol of buffer containing 2 mM Tris-HCl, pH 9, and 1 mM EGTA at 37°C for 30 min with gentle agitation. After centrifugation at 15,000 g for 30 min, the supernatant was titrated to pH 7.5. For “pull-down” assays, the solubilized tissue samples were incubated with control or GST fusion proteins linked to glutathione Sepharose 4B, washed three times with buffer containing 0.5% Triton X-100 and 250 mM NaCl, and proteins were eluted with SDS loading buffer. The GST–nNOS fusion protein was purified as described previously (Brennan et al., 1995). For immunoprecipitation, polyclonal antibodies (1 µg) to ALP or preimmune serum were added to 0.5-ml aliquots of solubilized skeletal muscle extract, and samples were incubated on ice for 1 h. Protein A Sepharose
actin-associated LIM Protein

(50 µl) was used to precipitate antibodies. Protein A pellets were washed three times with buffer containing 100 mM NaCl and 1% Triton X-100. Immunoprecipitated proteins were denatured with loading buffer and resolved by SDS-PAGE.

**In Situ Hybridization**

In situ hybridization used 35S-labeled RNA probes exactly as described (Sassoon and Rosenthal, 1993). Sense and antisense probes to ALP (full length) were synthesized from a pBluescript vector using T3 and T7 polymerases.

**Human Chromosome Mapping**

Two P1 clones corresponding to human ALP (Genome Systems, St. Louis, MO) were used to determine the location of ALP on human chromosomes by fluorescence in situ hybridization (FISH). The hybridized signal was detected by antidigoxigenin conjugated with FITC, as described (Sakamoto et al., 1995; Stokke et al., 1995).

Fine mapping of ALP was performed by PCR amplification of human hamster somatic cell hybrids and radiation-derived hybrids containing all or portions of human chromosome 4q35. The somatic cell hybrids included HHW986, containing intact human chromosome 4 (Carlock et al., 1986), HHW986, retaining only 4q35 translocated to a derivative 5p, and HHW1372, in which only the telomeric region of 4q35 (distal to D4S187) is retained on a derivative X (Bodrug et al., 1990). The radiation hybrids were derived from HHW416 and retain varying fragments of 4q35 (Wink et al., 1993). Negative controls included a human lymphoblastoid cell line GM7057 (NIGMS) and a Chinese hamster fibroblast cell line UCW 104.

Hybrids were screened by PCR for the presence of the ALP gene. The oligonucleotides (sense: GGGAGCTGTACTGCGAAA) and (antisense: CGATTGTGGTTCCTCTGTA) amplify a 150-bp product within the 3′ untranslated region of ALP. Amplifications with a 51°C annealing temperature were done in 25 µl containing 250 ng genomic DNA, 50 µl each oligonucleotide, 1.25 mM each dNTP, 16.6 mM ammonium sulfate, 67 mM Tris-HCl, pH 8, 16.6 mM MgCl2, 10 mM β-mercaptoethanol, and 2 U Taq polymerase.

**Results**

**Molecular Cloning of ALP and Characterization of mRNA Expression**

To identify novel PDZ proteins expressed in skeletal muscle, we performed RT-PCR with degenerate primers that amplify amino acids GLGF (sense) and GDXIL (antisense; see Materials and Methods for experimental details). These sequences are conserved among many PDZ domains. A library of these cDNA fragments was constructed and processed to remove the syntrophins leading to the identification of novel cDNAs encoding PDZ sequences. Northern analysis was then used to identify gene products that were enriched in skeletal muscle. The mRNA for one of the products, SK-2 (hereafter called ALP), migrated at 1.6 kb and occurred at high levels in skeletal muscle tissue, at low levels in the heart, and was undetectable in other tissues examined (Fig. 1 A). Human ALP showed a similar tissue-specific expression pattern (Fig. 1 B). To better understand mechanisms that regulate ALP during muscle development, we evaluated expression in myogenic cultures. ALP expression was dramatically induced after myotube fusion in culture (Fig. 1 C).

In situ hybridization of embryonic day (E15) mouse embryo with antisense ALP probe demonstrated the highest levels of ALP expression in developing skeletal muscle and heart (Fig. 1 D). ALP mRNA also occurred in embryonic intestine. No specific hybridization was observed using sense RNA control (Fig. 1 E).

**Structural Features of ALP and Comparison with Its Homologues**

cDNA clones encoding the full 1.6-kb mRNA were obtained from a rat skeletal muscle cDNA library. The mRNA contains a single ORF of 1,086 bp encoding a protein of 39 kD. Sequence analysis shows that ALP has a homology at the NH2 terminus to PDZ domains. Alignment of ALP with other PDZ domains is shown (Fig. 2 A). The presence of histidine at amino acid 62 suggests that the PDZ domain may be in group I (Brenman and Brecht, 1997; Songyang et al., 1997).
The central region of ALP showed no homology to any other cloned gene while the COOH terminus encodes a LIM domain. Though ALP has not previously been reported, other proteins with a similar domain structure have been described. A database homology search with BLAST indicated that ALP shares high homology to a number of newly identified transcripts including CLP-36, RIL, and enigma (Fig. 2B). CLP-36 was identified as a cDNA whose expression in the heart is downregulated by hypoxia (Wang et al., 1995). RIL, short for reversion-induced LIM protein, is downregulated in H-ras–transformed cells (Kiess et al., 1995). Enigma was identified as an insulin receptor–interacting protein (Wu et al., 1996). These investigators, however, did not recognize the homology of the NH2-terminal regions of CLP-36, RIL, or enigma with the PDZ domain. The PDZ domain of ALP shares 55, 48, and 45% amino acid identity with PDZ domains from CLP-36, PSD95, a1-syntrophin, (a1syn), nNOS, and INAD. Histidine 62 of ALP is marked with an asterisk and leucine 78 with a pound sign.

Figure 2. Sequence analysis of ALP isoforms. (A) Amino acids 5–80 of ALP encode a consensus PDZ domain. Alignment of ALP with PDZ domains from CLP-36, PSD95, a1-syntrophin, (a1syn), nNOS, and INAD. Histidine 62 of ALP is marked with an asterisk and leucine 78 with a pound sign. (B) Predicted sequences of rat ALP (GenBank/EMBL/DDBJ accession no. AF002281) and human ALP (hALP) are aligned with two homologous proteins, CLP-36 and RIL. (C) An alternative ALP isoform is expressed in the heart. Schematic model shows the domain structure of ALP and the divergence of ALP between skeletal muscle (hALPsk; accession no. AF002280) and heart (hALP; accession no. AF002280). The alignment shows that the central region of ALP is different between skeletal muscle and heart. The accession numbers for ESTs used to construct hALPsk are F12229, R20192, AA147575, AA211287, and D56502. The accession numbers for ESTs encoding the skeletal muscle–specific splice for hALPsk are Z28845, Z19288, and Z28703.

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acid identity with the PDZ domains of CLP36, RIL, and enigma, respectively. The LIM domain of ALP shares even stronger homology (67% identity) with CLP36 and RIL. While ALP, CLP36, and RIL all only have one LIM domain, enigma has three LIM domains. The sequence homology indicates that ALP, CLP36, and RIL constitute a new family of proteins containing an NH$_2$-terminal PDZ domain and a COOH-terminal LIM domain.

Our analysis of the expressed sequence tag (EST) database showed that overlapping cDNAs corresponding to human ALP have been deposited. The human ALP is 91% identical to the rat sequence. We noted that EST clones from human heart libraries were consistently different in the central region from those in human skeletal muscle libraries (Fig. 2C). Exons encoding the central 112 amino acids of skeletal muscle ALP are likely to be spliced out in the heart and replaced by exons encoding 64 different amino acids. To confirm this differential expression, we amplified the region that was unique to heart transcripts and reprobed the Northern blot. As expected, we found heart-specific expression of this region of ALP (data not shown). We therefore define two subtypes ALP$_{SK}$ and ALP$_{H}$ for the alternative transcripts that occur in skeletal muscle and heart, respectively.

The PDZ Domain of ALP Binds $\alpha$-Actinin-2

Previous studies have shown that PDZ domains participate in protein–protein interactions. To determine potential targets for the PDZ domain of ALP, we used the yeast two-hybrid system. We screened $10^6$ clones from an adult skeletal muscle library (Clontech) and obtained 120 positive clones, 35 of which were recovered and analyzed. All positive clones encoded fragments of $\alpha$-actinin-2, a muscle-specific cytoskeletal protein that contains an NH$_2$-terminal actin-binding domain, four central spectrin-like repeats, and a COOH-terminal region homologous to calcium-binding EF hands (Beggs et al., 1992). ALP interacts only with the spectrin-like repeat region of $\alpha$-actinin-2, and all interacting clones encode spectrin repeat three (Fig. 3). However, a deletion construct (9-5N) containing repeat three did not interact with ALP, indicating that repeat three is necessary but not alone sufficient for binding.

Interaction of a PDZ domain with spectrin-like repeats is unprecedented. We therefore asked whether this interaction was specific. We found that the PDZ domains of nNOS, $\alpha$-syntrophin, and the three PDZ domains of PSD-95 (Brenman et al., 1996) did not interact with $\alpha$-actinin-2 in the yeast two-hybrid system. We previously identified a

**Figure 3.** The PDZ domain of ALP binds to the spectrin repeats of $\alpha$-actinin-2. The sequence encoding amino acids 1–128 of ALP was fused to the GAL4 DNA-binding domain. Clones 9-2, 4, 5, 6, 7, and 12, which were rescued from a yeast two-hybrid screen of a human skeletal muscle library, encode different fragments of $\alpha$-actinin-2. Clone 9-5 was truncated with restriction enzymes to yield clones 9-5X, N, and B. All ALP-interacting clones encoded at least two complete spectrin-like repeats, one of which was the third repeat. nNOS, PSD-95, and $\alpha$-syntrophin did not interact with $\alpha$-actinin-2. Mutation of ALP leucine 78 to lysine abolished interaction with $\alpha$-actinin-2.
prominent band of 39 kD in skeletal muscle and a much stronger band in tissue extracts. As expected, the antiserum recognized a physiologically important protein in localizing ALP to specific cytoskeletal structures.

"pulled-down" experiments with a column containing the PDZ domain of nNOS revealed that ALP binds to this domain. Skeletal muscle extracts show selective coimmunoprecipitation of a-actinin-2 with ALP antisera but not with preimmune serum. By contrast, two control proteins, nNOS and syntrophin, were not coimmunoprecipitated.

To determine whether the interaction with a-actinin-2 occurs in a protein complex, we performed immunoprecipitation studies. We found that the antiserum to ALP specifically coimmunoprecipitated a-actinin-2 from solubilized cytoskeletal extracts from skeletal muscle. By contrast, neither nNOS nor syntrophin, cytoskeletal components of the dystrophin complex, were coimmunoprecipitated with ALP. We next compared the cellular distribution of ALP with a-actinin-2 in skeletal muscle. Immunofluorescent staining showed that ALP colocalizes with a-actinin-2 on the Z lines (Fig. 5 C). No ALP immunoreactivity was found at the sarcolemma, nucleus, or other structures of the myofiber.

**ALP Colocalizes with a-Actinin-2 at the Z Lines in Skeletal Muscle**

To determine whether the interaction with a-actinin-2 is physiologically important in localizing ALP to specific cytoskeletal domains, we developed an affinity-purified antiserum (see Materials and Methods). We evaluated the specificity of the serum by Western blot analysis of crude tissue extracts. As expected, the antiserum recognized a prominent band of 39 kD in skeletal muscle and a much less intense band of 35 kD in the heart (Fig. 5 A). No immunoreactive bands were noted in the spleen, kidney, brain, or liver. Western blotting of myogenic cell extracts showed that ALP is absent from myoblasts but is induced within 3 d after myotube fusion (Fig. 5 B).

To determine whether ALP and a-actinin-2 occur together in a protein complex in skeletal muscle, we performed immunoprecipitation studies (Fig. 4 B). We found that the antiserum to ALP specifically coimmunoprecipitated a-actinin-2 from solubilized cytoskeletal extracts from skeletal muscle. By contrast, neither nNOS nor syntrophin, cytoskeletal components of the dystrophin complex, were coimmunoprecipitated with ALP. We next compared the cellular distribution of ALP with a-actinin-2 in skeletal muscle. Immunofluorescent staining of longitudinal sections of adult skeletal muscle showed that ALP colocalized with a-actinin-2 on the Z lines (Fig. 5 C). No ALP immunoreactivity was found at the sarcolemma, nucleus, or other structures of the myofiber.

**The ALP Gene Maps to Human Chromosome 4q35**

To determine whether ALP might map close to any known genetic mutations that are associated with muscular dystrophy, we determined the chromosomal location of human ALP. We performed FISH with two independent digoxigenin-labeled genomic ALP probes on normal human metaphase chromosomes. Hybridization with these probes resulted in specific labeling of only chromosome 4q. The location of the probes was determined by digital image microscopy after FISH and was localized by the fractional length from the p terminus (FLpter) as described previously (Sakamoto et al., 1995; Stokke et al., 1995). Both clones mapped to the most telomeric region of chromosome 4, at 4q34-pter with FLpter values of 0.962 ± 0.004 and 0.959 ± 0.005.

Interestingly, FSHD, the most common autosomal dominant muscular dystrophy, maps to the telomeric region of chromosome 4, at 4q35. Since FSHD is associated with deletions of a subtelomeric repeat sequence (van Deutekom et al., 1993), the localization of ALP relative to the 4q telomere was of interest. We therefore used somatic cell and radiation hybrid panels to map ALP within chromosomal band 4q35. PCR analysis of the somatic cell hybrids revealed that ALP maps within the proximal portion of this band (Fig. 6 B). ALP is not present in HHW1372, which contains only the telomeric 2 Mb of chromosome 4q35 proximal to D4S187. Analysis of the radiation hybrid panel DNAs, which contain independent fragments of 4q35, localized ALP to the interval between D4S171 and the Factor XI gene (Fig. 6 C). Thus, the approximate distance of ALP from the telomere is 7–10 Mb.

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**Figure 4.** Association of ALP and a-actinin-2 and specificity of the PDZ-spectrin-repeat interaction. (A) Affinity chromatography demonstrates that a-actinin-2 is selectively retained by an immobilized ALP fragment (amino acids 1–128) fused to GST, not by GST-NOS fusion protein, which selectively binds syntrophin. The load is 20% of the input used for affinity chromatography experiment. (B) Immunoprecipitation of skeletal muscle extracts shows selective coimmunoprecipitation of a-actinin-2 with ALP antiserum but not with preimmune serum. By contrast, two control proteins, nNOS and syntrophin, were not coimmunoprecipitated. Immunoprecipitated proteins were resolved on four replicate gels and probed with antisera to a-actinin, ALP, nNOS, and syntrophin. Load is 10% of the input used for the immunoprecipitation.

**Figure 6.** Human ALP maps to chromosome 4q35. (A) FISH of PI clones to human metaphase chromosomes. Hybridizing signals (arrows) were detected by FITC (green), and the chromosomes were counterstained with propidium iodide and DAPI (purple, combined color). Inset on the lower left corner shows chromosome 4 with P1 hybridization aligned with a black and white image of DAPI-stained chromosome 4. (B) PCR analysis of human hamster somatic cell and radiation hybrids containing various portions of chromosomal band 4q35. The 150-bp amplification product from the ALP gene is present only in somatic cell hybrids containing the portion of 4q35 proximal to D4S187. Only those radiation hybrids that contain a portion of the interval between D4S171 and FXI were positive for ALP. (C) Schematic of the 4q35 locus contained within each somatic cell and radiation hybrid. The order and retention of the 12 loci between IRF2 (centromeric) and D4S809 (telomeric) in the radiation hybrids were determined previously (Winokur et al., 1993).
Figure 5. ALP protein is enriched in skeletal muscle and colocalizes with α-actinin-2 at the Z lines. (A) Rat tissue extracts (100 μg/lane) from rat kidney (K), spleen (S), liver (L), heart (H), skeletal muscle (M), and brain (B) was run on SDS-PAGE gel, transferred to a polyvinylidene fluoride membrane, and then probed with a polyclonal antibody against GST–ALP fusion protein. (B) Western blotting of protein extracts from C2 myogenic cultures shows that ALP is absent from myoblasts and is present in myotubes 3 and 5 d after fusion. (C) Immunofluorescent staining of rat skeletal muscle longitudinal sections shows that ALP (red) occurs at the α-actinin-2-rich (green) Z lines.
Discussion

The primary finding in this study is the identification of a functional interaction between a PDZ domain and the spectrin-like repeats of α-actinin-2. This association targets a novel LIM protein, ALP, to the actinin-rich Z lines of skeletal muscle fibers. PDZ domains are recently recognized protein–protein interaction motifs that are implicated in protein association with the cytoskeleton (Marfai et al., 1996) and in signal transduction (Brenman and Bredt, 1997; Sheng, 1996). Previous studies demonstrated that the two PDZ proteins in skeletal muscle, nNOS and the syntrophins, are constituents of the dystrophin complex (Adams et al., 1993; Brenman et al., 1995). Our work here shows that the PDZ protein ALP does not associate with the dystrophin complex, but instead binds to α-actinin-2, which is in the dystrophin superfamily of cytoskeletal proteins.

Interaction with the spectrin-like repeat represents a new mode of binding for a PDZ domain. Previous work has shown that PDZ domains of the postsynaptic density protein, PSD-95, bind to certain glutamate receptors and K+ channels in the brain that terminate with a consensus of E-T/S-X-V/I (Cohen et al., 1996; Kim et al., 1995; Kornau et al., 1995). These interactions appear to anchor ion channels to synaptic sites in neurons. Interaction with specific COOH-terminal peptides may be a general property of PDZ domains, and two recent studies demonstrate that distinct PDZ domains, bind to specific COOH-terminal peptide sequences (Songyang et al., 1997; Stricker et al., 1997). Certain PDZ domains can also associate with each other in a homotypic-type interaction (Brenman et al., 1996). The PDZ domain of nNOS binds to the second PDZ domain of PSD-95 in the brain and to the PDZ domain of α-syntrophin in skeletal muscle.

The binding interface between the PDZ domain of ALP and the spectrin-like repeats of α-actinin-2 represents a third mode for protein interactions mediated by PDZ domains. We suspect that this type of interaction is not unique to ALP and may explain cytoskeletal interactions of diverse PDZ proteins. Z-1 protein of epithelial tight junctions contains three PDZ domains and associates with spectrin in the cell cortex (Willott et al., 1993). Electron micrographic studies indicate that ZO-1 forms a complex with the central rodlike repeat domains of spectrin. It is not yet clear whether the PDZ domains of ZO-1 mediate this interaction. A complex ternary interaction between the spectrin-like repeats of dystrophin and the PDZ domains of nNOS/syntrophin may occur at the skeletal muscle sarcolemma (Chao et al., 1996). Thus, in vitro assays demonstrate that nNOS binds directly to syntrophin, but not to dystrophin. However, the nNOS/syntrophin interaction in skeletal muscle requires that certain spectrin-like repeats of dystrophin be intact. Also, nNOS is selectively absent from skeletal muscle sarcolemma in patients with Becker muscular dystrophy who have mutations within the spectrin-like repeats of dystrophin (Chao et al., 1996).

We find that ALP expression is normal in Duchenne and Becker muscular dystrophies (Xia, H., and D.S. Bredt, unpublished data). On the other hand, certain inherited muscular dystrophies result from mutations in cytoskeletal proteins that do not interact with the dystrophin complex (Hoffman et al., 1995). Plectin, a cytoskeleton–membrane anchorage protein of hemidesmosomes, links intermediate filaments to the sarcolemma and also occurs at the Z lines in skeletal muscle (Wiche et al., 1983). Mutations in plectin do not affect the dystrophin complex, but they cause an autosomal recessive muscular dystrophy associated with skin blistering (Smith et al., 1996). It will be important to assess ALP expression in a variety of inherited muscular dystrophies to determine whether it may play a role in any of these diseases.

Our chromosomal mapping studies indicate that ALP occurs on human chromosome 4q 35. Interestingly, the location is ~7–10 Mb from the subtelomeric region that is mutated in FSHD, an autosomal dominant disease (Wijmenga et al., 1992). The specific genetic defect in FSHD disease appears to be a deletion of heterochromatin (Lyle et al., 1995; Winokur et al., 1994). It is not clear how this mutation results in muscular dystrophy. It is postulated that the telomeric mutation mediates a “position effect” that alters the expression of a nearby muscle-specific gene (Altherr et al., 1995). Genes separated by genomic distances >2 Mb from heterochromatin have been reported to be affected by position effect variegation in Drosophila (Bedell et al., 1996). Therefore, ALP should be considered a candidate gene for FSHD. In preliminary studies, we have not detected consistent changes in ALP expression in muscle biopsies from FSHD tissues. However, the muscle samples from FSHD patients analyzed for ALP expression may not have been from the critically affected muscle groups or from appropriate developmental stages.

What might be the normal function of ALP? Determining the role of the LIM motif in ALP remains a critical question. LIM motifs were first identified in protein products from three different genes, lin11 (Freyd et al., 1990), isl1 (Karlsson et al., 1990), and mec3 (Way and Chalfie, 1988), which all contain two LIM domains in association with a homeodomain DNA binding motif. These transcription factor LIM proteins participate in cell fate determination. Many distinct classes of LIM proteins have now been identified that do not have a homeodomain but still participate in cell fate determination (Sanchez-Garcia and Rabbitts, 1994). At the biochemical level, LIM motifs are implicated in protein–protein interactions. LIM motifs have been shown to interact with basic helix-loop-helix transcription factors (Wadman et al., 1994), protein kinase C (Kuroda et al., 1996), receptor tyrosine kinase tight turn structure (Wu et al., 1996), and other LIM motifs (Schmeichel and Beckerle, 1994). In striated muscle, the LIM-only protein, MLP, occurs at the Z lines and is an essential regulator of myogenic differentiation (Arber et al., 1994). Targeted disruption of MLP results in a disorganization of the myocyte cytoarchitecture (Arber et al., 1997). It will be interesting to determine whether the LIM domain of ALP interacts with MLP or other LIM proteins at the Z lines. To decisively determine the role of ALP in cellular physiology and muscular dystrophy, it will be important to delete the function of ALP by knockout and dominant negative approaches.

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