Communication

Identification of a Chromosome 6-encoded Dystrophin-related Protein*

(Received for publication, April 11, 1990)

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Dystrophin is the protein product of an X-linked locus which is disrupted to yield the phenotypes of Duchenne and Becker muscular dystrophies. Recently an autosomal transcript was identified (Love, D. R., Hill, D. F., Dickson, G., Spurr, N. K., Byth, B. C., Marsden, R. F., Walsh, F. S., Edwards, Y. H., and Davies, K. E. (1989) Nature 339, 55–58) with a carboxyl-terminal sequence similar to dystrophin. We have isolated part of this chromosome 6-encoded cDNA by polymerase chain reaction cloning and expressed it as a recombinant bacterial protein. Antibodies against this recombinant protein detected a large protein that exactly co-migrates with dystrophin yet is detectable in patients suffering from Duchenne and Becker muscular dystrophies. This protein of similar size to dystrophin may play a functional role similar to dystrophin, but unlike dystrophin, the protein is detected in tissues other than muscle and nerve. This newly identified protein is presumably a member of the spectrin/α-actinin/dystrophin family of proteins.

Duchenne/Becker muscular dystrophy is one of the most common inherited neuromuscular disorders in humans. The etiological basis of this disorder is a quantitative or qualitative defect in dystrophin, which is the protein product of the X-linked DMD locus (1–3). Dystrophin is a large cytoskeletal protein that forms a network beneath external membranes (4). Extensive sequence similarities exist between dystrophin and the spectrins and α-actinins (5). Much of the sequence similarity is across the central “rod” domain of each protein and the spectrins and α-actinins (5). A-Actinin, β-spectrin, and dystrophin sequences have immunological cross-reactivity data (9).

The spectrins and α-actinins are themselves families of proteins that contain multiple isoforms (8, 10). Spectrin usually exists as a heterodimer of α and β subunits associated in an antiparallel manner. The functional multimeric state, however, is thought to be a tetramer of two dimers associated end-to-end. The spectrins are components of membrane cytoskeleton and have been best characterized in erythroid cells; however, many specific non-erythroid isoforms exist (10). The spectrins have been shown to bind F-actin, and at least in the case of the sea urchin this binding is modulated by calcium (11). The spectrin heterodimer usually consists of large M, 220,000 265,000 subunits. The α-actinins are also F-actin binding proteins, many isoforms of which have been defined in a number of species and cell types based upon their molecular mass and structure (8). Muscle and non-muscle α-actinin isoforms appear to be somewhat functionally divergent based upon the calcium sensitivity of non-muscle isoforms toward binding actin and differential subcellular tissue distribution of isoforms (9). Non-muscle α-actinin appears to consist of a homodimer with M, 95,000 subunits.

Since the spectrins and α-actinins have many isoforms, at least some of which seem functionally diverse, it is possible that proteins exist that are similar in structure and size to dystrophin. A strong candidate for such a dystrophin-related protein has come from the recent identification of an autosomally encoded partial cDNA (B3 cDNA) corresponding to part of a 13-kilobase mRNA species (12). Over the short stretch sequenced, the predicted protein shares 83% amino acid identity with the carboxyl terminus of dystrophin. To identify and characterize the putative protein product encoded by the B3 cDNA, we have amplified by polymerase chain reaction a 646-bp region of the B3 cDNA and have directly cloned the amplified product in a bacterial protein expression vector. Antisera were raised against the bacterial fusion proteins and affinity-purified antibodies prepared which were specific for these fusion proteins. These antibodies were then used to study this protein.

We have found this newly identified protein to share the molecular weight and low level of cellular abundance of dystrophin (M, 427,000 and <0.01% total protein). However, unlike dystrophin this large dystrophin-related protein (DRP) is present at normal levels in muscle obtained from both normal and Duchenne/Becker dystrophy-affected individuals. Interestingly, this protein, unlike dystrophin, is detected in all tissues studied and thus does not share the muscle and neuron specificity of dystrophin. Based on the previously determined sequence homology with dystrophin, together with our demonstration of similar size and abundance in muscle, we have named this new protein DRP.

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* This work was supported by grants from the National Institutes of Health (to L. M. K.), Muscular Dystrophy Associations of U. S. A. (to L. M. K. and E. P. H.), and Division of Medical Sciences, Harvard Medical School (to T. S. K.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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EXPERIMENTAL PROCEDURES

Cloning and Expression of Recombinant Proteins—cDNA was synthesized by reverse transcription from human fetal muscle RNA. This was used as a template, and polymerase chain reaction was performed using standard conditions as recommended by the manufacturers (Perkin-Elmer Cetus). The primers were designed so as to amplify a region capable of directing the synthesis of TrpE fusion proteins.

1 The abbreviations used are: bp, base pair(s); DRP, dystrophin-related protein; SDS, sodium dodecyl sulfate.
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Bluescribe and PATH (13) vectors for sequencing and protein expression to generate restriction sites, and ligated into excised and purified using Geneclean (Bio 101). Clones were treated with ethidium bromide staining of agarose gels. Bands were visualized by ethidium bromide staining of agarose gels. Bands were visualized by ethidium bromide staining of agarose gels.

The amplified 646-bp region of B3 cDNA (12) corresponds to domain IV of dystrophin (5). Polymerase chain reaction products were visualized by ethidium bromide staining of agarose gels. Bands were visualized by ethidium bromide staining of agarose gels.

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tional localization to a panel of somatic cell hybrids. The primers were designed to maintain an open reading frame in the pATH 2 vector, thus obviating the need for engineering sites and allowing rapid production of large amounts of fusion protein. Fusion protein was processed for antibody production in rabbits (Hazelton Laboratories) by methods described previously (1, 13). Antibodies specific for DRP were affinity-purified using an Affi-Gel 10 column (Bio-Rad) to which the fusion proteins had been cross-linked covalently, using methods similar to those suggested by the manufacturer (14). The globulin fraction was precipitated using ammonium sulfate. Antibodies to trpE were removed by incubating the antibodies with trpE lysate and centrifuging the precipitated antigen-antibody complex.

Immuno blotting—Tissue was processed for immuno blotting as previously described (1). Briefly, tissues were solubilized in sample buffer (10% SDS, 0.1 M Tris, pH 8.0, 10 mM EDTA, 50 mM diethio reitol (1), boiled, and aliquots fractionated on a 3.5–12.5% gradient SDS-polyacrylamid gel. After electrophoresis, proteins were transferred to nitrocellulose filters. Post-transfer gels were stained with Co massie Blue to control for gel loading, and some filters were subjected to staining with Ponceau S solution, which along with transfer of prestained molecular weight standards serves as a control for the efficiency of transfer.

RESULTS AND DISCUSSION

In order to identify and characterize the protein product of the previously identified “B3” cDNA (12), we used polymerase chain reaction-mediated cloning and fusion protein expression to generate antibodies to the encoded protein species. The resulting antibodies were affinity-purified on antigen columns and used in immuno blot analyses.

The affinity-purified antibodies recognized 10 ng of fusion protein on an immuno blot (Fig. 1), indicating that they were a sensitive tool for further characterization of the putative protein product of the B3 cDNA. Immunoblotting of total human skeletal muscle in the same experiment revealed a strong immunoreactive band of very large molecular weight along with some minor low molecular weight cross-reactive bands of variable intensity (Fig. 1). Thus, the affinity-purified antibodies, much like those directed against dystrophin, chiefly detect a high molecular weight component of muscle, and the large protein was presumed to be the product of B3 cDNA.

The antibodies used were generated against a fusion protein having significant sequence homology to dystrophin. Thus, it was possible that the protein recognized by the antibodies might actually be dystrophin rather than the putative product of B3 cDNA. To test this possibility, we utilized these antibodies on immunoblots of muscle biopsies from both Duchenne dystrophy and Becker dystrophy patients who show dystrophin deficiency and abnormal molecular weight dystrophin, respectively. Parallel immunoblots containing these patient muscle samples were processed using either affinity-purified anti-dystrophin antibodies (1) or the anti-B3 antibodies described here. As shown in Fig. 2, dystrophin is visualized as the expected Mr, 427,000 protein species which is deficient in Duchenne patients and of abnormal molecular weight in Becker patients. On the other hand, the anti-B3 antibodies recognized a single large molecular weight protein which was of similar size and intensity in all of these patients (Fig. 2). In addition, this newly identified protein differs from dystrophin in that it appears to be expressed more highly in fetal muscle tissue than in adult muscle (Fig. 2). To completely eliminate the possibility that we might be observing a cross-reaction to dystrophin we tested a Duchenne dystrophy patient having a genetic deletion spanning the entirety of his dystrophin gene (15). This patient with the complete deletion also expressed apparently normal levels of this newly identified protein species (data not shown). Thus, we conclude that the protein species detected by our anti-B3 antibodies is in fact distinct from dystrophin. Given that this protein is large in size and has extensive sequence homology to dystrophin, we have named this protein DRP.

The cellular abundance of the transcript encoding DRP has been previously compared with that of dystrophin and found to be similar by Northern blot experiments (12); since previous reports have suggested that the abundance for dystro-
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We have identified a new protein species which shares the molecular weight, cellular abundance, and at least partial sequence homology with dystrophin but differs significantly in its tissue distribution. It will be important to determine if DRP is homologous to dystrophin over its entire length, and, therefore, a true member of the spectrin/α-actinin/dystrophin family. In this respect, it is interesting to note a recent publication which described a cross-reactive protein recognized by anti-dystrophin antibodies raised against either the amino or carboxyl termini of dystrophin (15). This cross-reactive protein was of identical molecular weight as dystrophin yet, unlike dystrophin, it was present in Duchenne dystrophy muscle, mdx mouse muscle, and many non-muscle tissues. We feel that it is very likely that this cross-reactive protein detected by antibodies against dystrophin and DRP is not muscle-specific. Tissue aliquots were individually weighed and prepared for immunoblot analysis (as described in Fig. 1) from mdx and normal mouse tissues and incubated with anti-DRP antibodies. DRP is seen in all tissues of both normal and mdx mice. A parallel blot incubated with anti-dystrophin antibodies showed a strong dystrophin signal only in the normal skeletal muscle lane (not shown).

Previously the B3 transcript had been reported to be present in muscular tissue and gut smooth muscle (12). With the ability to detect the encoded protein directly we attempted to see if the tissue distribution was similar to dystrophin or different. Since the DRP antisera detected the expected large protein in (dystrophin-deficient) mdx mice, we used tissues of both the mdx mouse and normal mice to study tissue distribution of this protein, thereby eliminating the possibility of dystrophin complicating the analysis. A protein of M, similar to DRP was detected, being equally abundant in muscle, brain, kidney, and gut in both normal and mdx mice (Fig. 4). This protein was also detected in the spleen, liver, and testis (data not shown). Thus the large molecular weight protein detected by DRP antisera has a tissue distribution different than that found for dystrophin.

We have identified a new protein species which shares the molecular weight, cellular abundance, and at least partial sequence homology with dystrophin but differs significantly in its tissue distribution. It will be important to determine if DRP is homologous to dystrophin over its entire length and, therefore, a true member of the spectrin/α-actinin/dystrophin family. In this respect, it is interesting to note a recent publication which described a cross-reactive protein recognized by anti-dystrophin antibodies raised against either the amino or carboxyl termini of dystrophin (15). This cross-reactive protein was of identical molecular weight as dystrophin yet, unlike dystrophin, it was present in Duchenne dystrophy muscle, mdx mouse muscle, and many non-muscle tissues. We feel that it is very likely that this cross-reactive protein detected by antibodies against dystrophin (15) is in fact the DRP species described in this communication. If
DRP and the cross-reactive protein are indeed identical, then the fact that amino-terminal dystrophin antibodies appeared to recognize the same protein implies that the sequence homology between dystrophin and DRP exists not only at the carboxyl terminus (12) but might in fact extend through the amino terminus.

Our present findings concerning the identification of DRP and the previous findings concerning cross-reaction of some dystrophin antisera suggest due caution be applied to the interpretation of dystrophin abnormalities in neuromuscular disease patients. Specifically, the inadvertent visualization of dystrophin-related protein could complicate the diagnostic interpretation of these biochemical assays, which are currently in widespread use. For example, if a poorly characterized anti-dystrophin antibody is used on muscle from a Duchenne patient and DRP is visualized, then the patient might be incorrectly interpreted as having significant levels of dystrophin and, therefore, diagnosed as a non-Duchenne patient. In this regard, it is important to note that a few recent papers using new previously uncharacterized dystrophin antisera have reported what was interpreted as dystrophin in Duchenne dystrophy patients (17, 18). It is quite possible that these investigators were in fact visualizing the DRP we have described in this communication. Additionally, in view of this type of immunological cross-reactivity it is possible that DRP antisera may recognize other members of the superfamily such as dystrophin or hitherto uncharacterized proteins such as the 400-kDa protein from Torpedo electrolyte (24, 25) or those visualized at the myotendinous junctions in mdx mice (26).

The identification of DRP brings to mind a number of questions concerning the possible role of this protein during development and health and its presence or absence during the continuum of disease. Based solely upon the limited sequence similarity reported, a role for DRP in modulating the dystrophin and, therefore, diagnosed as a non-Duchenne patient. In this regard, it is important to note that a few recent papers using new previously uncharacterized dystrophin antisera have reported what was interpreted as dystrophin in Duchenne dystrophy patients (17, 18). It is quite possible that these investigators were in fact visualizing the DRP we have described in this communication. Additionally, in view of this type of immunological cross-reactivity it is possible that DRP antisera may recognize other members of the superfamily such as dystrophin or hitherto uncharacterized proteins such as the 400-kDa protein from Torpedo electrolyte (24, 25) or those visualized at the myotendinous junctions in mdx mice (26).

The identification of DRP brings to mind a number of questions concerning the possible role of this protein during development and health and its presence or absence during the continuum of disease. Based solely upon the limited sequence similarity reported, a role for DRP in modulating dystrophic phenotypes has been suggested (19, 20). Though it is tempting to speculate that DRP deficiency could lead to a dystrophic phenotype, its apparent widespread tissue distribution would suggest that a disorder caused by abnormal expression of DRP might manifest by involving more tissues than usually involved in most inherited neuromuscular disorders. We are currently screening patient biopsies to study these questions. It has been asked if DRP might interact with dystrophin by forming a heterodimer (19) much like the homologous α- and β-spectrins do. Dystrophin and DRP are indeed similar in size and possibly structure, but we have demonstrated differences in tissue distribution of DRP and dystrophin. The α- or β-spectrins which are normally found as heterodimers are usually co-expressed in mammalian tissues (10). However, the mismatch of tissue distribution does not exclude this possibility, since it has recently been reported that only β-spectrin is detectable postsynaptically at rat myotubular neuromuscular junctions (21). In addition, in chicken it has been shown that tissue-specific β-spectrin isoforms associate with the same α-spectrin in different tissues (10); a process similar to this may be occurring with respect to dystrophin and DRP. Clearly, more information is needed about the functions of dystrophin, DRP, and other members of the spectrin superfamily before conclusions can be drawn about their functional relationships.

Acknowledgments—We wish to thank Dr. R. L. Neve for suggesting the use of Geneclean and all members of the laboratory for support. We thank Dr. Tim Byers for a valuable critical review of the manuscript.

REFERENCES
1. Hoffman, E. P., Brown, R. H., Jr., and Kunkel, L. M. (1987) Cell 51, 919-928
2. Hoffman, E. P., Fischbeck, K. H., Brown, R. H., Jr., Johnson, M., Medori, R., Loike, J. D., Harris, J. B., Waterston, R., Brooke, M., Specht, L., Kupsky, W., Chamberlain, J., Gaskey, C. T., Shanorro, F., and Kunkel, L. M. (1988) N. Engl. J. Med. 318, 1363-1368
3. Koenig, M., Hoffman, E. P., Bertelson, C. J., Monaco, A. P., Feener, C., and Kunkel, L. M. (1987) Cell 50, 508-517
4. Hoffman, E. P., Hadeck, M. S., Rosenberg, P. A., Pollina, C. M., and Kunkel, L. M. (1988) Neuron 1, 411-420
5. Koenig, M., Monaco, A. P., and Kunkel, L. M. (1988) Cell 53, 219-228
6. Byers, T. J., Husain-Chishti, A., Dubreuil, R. R., Branton, D., and Goldstein, L. S. B. (1989) J. Cell Biol. 109, 1033-1042
7. Davison, M. D., and Critchley, D. R. (1988) Cell 52, 159-160
8. Blanchard, A., Ohanian, V., and Critchley, D. R. (1989) J. Muscle Cell Motil. 10, 280-289
9. Hoffman, E. P., Watkins, S. C., Slayter, H. S., and Kunkel, L. M. (1989) J. Cell Biol. 108, 503-510
10. Coleman, T. R., Fishkind, D. J., Mooskeller, M. S., and Morrow, J. S. (1989) Cell Motil. Cytoskeleton 12, 225-247
11. Fishkind, D. J., Bondur, E. M., and Begg, D. A. (1987) Cell Motil. Cytoskeleton 7, 304-314
12. Love, D. R., Hill, D. F., Dickson, G., Spurr, N. K., Byth, B. C., Marsden, R. F., Walsh, F. S., Edwards, Y. H., and Davies, K. E. (1989) Nature 339, 59-66
13. Diekmann, C. L., and Tzagoloff, A. (1985) J. Biol. Chem. 260, 1513-1520
14. Hoffman, E. P., Morgan, J. E., Watkins, S. C., and Partridge, T. A. (1990) J. Neural. Sci. in press
15. Hoffman, E. P., Begg, A. H., Koenig, M., Kunkel, L. M., and Angelini, L. (1989) Lancet ii, 1211-1212
16. Hoffman, E. P., Monaco, A. P., Feener, C. C., and Kunkel, L. M. (1987) Science 238, 347-350
17. Ginjier, J. L., Bakker, E. de, Denunen, J. T., van Pason, M. M. B., van Ommen, G. B., Zubrzyacka-Gaarn E., Kooosterman, M. D., Wessels, A., and Moorman, A. P. M. (1989) Lancet 11, 1212-1213
18. Nicholson, L. V. D., Davison, K., Johnson, M. A., Slater, C. R., Young, C., Bhattacharya, S., Gardner-Medwin, D., and Harris, J. B. (1989) J. Neural. Sci. in press
19. Mandel, J. L. (1988) Nature 335, 584-586
20. England, S. B., Nicholson, L. V. D., Johnson, M. A., Forrest, S. M., Love, D. R., Zubrzyacka-Gaarn, E., Bulman, D. E., Harris, J. B., and Davies, K. E. (1990) Nature 343, 180-182
21. Rloch, R. J., and Morrow, J. S. (1989) J. Cell Biol. 108, 481-493
22. Koenig, M., and Kunkel, L. M. (1990) J. Biol. Chem. 265, 4590-4596
23. Hoffman, E. P., Kunkel, L. M., Angelini, C., Clarke, A., Johnson, M., and Harris, J. B. (1989) Neurology 39, 1011-1017
24. Chang, H. W., Bock, E., and Bonilla, E. (1989) J. Biol. Chem. 264, 25831-25834
25. Jasmin, B. J., Cartaud, A., Ludosky, M. A., Changeux, J. P., and Mirsat, C. E., and Bonilla, E. (1990) Muscle & Nerve 13, 493-500
26. Towham, H., Stachelin, T., and Gordon, J. (1979) Proc. Natl. Acad. Sci. U. S. A. 76, 4350-4354
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J. Biol. Chem. 1990, 265:16717-16720.

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