Deletion analysis of the dystrophin–actin binding domain

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Abstract

Three sequence motifs at the N-terminus of dystrophin have previously been proposed to be important for binding to actin. By analyzing a series of purified bacterial fusion proteins deleted for each of these sites we have demonstrated that none of the three are critical for dystrophin–actin interactions. Instead, our data suggest that sequences in the N-terminal 90 amino acids of dystrophin, excluding a conserved KTFT motif, contain the major site for interaction with actin.

Key words: Dystrophin; Actin binding site; Duchenne muscular dystrophy; α-Actinin

1. Introduction

Duchenne and Becker muscular dystrophies (DMD/BMD) are progressive, lethal muscle disorders caused by defects in the dystrophin gene [1]. Although complete cDNA sequences have been determined for the human, mouse, and chicken dystrophins [2–4], little is known of the precise role that this protein plays in cells. Many mildy affected BMD patients display in-frame deletions of various portions of the gene, suggesting that some portions of dystrophin are not critical for its function [5]. A complete understanding of the functional domains of dystrophin would clarify the role this protein plays in cells and the mechanisms by which mutations in the gene lead to progressive muscle degeneration. In addition, identification of sequences less important for dystrophin activity could facilitate the design of truncated dystrophin expression vectors small enough for insertion into viral delivery vehicles being considered for gene therapy of DMD [6,7].

Dystrophin is localized to the cytoplasmic face of the sarcolemma where it has been proposed to act as a structural component of the membrane cytoskeleton [8]. Biochemical analysis indicates that dystrophin binds to an integral membrane complex of glycoproteins associated with the laminin receptor, suggesting that dystrophin acts as a link between the sarcoplasmic cytoskeleton and the extracellular matrix [9]. The N-terminus of dystrophin displays a high degree of sequence identity with the spectrin family of F-actin binding proteins, including β-spectrin, α-actinin, filamin, and ABP120 [2]. Recent studies have shown that native dystrophin binds F-actin in vitro [10]. It has also been shown that fusion proteins containing at least the N-terminal 233 amino acids of dystrophin bind actin in in vitro pelleting assays, and actin-stress fibers in situ when expressed in non-muscle cells [11,12]. In addition, two putative actin binding sites within the N-terminus have been identified by NMR spectroscopy experiments using synthetic dystrophin peptides [13,14]. To delineate further the actin binding domains of dystrophin, we have expressed and isolated various bacterial fusion proteins containing deletions of the N-terminal domain of dystrophin and have tested their ability to bind actin in vitro. Our results demonstrate that a fusion protein containing only the first 90 amino acids of dystrophin retains the ability to bind actin in in vitro binding assays. Furthermore, within these 90 amino acids, the conserved amino acids KTFT are not critical for the observed binding activity. These results suggest that additional sequences not previously implicated in the binding of actin must be the major determinants of dystrophin–actin interactions.

2. Materials and methods

Actin was prepared from rabbit muscle acetone powder as described by Spudich and Watt [15]. A series of maltose-binding protein (MBP) fusion constructs were generated by PCR using *Pfu* polymerase (Stratagene) with murine dystrophin cDNA clones as templates [16]. PCR reaction conditions for plasmid amplification were essentially as described previously [17] except that the buffer recommended by Stratagene was used. *Pfu* polymerase was utilized due to the 12-fold higher

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fidelity rate claimed by the manufacturer in comparison with *Taq* polymerase. To facilitate subcloning, the 5' oligonucleotide primer was designed to include an *Eco*RI restriction site. The 3' oligonucleotide primers were designed with an in-frame translation stop codon and an *Xba*I restriction site. The PCR products were digested with *Eco*RI and *Xba*I and then cloned into *Eco*RI/*Xba*I cut pMALCRII (New England Biolabs) so that the MBP was fused in-frame with the second amino acid of dystrophin. The DYS*Δ*90 construct was constructed from DYS90 using divergent primers and recombinant PCR [18] to create an exact deletion of amino acids 19–22. The sequences of clones DYS90 and DYS*Δ*90 were confirmed by dideoxy sequencing. PCR primers used to construct the MBP–dystrophin fusion proteins are as follows:

5' CGGAATTCCTTTGGTGGGAAGAAGTAG 3'	forward
5' GCTCTAGACTATTCAATGCTCACTTGTTGTGGC 3'	reverse DYS246
5' GCTCTAGACTATCCAGCCATGATAGTTTTC 3'	reverse DYS130
5' GCTCTAGATTAATCAACATTATTTTTCTG 3'	reverse DYS90
5' GAAGATGTTCAAAAGAAATGGATAAATGCAC 3'	forward *Δ*90
5' GTGCATTTATCCATTTCTTTTGAACATCTTC 3'	reverse *Δ*90

The C-DYS-MBP fusion plasmid was constructed by subcloning the 3' end of the murine dystrophin cDNA coding sequence [16] as an *Nru*I (blunt ended)–*Not*I fragment into *Eco*RI (blunt ended)–*Not*I cut pMAlcR plasmid so that the MBP was fused in-frame to amino acids 3364–3678 of dystrophin ([3]; Genbank accession no. M66859). This fragment contains the adult mouse dystrophin stop codon ([3]; G. Cox, J.S.C., manuscript in preparation).

The fusion proteins were grown and purified essentially following the protocol of the vector supplier (New England Biolabs). DH5αF' cells containing dystrophin–MBP fusion vectors were induced by addition of IPTG to 0.3 mM and grown for 2.5 h at 37°C with continuous shaking. Cells were pelleted at 4,000 × *g* for 20 min at 4°C, resuspended in CL buffer (20 mM Tris-Cl, pH 7.4, 200 mM NaCl, 1 mM EDTA, 10 mM *β*-mercaptoethanol), and frozen overnight at −20°C. Upon thawing, the protease inhibitors aprotinin, leupeptin and PMSF were added to a final concentration of 0.3 μM, 1.0 μM and 400 μM, respectively. The cells were then lysed by sonication and spun at 14,000 × *g* for 20 min at 4°C. The supernatant was diluted 1:5 with CL buffer and loaded onto an amylose-resin column. Bound fusion protein was eluted with 10 mM maltose in CL buffer and pooled protein-containing fractions were concentrated using Centricon-30 filters (Amicon). The concentration of protein was determined using a protein assay kit with BSA as a standard (Pierce). The purity of the fusion proteins was monitored by SDS-PAGE.

2.2. Actin binding

The solid-phase immunoassays were performed essentially as described by Jin and Wang [19]. F-actin (100 μg/ml) or BSA (100 μg/ml) diluted in AB buffer (10 mM Tris-Cl, pH 7.5, 100 mM KCl, 10 mM MgCl₂, 0.2 mM ATP and 0.02% sodium azide) were adsorbed to microtiter plates by incubating 100 μl/well at 4°C overnight. After three rinses with AB buffer, the plate was blocked with 0.15% BSA at 4°C overnight and subsequently washed three times with ABT buffer (AB buffer + 0.1% Tween-20). All subsequent steps were carried out at room temperature. Purified fusion proteins or purified maltose binding protein were serially diluted in ABT buffer and incubated (100 μl/well) for 2 h followed by three 10 min washes with ABT buffer. The wells were then incubated for 1 h (100 μl/well) with anti-MBP antiserum (New England Biolabs) diluted 1:1000 in ABT + 0.015% BSA followed by three 10 min washes with ABT. The wells were then incubated with alkaline phosphatase-conjugated anti-rabbit secondary antibody diluted 1:1000 in ABT + 0.015% BSA followed by one 10 min wash and two 20 min washes in ABT. After rinsing once with AB and twice with 10 mM diethanolamine/0.5 mM MgCl₂, PNPP substrate (Sigma) was added (100 μl/well). The reaction was stopped after 30 min by addition of 100 μl of 100 mM EDTA and the absorbance was measured at 405 nm.

For the centrifuge binding assays, purified DYS246, DYS90, C-DYS, or MBP was mixed with purified rabbit muscle F-actin and incubated in binding buffer (10 mM Tris-Cl, pH 8.0, 100 mM KCl, 3 mM MgCl₂, 0.2 mM ATP, 0.1% Triton X-100, 0.02% sodium azide) for 1 h at room temperature and then subjected to centrifugation in a Beckman airfuge at 30 psi for 30 min. The pellets were recovered, mixed with 2 × loading dye, and an aliquot was separated by SDS-PAGE on an 8% gel, and stained with Coomassie blue.

Fig. 1. Amino acid sequence of the actin binding domain of dystrophin. The N-terminal 251 amino acids of the murine dystrophin protein. Underlined are the three putative sites of interaction between dystrophin and actin based on experimental results with human dystrophin or with similarities to other actin binding proteins. ABS1 and ABS2 were identified by Levine et al. [13,14] using NMR spectroscopy to identify interactions between synthetic dystrophin peptides and actin. ABS3 is a highly conserved stretch of amino acids shown to be important for actin binding of ABP-120, Bcr-Abl, and α-actinin [20–23]. Asterisks indicate the 3' end portion of constructs used in this study.

3. Results

The N-terminal 251 amino acid actin binding domain of murine dystrophin is shown in Fig. 1. Three sites previously proposed to interact with actin are underlined and designated ABS1, ABS2, and ABS3. ABS1 (amino acids 18–27) and ABS2 (amino acids 131–148) are putative sites of interaction between actin and dystrophin identified by NMR spectroscopy experiments with synthetic dystrophin peptides [13,14]. ABS3 (amino acids 91 117) is highly conserved strech of amino acids shown to be important for the actin binding activity of ABP120, Bcr-Abl, and α-actinin [20 23]. To determine whether these sites are critical for actin binding to dystrophin we constructed a series of C-terminal deletions that sequentially removed these putative actin binding sites and tested their ability to bind actin in solid-phase binding assays. Three fusion proteins were analyzed for their ability to bind actin: DYS246 (amino acids 2–246) includes all three putative binding sites; DYS130 (amino acids 2–130) contains ABS1 and ABS3 but is deleted for ABS2; and DYS90 (amino acids 2–90) which is deleted for ABS2 and ABS3 but contains ABS1 (Fig. 1). Fig. 2 shows that all three fusion proteins bound actin in a saturable manner, whereas the control MBP exhibited only non-specific binding. As a second control, a fusion protein containing the C-terminal 315 amino acids of dystrophin was found to display non-specific binding similar to the MBP (Fig. 2C). Apparent 50% saturation for all three N-terminal proteins occured in the range of 100 nM (Fig. 2A). It was also noted that at the lower concentrations of dystrophin, DYS130 reproducibly showed a slightly lower binding affinity to actin than DYS246 and DYS90 (Fig. 2B, and data not shown).

The observation that DYS90 retained the ability to bind actin was unexpected since it lacks the highly conserved binding site, ABS3. To confirm this result, we assayed the ability of DYS246, DYS90, and C-DYS to bind actin in a conventional co-sedimentation assay
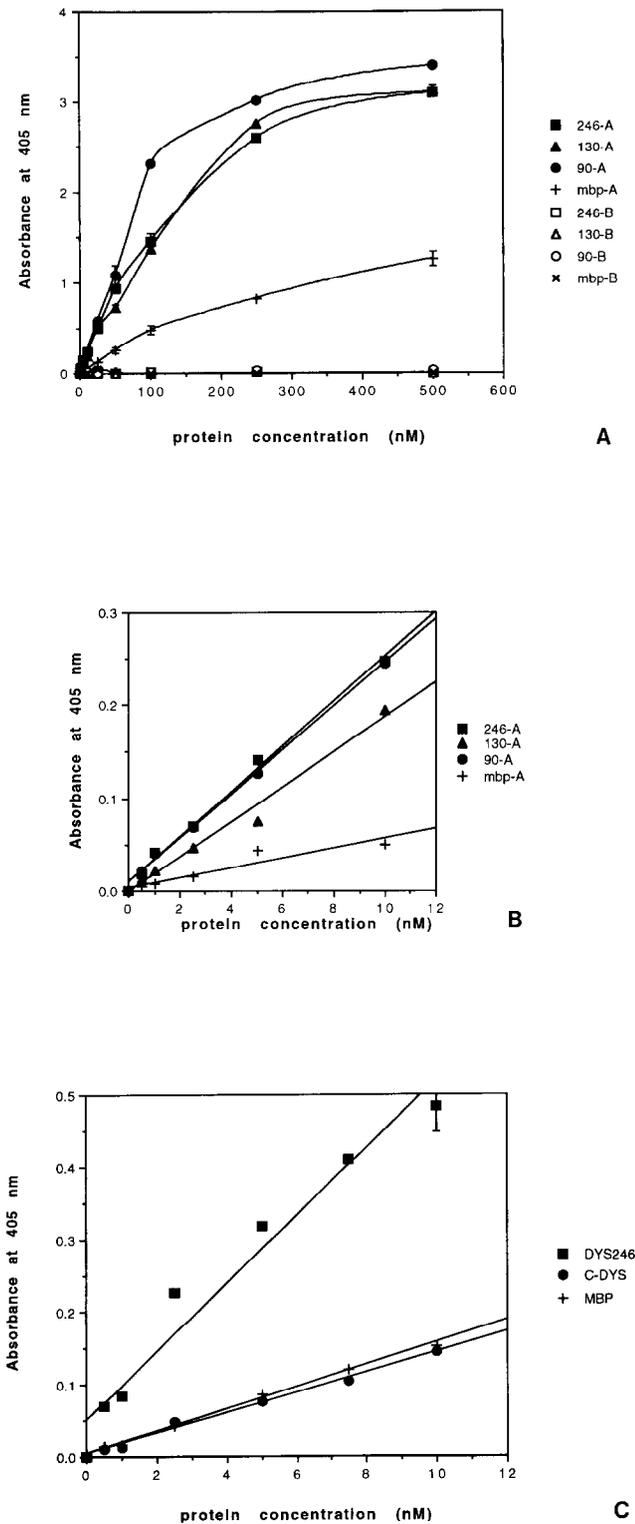
Fig. 2. Solid-phase immunoassay. (A) The fusion proteins DYS246, DYS130, DYS90 and maltose binding protein (MBP) (see text) were analyzed for their ability to bind actin (-A) or BSA (-B) as described in section 2. (B) Same as in A except only the linear region of the binding curves are shown. (C) The fusion proteins DYS246, C-DYS, and MBP were analyzed for their ability to bind actin. All points are the average of duplicate determinations. Error bars reflect the actual data points in cases where the difference between the duplicate points was greater than 0.05 absorbance units.

(Fig. 3). Consistent with the data from the plate binding assay, both DYS246 and DYS90, but not C-DYS, were found in the pellet in the presence of actin while very little was found in the pellet in the absence of actin, indicating that both constructs are soluble and bind actin: the control MBP alone, like C-DYS, did not bind actin in this assay (data not shown). In an alternate assay, we tested whether a synthetic peptide of the 27 amino acid ABS3 sequence could act as a competetive inhibitor in a plate binding assay or in a co-sedimentation assay. This peptide did not inhibit the binding of DYS246 to actin in either assay (data not shown). Together these results indicate that neither ABS2 nor ABS3 is required for dytrophin binding to actin. It therefore seemed possible that the binding activity of DYS90 was due to the remaining site, ABS1, which has been suggested as a potential binding site by Levine et al. [13,14]. To test this hypothesis, we constructed a fusion protein (DYSΔ90) containing the first 90 amino acids of dystrophin but deleted for 4 amino acids, KTFT, the amino acids in ABS1 proposed to be involved in actin interactions [14], and tested the ability of this protein to bind actin in the solid-phase binding assay. Suprisingly, DYSΔ90 displayed only a slight decrease in its ability to bind actin compared with the DYS90 protein (Fig. 4). This result indicates that an actin-binding site other than ABS1 exists within the first 90 amino acids of murine dystrophin.

4. Discussion

The high degree of sequence similarity between the N-terminal domain of dystrophin and the spectrin family of F-actin binding proteins suggests that this region is involved in binding to the actin cytoskeletal network of muscle cells. Recent studies have shown that both native dystrophin and fusion proteins containing the N-terminus of dystrophin bind actin in vitro [10–12]. Delineation of the regions neccessary for the dystrophin-actin inter-
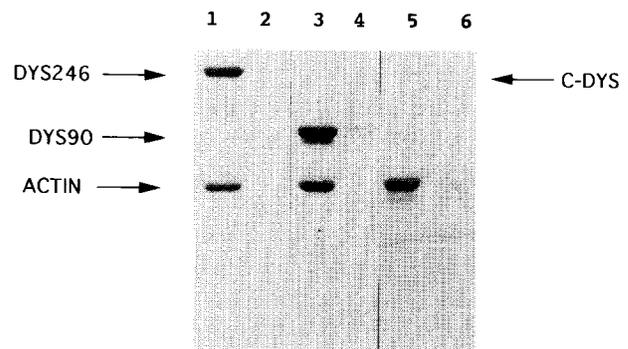

Fig. 3. Co-sedimentation assay. Binding assays were carried out as described in section 2. Lanes 1 and 2, DYS246; lanes 3 and 4, DYS90; lanes 5 and 6, C-DYS. Lanes 1, 3, and 5 are in the presence of actin and lanes 2, 4, and 6 are in the absence of actin. The concentrations of DYS246, DYS90 and C-DYS were 9 μM; actin was present at 3 μM.
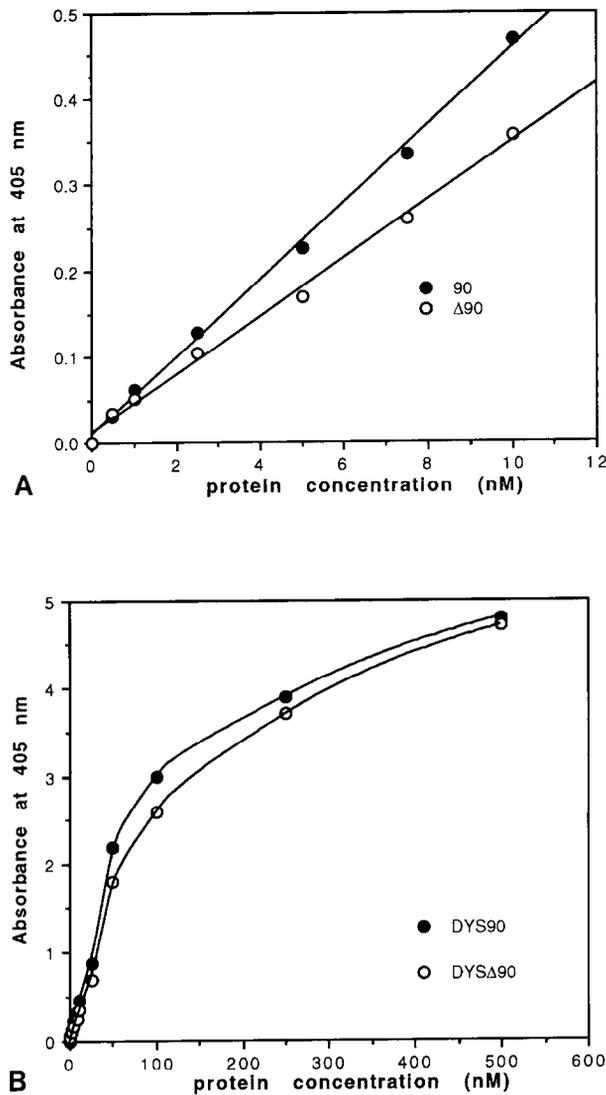
Fig. 4. Solid-phase immunoassay. (A) The fusion proteins DYS90 and DYS*Δ*90 were analyzed for their ability to bind actin as described in materials and methods. (B) Same as in A except only the linear region of binding curves are shown. All points are the average of duplicate determinations. Error bars reflect the actual data points in cases where the difference between the duplicate points was greater than 0.05 absorbance units.

action will facilitate an understanding of the function of dystrophin. Based on NMR spectroscopy experiments and sequence similarity with other actin binding proteins, it has been proposed that dystrophin might contain three actin binding sites: ABS1 (amino acids 18–27), ABS2 (amino acids 131–148) and ABS3 (amino acids 91–117) [13,14,18,21]. To explore the role of these sites we have constructed four N-terminal dystrophin fusion proteins containing successive deletions that specifically eliminate the proposed actin binding sites and have assayed their ability to bind actin. We have shown that a fusion protein containing the N-terminal 246 amino acids of dystrophin binds actin in a specific manner in

in vitro solid-phase binding assays and in actin co-sedimentation assays. In addition, we have shown that a synthetic 27 amino acid peptide of ABS3 sequences fails to inhibit this binding and, more dramatically, that a construct deleted for both ABS2 and ABS3 retains the ability to bind actin.

These results suggest that unlike the actin binding proteins ABP-120 and Bcr-Abl, neither ABS2 nor ABS3 is critical for dystrophin binding to actin. Our results are similar to those observed for α-actinin in which the first 107 amino acids were shown to bind actin in the absence of ABS3 [23]. However, in the case of α-actinin, the binding between ABS1 and actin appears to be weaker than that of ABS3 and actin, whereas for dystrophin, the construct containing ABS1 alone (DYS90) exhibits an actin binding activity similar to that of the construct containing all three binding sites (DYS246) (Fig. 2). Furthermore, while the experiments of Levine et al. [13,14] have suggested that the highly conserved amino acids, KTFT, of ABS1 in dystrophin interact with actin, we have shown that deleting these 4 amino acids has little effect on the ability of DYS90 to bind actin. This observation is consistent with results from mutational analysis of this same region of α-actinin where it was shown that the amino acids, KTFT, were not essential for its binding to actin [12]. It should be noted, however, that in the α-actinin study, the actin binding site ABS3 was still present in the KTFT deletion construct, possibly accounting for its actin binding ability. Since DYS90 contains only ABS1, our results (Fig. 4) suggest that these 4 amino acids are not the critical amino acids involved in the contact sites between dystrophin and actin and/or that the actin binding site resides somewhere else in the first 90 amino acids of dystrophin.

While this manuscript was in preparation, Fabbrizio et al. [24] reported the results of co-sedimentation experiments designed to delineate the actin binding site of human dystrophin. Their results were consistent with ours and demonstrated that a fusion protein joining the MBP with amino acids 1–68 of dystrophin was capable of binding actin. Hence, by two independent in vitro assays, it has been shown that neither ABS2 nor ABS3 is critical for dystrophin binding to actin. In addition, a comparison of the dissociation constants of the various constructs used by Fabbrizio et al. [24] suggests that there is an inhibitory effect of the ABS3 site on the ABS1 site. This result is also consistent with our observation that a construct containing just ABS1 (DYS90) appeared to bind actin with a greater affinity than a construct containing ABS1 and ABS3 (DYS130) (Fig. 2B). The fact that DYS90 displays a greater binding activity than DYS130 suggests that the latter may contain a region that inhibits binding to actin in the absence of amino acids 131–246. However, it is also possible that this result is due to subtle changes in the conformation of the fusion proteins.

From the experimental data observed to date, it appears that a previously unidentified actin binding site resides within the first 68 amino acids of dystrophin and that this binding activity does not require amino acids 19–22, the conserved KTFT motif. Comparing the dystrophin actin binding results with those of other members of this family of proteins, it is becoming clear that the sites identified by sequence homology may not all behave similarly with respect to their actin binding activity. In support of this idea, it has been shown that α-actinin does not inhibit the binding of purified dystrophin to actin [10]. This observation suggests that the sites on actin with which the two proteins interact are independent and presents the possibility that dystrophin and α-actinin interact with actin by different mechanisms, perhaps requiring additional binding sites and/or other interacting proteins. One possible candidate for a dystrophin interacting protein is the actin binding protein, talin, which has been shown to bind dystrophin in vitro [10]. A new putative actin binding site has also been suggested from the recent identification of a severely affected DMD patient carrying a point mutation in the actin binding domain of dystrophin [25]. The mutated amino acid (Leu^{54}→Arg) does not reside in any of the conserved binding sites, which suggests that amino acid 54 may be important for the function of dystrophin, possibly due to an interaction with actin. This amino acid is present in each of the fusion proteins that we have shown to bind actin in vitro.

A variety of studies have now been reported that demonstrate binding of bacterial expressed dystrophin protein fragments with F-actin in vitro. The primary discrepancy between these studies is the apparent dissociation constant for the dystrophin–actin interactions. Way et al. proposed a K_d of 44 μM [11], Fabbrizio et al. proposed a K_d in the range of 1–5 μM [24], while our studies suggest an even greater affinity in the range of 100 nM (Fig. 2). These discrepancies are most likely reflecting variations in the experimental systems employed. Our study and that of Fabbrizio et al. [24] used fusion proteins, which could affect the conformation of the dystrophin fragment, whereas Way et al. [11] used bacterial expressed dystrophin fragments not part of a fusion protein. Fabbrizio et al. [24] and the present study both used MBP fusions, however, we also used a solid-phase binding assay that may be more sensitive that the co-sedimentation assays. Hemmings et al. also used bacterial fusion proteins to demonstrate actin-dystrophin binding, but those authors did not estimate a K_d [12]. Several groups have studied the binding of full-length dystrophin to actin. Using a solid-phase assay Senter et al. [10] detected a K_d for purified dystrophin in the nM range. Fabrizio et al. [24] and Ervasti and Campbell [26] both demonstrated that dystrophin-enriched membranes or the dystrophin–glycoprotein complex, respectively, are able to bind actin in co-sedimentation assays. While each of

these studies support the idea that actin and dystrophin form a direct interaction, none rule out the possibility that other proteins facilitate this interaction.

Elucidation of the precise sequences required for the actin–dystrophin interaction will assist in our understanding of the pathogenesis of DMD and could aid in the development of therapeutic approaches for this disease. While important insights into the structural basis for actin binding can be gained by studies of patient mutations, analysis of mutant and truncated dystrophins both in vitro and in vivo will most likely be required to resolve remaining ambiguities related to the mechanism by which dystrophin interacts with the sarcoplasmic cytoskeleton. For example, multiple studies have demonstrated that deletion of exons 2–7, 3, and, often, 3–7, in a variety of patients typically results in BMD rather than DMD [27–29]. The larger of these deletions leads to production of truncated dystrophin molecules missing amino acids 11–217, which includes ABS 1–3 [2]. The exon 3–7 deletions should be mRNA frameshifting deletions, but these frequently generate low levels of dystrophin molecules that arise either by exon skipping (to restore the reading frame) or by translational initiation at an AUG codon within exon 8 [28,29]. The model proposed by Ervasti et al. [9] for the structure of dystrophin in muscle postulates a firm interaction between dystrophin and actin filaments for maintenance of a link between the cytoskeleton and the extracellular matrix. Beggs et al. have demonstrated that deletions that remove portions of the dystrophin N-terminus generally lead to low levels of protein accumulation, suggesting that these truncated dystrophins may be unstable as a result of impaired interactions with the cytoskeleton [27]. In this regard, while Prior et al. have demonstrated that mutation of amino acid 54 can lead to DMD, it is not clear whether this mutation affected actin binding per se or rendered the mutant protein unstable [25]. Similarly, deletion of exon 3 results in BMD characterized by low levels of a dystrophin molecule missing amino acids 32–62 [27], which spans the region shared by each of our actin binding constructs (Fig. 1). The observed mild (BMD) phenotype in multiple patients with deletions spanning various combinations of exons encoding the actin binding domain suggests that dystrophin is capable of forming at least a weak interaction with the cytoskeleton even in the absence of a strong, direct link with F-actin mediated by sequences at the extreme N-terminus of dystrophin. Higher resolution analysis of the sequences needed for linking dystrophin to the cytoskeleton will be aided by studies in cell culture and in transgenic mice, experiments currently in progress. Clarification of the structural basis for dystrophin function will also facilitate the rational design of truncated mini-genes that could be delivered to muscles by viral vectors, and may suggest alternate approaches for therapy of DMD/BMD.

References

[1] Hoffman, E.P., Brown, R.H. and Kunkel, L.M. (1987) Cell 51, 919–928.

[2] Koenig, M., Monaco, A.P. and Kunkel, L.M. (1988) Cell 53, 219–226.

[3] Bies, R.D., Phelps, S.F., Cortez, M.D., Roberts, R., Caskey, C.T. and Chamberlain, J.S. (1992) Nucleic Acids Res. 20, 1725–1731.

[4] Lemaire, C., Heilig, R. and Mandel, J.-L. (1988) EMBO J. 7, 4157–4162.

[5] England, S.B., Nicholson, L.V.B., Johnson, M.A., Forrest, S.M., Love, D.R., Zubrycka-Gaarn, E.E., Bulman, D.E., Harris, J.B. and Davies, K.E. (1990) Nature 343, 180–182.

[6] Chamberlain, J.S. (1992) Curr. Opin. Neurol. Neurosci. 5, 610–614.

[7] Ragot, T., Vincent, N., Chafey, P., Vigne, E., Gilggenkrantz, H., Couton, D., Cartaud, J., Briand, P., Kaplan, J., Perricaudet, M. and Kahn, A. (1993) Nature 361, 647–650.

[8] Zubrycka-Gaarn, E.E., Bulman, D.E., Karpati, G. et al. (1988) Nature 333, 466–469.

[9] Ervasti, J.M. and Campbell, K.P. (1991) Cell 66, 1121–1131.

[10] Senter, L., Luise, M., Presotto, C., Betto, R., Teresi, A., Ceolodo, S. and Salvati, G. (1993) Biochem. Biophys. Res. Commun. 192, 899–904.

[11] Way, M., Pope, B., Cross, R.A., Kendrick-Jones, J. and Weeds, A.G. (1992) FEBS Letts. 301, 243–245.

[12] Hemmings L., Kuhlman, P.A. and Critchley, D.R. (1992) J. Cell Biol. 116, 1369–1380.

[13] Levine B.A., Moir, A.J.G., Patchell, V.B. and Perry, S.V. (1992) FEBS Lett. 298, 44–48.

[14] Levine B.A., Moir, A.J.G., Patchell, S.V. and Perry, S.V. (1990) FEBS Lett. 263, 159–162.

[15] Spudich, J.A. and Watt S. (1971) J. Biol. Chem. 246, 4866–4871.

[16] Lee, C.C., Pearlman, A., Chamberlain, J.S. and Caskey, C.T. (1991) Nature 349, 334–336.

[17] Maichele, A.J., Farwell, N.J. and Chamberlain, J.S. (1993) Genomics 16, 139–149.

[18] Higuchi, R. (1990) in: PCR Protocols: A Guide to Methods and Applications, (Innis, M.A., Gelfand, D.H., Sninsky, J.J., White, T.J. eds.) pp. 177–183, Academic, San Diego

[19] Jin, J. and Wang, K. (1991) FEBS Lett. 281, 93–96.

[20] Bresnick, A.R., Warren, V. and Condeelis, J. (1990) J. Biol. Chem. 266, 9236–9240.

[21] Bresnick, A.R., Janmey, P.A. and Condeelis, J. (1991) J. Biol. Chem. 266, 12989–12993.

[22] McWhirter, J.R. and Wang. J.Y.J. (1993) EMBO J. 12, 1533–1546.

[23] Kuhlman, P., Hemmings, L. and Critchley, D.R. (1992) FEBS lett. 304, 201–206.

[24] Fabbrizio, F., Bonet-Kerrache, A., Leger, J.J. and Mornet, D. (1993) Biochemistry 32, 10457–10463.

[25] Prior, T.W., Papp, A.C., Snyder P.J., Burghes, A.H.M., Bartolo, C., Sedra, M.S., Western L.M. and Mendell, J.R. (1993) Nature Genet. 4, 357–360.

[26] Ervasti, J. and Campbell, K.P. (1993) J. Cell Biol. 122, 809–823.

[27] Beggs, A., Hoffman, E.P., Snyder, J.R., Arahata, K., Specht, L., Shapiro, F., Angelini, C., Sugita, H. and Kunkel, L.M. (1991) Am. J. Hum. Genet. 49, 54–67.

[28] Gangopadhyay, S.B., Sherratt, T.G., Heckmatt, J.Z., Dubovitz, V.V., Miller, G., Shokier, M., Ray, P.N., Strong, P.N. and Worton, R.G. (1992) Am. J. Hum. Genet. 51, 562–570.

[29] Winnard, A.V., Klein, C.J., Coovert, D.D., Prior, T., Papp, A., Snyder, P., Bulman, D.E., Ray, P.N., McAndrew, P., King, W. et al. (1993) Hum. Mol. Genet. 2, 737–744.