Minireview

Dystrophin and utrophin: the missing links!

S.J. Winder[a,*], T.J. Gibson[b], J. Kendrick-Jones[a]

[a]*MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, UK*
[b]*EMBL, Postfach 10.2209, Meyerhofstrasse 1, D69012 Heidelberg, Germany*

Received 3 April 1995

Abstract There is considerable sequence homology between dystrophin and utrophin, both at the protein and DNA level, and consequently it was assumed that their domain structures and functions would be similar. As more of the detailed biochemical and cell biological properties of these two proteins become known, so it becomes clear that there are subtle if not significant differences between them. We review recent findings and present new hypotheses into the structural and functional properties of the actin-binding domain, central coiled-coil region and regulatory/membrane protein-binding regions of dystrophin and utrophin.

Key words: Utrophin; Dystrophin; Spectrin; Actin binding; Coiled-coil

1. Introduction

One of the crucial links involved in maintaining the integrity of the cell membrane during muscle contraction is the cytoskeletal protein dystrophin. Consequently mutations or deletions in the dystrophin gene, located on the X-chromosome, lead to fragility in the muscle membrane and necrosis associated cell death, as typified in the X-linked myopathies Becker and Duchenne muscular dystrophy [1]. Utrophin, the autosomal homologue of dystrophin, shares considerable sequence homology [2], suggesting that it may have similar functions and raising the possibility of utrophin being used as a therapeutic replacement for dystrophin in muscular dystrophy [3]. Dystrophin and utrophin also share sequence homology with other cytoskeletal proteins such as the spectrins and α-actinin with which they form a broad family. The overall domain structure of dystrophin and utrophin is shown in Fig. 1b. The NH_2-terminal regions of dystrophin and utrophin bind to the actin cytoskeleton, acting as the intracellular anchor whereas the COOH-terminal regions bind to a group of proteins anchored in the cell membrane (Fig. 2). These two regions are connected by a long, potentially flexible rod which comprises over 70% of the protein. The complex of proteins anchored in the membrane, the dystrophin/utrophin-associated proteins in turn bind to components of the extracellular matrix, mainly laminin. Thus dystrophin and utrophin form a link between the actin cytoskeleton and the dystrophin/utrophin-associated protein complexes which can be considered to be new additions to the growing family of cell anchoring molecules.

*Corresponding author. Fax: (44) (1223) 213556.
E-mail: sjw@mrclmb.cam.ac.uk

2. Actin binding domain

Although the NH_2-terminal actin binding regions in dystrophin and utrophin were initially identified on the basis of sequence similarity to the actin binding domain of α-actinin, recent work has shown that the bacterially expressed domains from dystrophin and utrophin do indeed bind actin in vitro [4,5] and in transfected or microinjected cells [5,6]. Furthermore, in vitro studies have demonstrated that purified dystrophin or dystrophin enriched fractions also cosediment with F-actin [7–9]. Actin binding has been demonstrated by NMR, cosedimentation and solid-phase assay, using peptides, expressed fusion and non-fusion proteins and native dystrophin [4,5,7–12]. Given the variety of techniques employed, the findings are in broad agreement, with binding affinities of dystrophin and utrophin for F-actin in the high nM to low μM range. In one comparative study [5] a bacterially expressed utrophin construct was shown to bind to skeletal muscle F-actin with ~2-fold higher affinity than the equivalent region of dystrophin, furthermore, both dystrophin and utrophin constructs bound to non-muscle (platelet) F-actin with ~4-fold higher affinity than to skeletal muscle actin. This clear demonstration that dystrophin and utrophin bind to non-muscle actin with higher affinity suggests that in muscle cells dystrophin binds to a cortical submembrane network of non-muscle actin and not directly to the sarcomeric actin. Whilst deletion analysis of the actin binding domain of dystrophin and utrophin has revealed three distinct regions important for actin binding (called ABS1, ABS2, ABS3 from NH_2- to COOH-terminal respectively [5], Fig. 1a), none appears to be absolutely essential for actin binding and it is likely that all three contribute in some way to the overall actin binding 'pocket' in the tertiary structure of these proteins [5,8].

One recently discovered difference in behaviour between the actin binding domains of dystrophin and utrophin, however, is the interaction with calmodulin. Although the bacterially expressed actin binding domains from both dystrophin and utrophin bound to calmodulin affinity columns in the presence of calcium [13,14], but not in the absence, only utrophin binding to actin was inhibited by calmodulin in a calcium-dependent manner [14]. Given the high degree of sequence conservation in this region in these two proteins, the reasons for this difference in behaviour may have to await the elucidation of their tertiary structures.

3. Coiled-coil region

The central region of the dystrophin and utrophin molecule consists of a series of weakly repeating units of ~110 amino acids

SSDI 0014-5793(95)00398-3

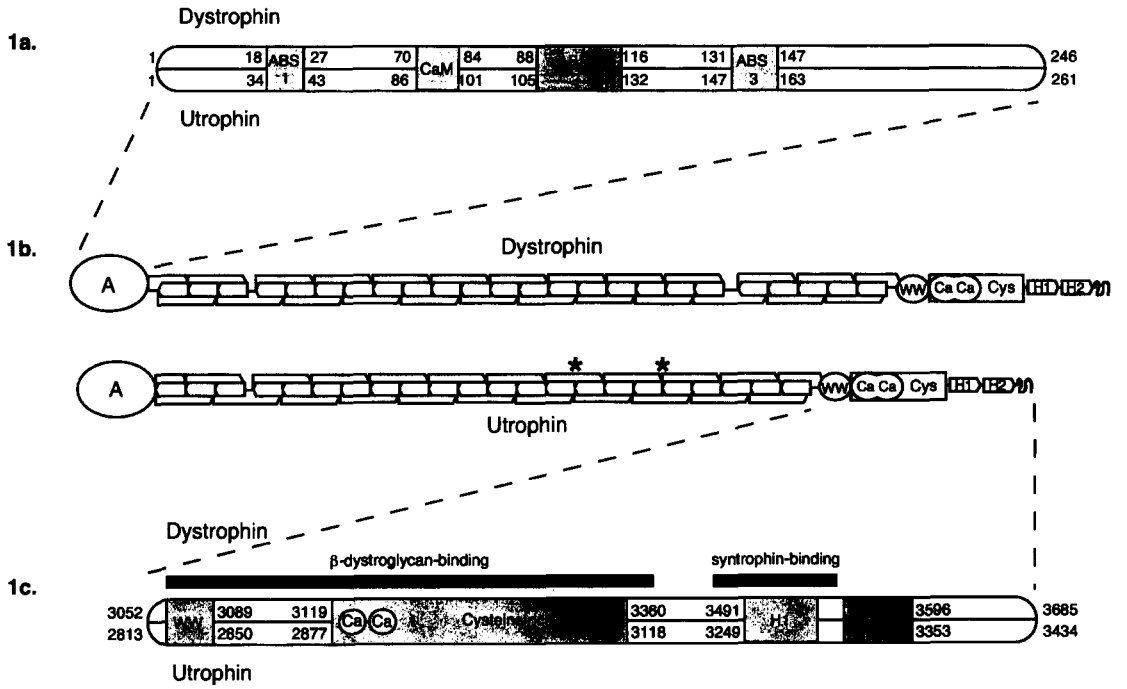


Fig. 1. Domain structure of Dystrophin and Utrophin. All diagrams are shown with the NH_2-terminus at the left. Numbers refer to amino acid residues delimiting the domains or regions in the respective sequences, dystrophin on top and utrophin on the bottom. (a) Schematic view of the actin binding domains of dystrophin and utrophin with principal regions shaded. ABS1–3 represent the major actin binding regions and CaM represents a putative calmodulin binding site. (b) Representation of the whole dystrophin and utrophin domain structure indicating the principal domains. A, actin binding; WW, WW or WWP domain; Ca Ca, calcium binding EF-hands; Cys, cysteine rich; H1 and H2, helices 1 and 2, respectively. The central coiled coil regions comprising 24 and 22 repeats in dystrophin and utrophin respectively are unlabelled; *above utrophin indicate the relative positions of missing repeats 15 and 19. (c) Schematic view of the COOH-terminal domains of dystrophin and utrophin with principal regions shaded. Nomenclature is as above, the shaded bars above the dystrophin sequence represent the regions involved in binding the dystrophin-associated proteins β-dystroglycan and syntrophin.

with similarity to the coiled-coil repeats of spectrin [15,16]. Analysis of the dystrophin sequence revealed 24 or 25 repeats separated by 4 proline-rich hinge regions [17,18], similarly utrophin is thought to contain 22 repeats and two hinges, see below. Dotplots reveal, that compared to dystrophin, utrophin is missing repeats 15 and 19 (conversely dystrophin could have gained repeats at these positions). Cross et al. [18] proposed that the structure of the coiled coil repeat would comprise one short helix and the NH_2- and COOH-terminal halves of separate flanking long helices. CD analysis of an expressed dystrophin repeat [19] and the recent solution of the crystal structure of a spectrin repeat [20] would agree with this model. This arrangement of nested repeats and hinges would suggest a highly elastic and flexible molecule. It should be stressed however, that the conservation between repeat units in dystrophin and utrophin is much weaker than that seen in spectrin, particularly when one compares the sequence alignments, and the numbers of conserved residues and insertions between the repeats of α- and β-spectrin with respect to dystrophin and utrophin (see Fig 3).

There are more conserved residues in the aligned spectrin sequences than in dystrophin and utrophin (compare the overall amount of colour in Fig. 3a vs. 3b). The lengths of the helices in the spectrins are more consistent and well defined and contain considerably fewer insertions than do those in dystrophin and utrophin. Compared to the spectrins the B helices in dystrophin and utrophin are less regular, repeats 10 and 14 in dystrophin and 14 in utrophin are truncated, there are large insertions after the B helix in repeats 23/21 of dystrophin/utrophin and large insertions within the A helix in repeats 3, 15 and 19 of dystrophin and 3, 13, and 17 of utrophin. The insertions in the A helix (helix C in one repeat is continuous with helix A in the next) would be especially unfavourable for antiparallel dimer formation due to the insertions putting the helices out of register. α- and β-spectrin are known to form anti-parallel dimers, so surface residues should be conserved in the dimer interface. There are 21 extra residues conserved in each spectrin repeat and they map to one external face of the domain structure, consisting of helix A and half of helix B: therefore this region is likely to be the dimer interface. The conservation pattern suggests that the NH_2-terminus of helix A packs in a groove formed by the COOH-terminus of helix A′ and the NH_2-terminus of helix B′ in the apposing dimer. By contrast, all of the conserved residues in dystrophin and utrophin map exclusively to core or interface positions with none available to form a surface for dimer interface as in spectrin.

Electron micrographs of rotary shadowed dystrophin molecules have revealed a heterogeneous population comprising mostly monomers, but also some dimers, end to end and side by side, and higher order structures [21,22]. Recently, however, Sato et al. suggested that the dumbbell shaped structures seen in their micrographs may have been collagen VI contaminants and not dystrophin [23]. Ervasti et al. [24] claimed that alkali treated dystrophin (which by their own admission was not completely pure) sedimented at a size consistent with a dystrophin dimer, though no data were presented. The lack of sequence conservation among the surface residues of the dystro-

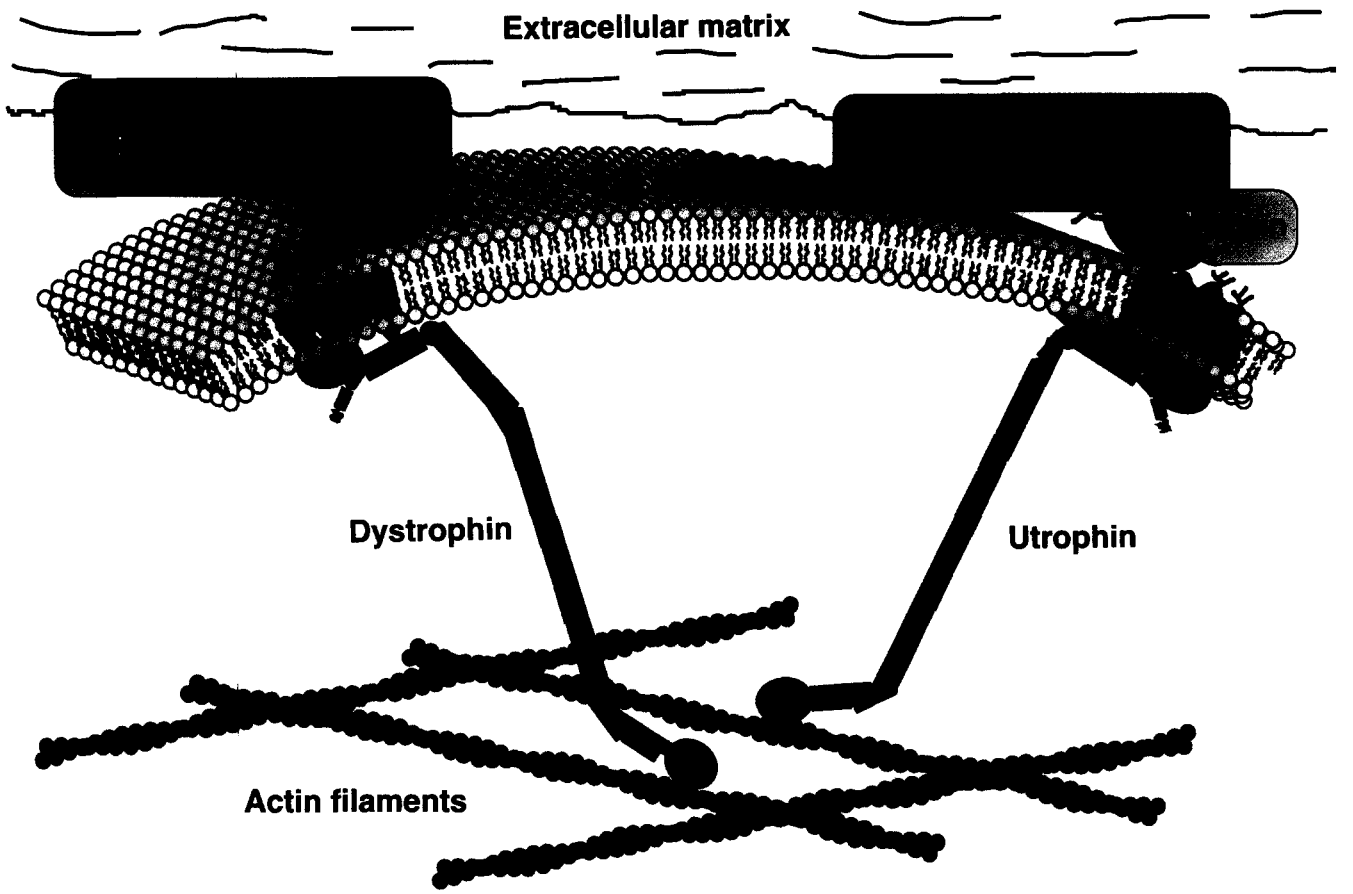


Fig. 2. A representation of the organisation of dystrophin and utrophin and associated proteins in a hypothetical cell. Ad, adhalin; 35, 35 kDa dystrophin- (utrophin-)associated glycoprotein (DAG); 25, 25 kDa dystrophin- (utrophin-)associated protein (DAP); β, β-dystroglycan; α, α-dystroglycan; syn, syntrophin.

phin and utrophin coiled coil regions and the insertion of loops between some of these regions argues strongly against side by side dimerisation along the whole length of these molecules. It is possible that dystrophin and utrophin may dimerise in an end to end arrangement via the COOH-terminal coiled coil regions [25] or possibly by a staggered overlap involving a short region of dimerisation in the coiled coil region. There is at present, however, no direct biochemical evidence to support any of these hypotheses.

4. Regulatory/membrane protein-binding regions

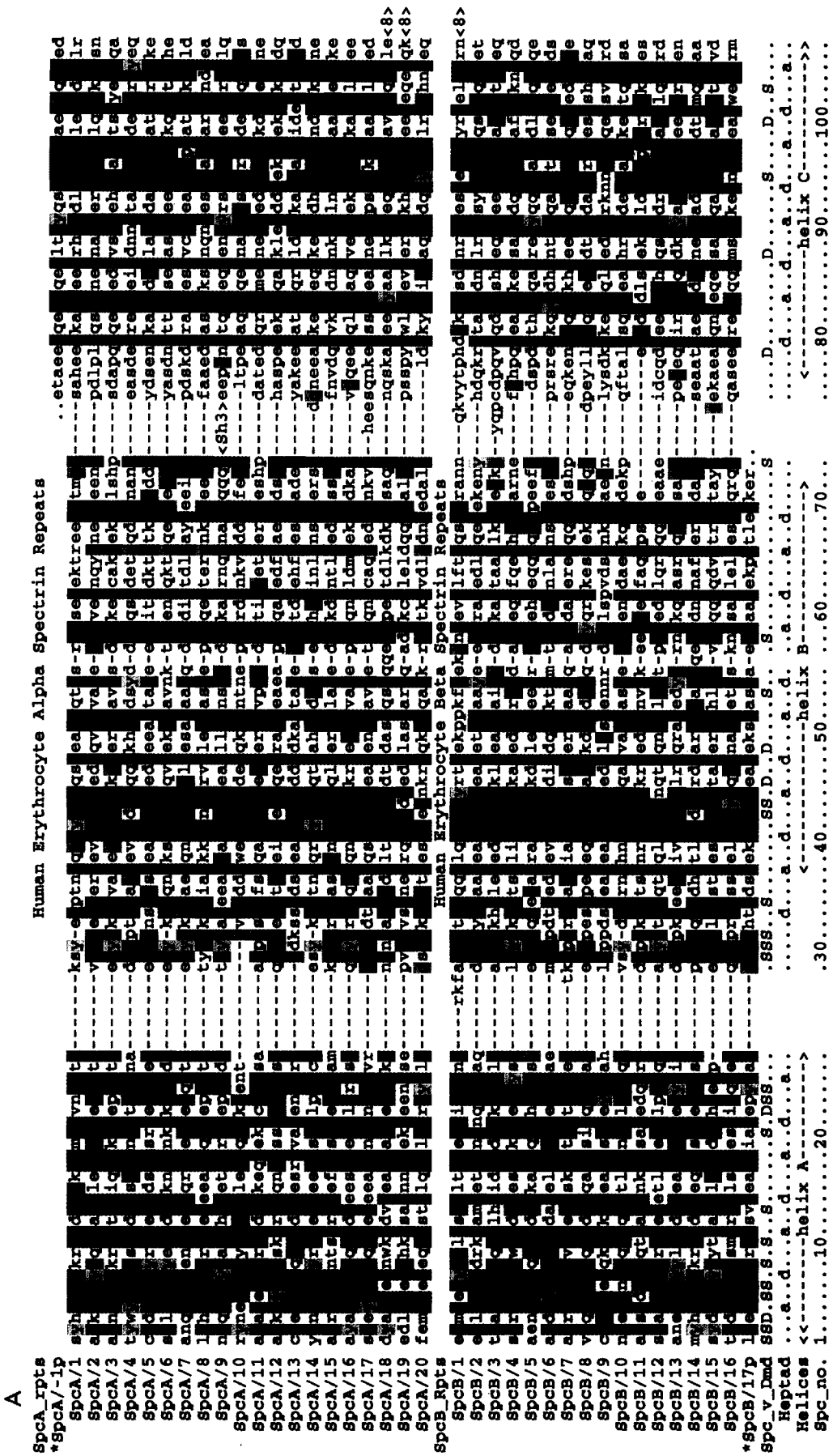
The carboxy-terminal regions of dystrophin and utrophin present a complex arrangement of domains (Fig. 1c) whose functions are only just beginning to be elucidated. The COOH-terminus is clearly involved in binding to the membrane glycoprotein complex [26,27] and possibly to other proteins. These interactions may be regulated by other domains within the COOH-terminus: namely the WW/WWP domain [28,29] or the Ca^{2+}-binding domain [16], alternatively these two regulatory domains may be involved in other as yet uncharacterised functions.

4.1. Membrane protein-binding regions

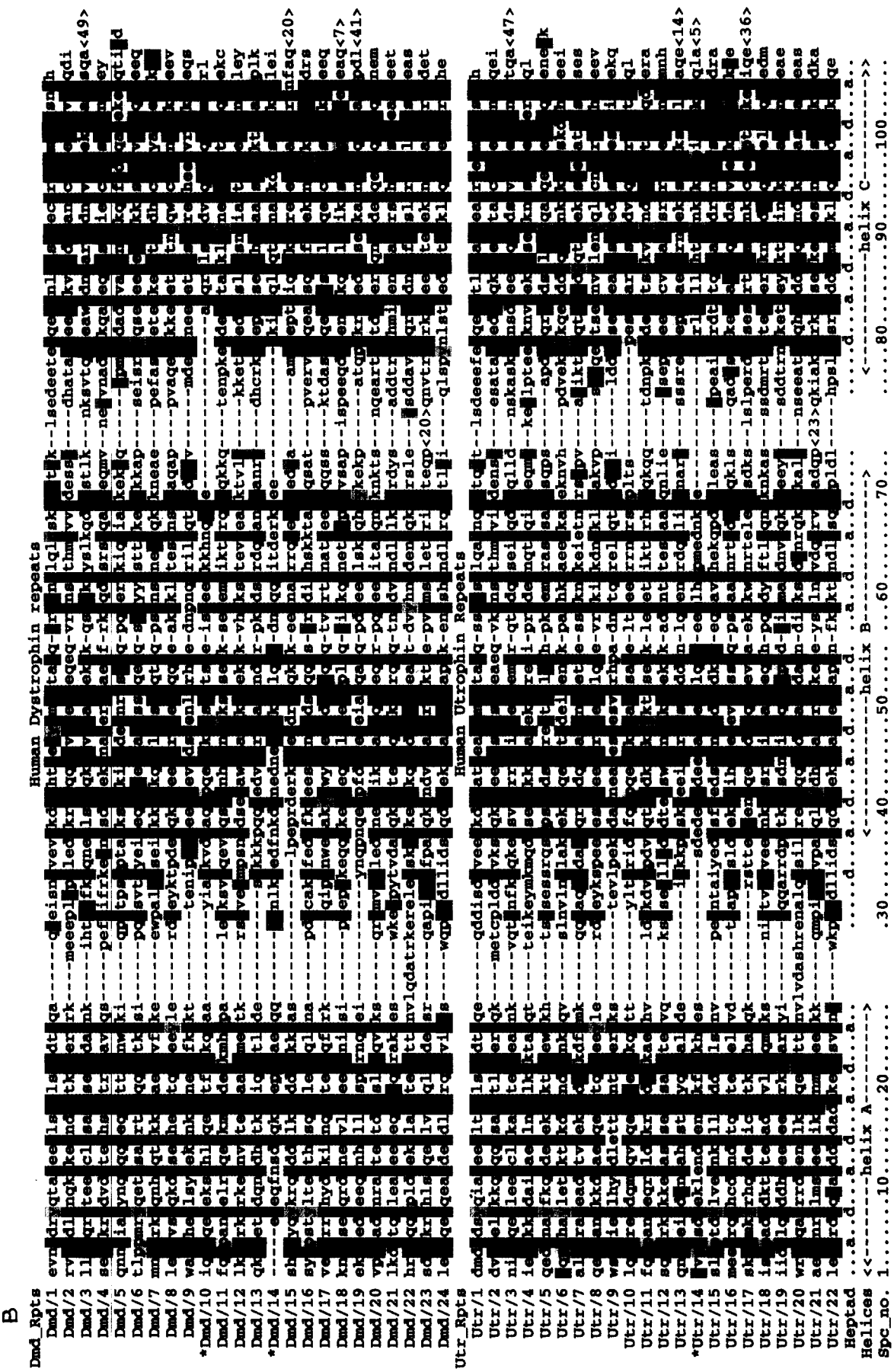
A full discussion of the dystrophin- and utrophin-associated proteins is beyond the scope of this short article, and readers are directed to a recent review [30]. Two of these proteins, however are pertinent to this discussion: the 43 kDa transmem-

(*Figure see pp. 4 and 5*)

Fig. 3. Alignment of spectrin-type triple-helical repeats in α-spectrin, β-spectrin, dystrophin and utrophin, colour coded to highlight conserved features. (a) Repeats from α- and β-spectrins. *Denotes the partial terminal repeats thought to mediate end-to-end association of α- and β-spectrin. Gaps in unconserved loops have been minimised and deleted sequences are recorded within angle brackets: $\langle n \rangle$. Comment lines below the sequence annotate the 3 α-helices; Spc v Dmd, the positions showing greater conservation in spectrins (S) or in dystrophin/utrophin (D); heptad, the helical heptad periodicity; helices, helix name for identification purposes, it should be noted that helix C is continuous with helix A in the following repeat; Spc no., residue number as for the solved structure of a Drosophila repeat [20]. All G (orange) and P (yellow) residues are coloured. Other colouring is by conserved property in >55% of any column: uncoloured residues lack a sufficiently conserved property. Blue, hydrophobic; light blue, partially hydrophobic; red and pink, positive; purple, negative; green, hydrophilic. The figure was prepared with the GDE alignment editor (S. Smith, Harvard) and COLORMASK (J. Thompson, EMBL). (b) Repeats from dystrophin and utrophin displayed as in (a). *Indicates aberrant repeats with truncated helices.


A
Human Erythrocyte Alpha Spectrin Repeats
Human Erythrocyte Beta Spectrin Repeats
SpcA_rpts
*SpcA/-1p
SpcA/1
SpcA/2
SpcA/3
SpcA/4
SpcA/5
SpcA/6
SpcA/7
SpcA/8
SpcA/9
SpcA/10
SpcA/11
SpcA/12
SpcA/13
SpcA/14
SpcA/15
SpcA/16
SpcA/17
SpcA/18
SpcA/19
SpcA/20
SpcB_Rpts
SpcB/1
SpcB/2
SpcB/3
SpcB/4
SpcB/5
SpcB/6
SpcB/7
SpcB/8
SpcB/9
SpcB/10
SpcB/11
SpcB/12
SpcB/13
SpcB/14
SpcB/15
SpcB/16
*SpcB/17p
spc_v_Dmd
Heptad
Helices
Spc_no.

B


Human Dystrophin repeats
Human Utrophin Repeats
Dmd_Rpts
Utr_Rpts
Heptad
Helices
Spc_no.

brane glycoprotein, β-dystroglycan [31] and a heterologous group of 58–59 kDa intracellular proteins [32–34] known as the syntrophins. Recent elegant studies from Ozawa's group [27,35,36] have delineated the regions in dystrophin involved in the binding of β-dystroglycan and the α1- and β1-syntrophins; they are the cysteine-rich region and first helical region (H1) of the COOH-terminus (Fig. 1c). β-dystroglycan binds tightly to the proximal part of the cysteine-rich region even overlapping into the WW/WWP domain, with a weaker interaction at the distal end of this region [36]. α1-syntrophin binds to a region between the end of the cysteine-rich region and start of the first COOH-terminal helical region, and β1-syntrophin binds to the first helical region itself [27].

It has been demonstrated, using the yeast two-hybrid system, that this first helical region of dystrophin interacted with the fast skeletal muscle isoform of troponin T [37]. It is difficult, however, to reconcile this latter finding in terms of our current understanding of the organisation of dystrophin and the dystrophin-associated proteins in the submembrane cytoskeleton of muscle and non-muscle cells.

We have proposed [25] that the helical regions at the COOH-termini of dystrophin and utrophin may be involved in protein–protein interactions. Analysis of these helical regions reveals a propensity for parallel dimeric coiled-coil interactions, possibly involved in dimerisation of dystrophin or utrophin molecules, or interaction with other proteins. Interestingly, using the Lupas algorithm [38] none of the syntrophins are predicted to contain regions that will form coiled coils, suggesting an alternative mode of binding to helix 1 of dystrophin/utrophin. Nevertheless, if syntrophins do bind to the first helical region, there is still the potential for other protein–protein interactions with the second helix H2 (Figs. 1c, 2).

4.2. Regulatory regions

Analysing the sequences of the two potential EF-hand Ca^{2+}-binding motifs identified in the dystrophin and utrophin sequences [2,16] suggests, that the absence of crucial amino acids in the co-ordinating positions within the binding loops would mean that these sites should not bind calcium. Recent structural information has revealed, however, that despite the apparent absence of essential amino acids in the Ca^{2+}-coordinating positions, the scallop myosin essential light chain is still able to bind calcium. In this case the missing ligating side chains are contributed by the other light chain and the myosin heavy chain which interact to form a functional binding loop [39]. These observations may explain why Milner et al. [40] were able to detect calcium binding in a GST-fusion construct comprising residues 3,107–3,400 of dystrophin, whereas Ervasti and Campbell failed to detect calcium binding in [^{45}Ca] gel-overlays of blotted whole dystrophin [7], possibly due to the inability of whole dystrophin to re-fold correctly after blotting. Furthermore, calmodulin overlays with whole dystrophin failed to detect calmodulin binding [7] despite positive results being obtained with discrete expressed domains [13,14]. Negative results in gel-overlay experiments therefore, cannot necessarily be construed as evidence of lack of function. Given the degree of conservation in the EF-hand regions of dystrophin and utrophin it is quite likely that utrophin also binds calcium. The precise function, structural or regulatory, of calcium binding in dystrophin and utrophin is not known; it may regulate binding to the membrane-associated protein complex, it may even regulate actin binding as in α-actinin and spectrin [14] or it may be involved purely in a structural role (as a Mg^{2+}/Ca^{2+} binding site). Further experiments are needed to clarify these points.

The most recently identified domain within dystrophin is the WW or WWP domain [28,29], so called because of two conserved tryptophan (single letter code, W) and a proline (P) residues flanking the ~30 amino acid region. It has been proposed that the WW/WWP domain is somehow involved in protein–protein interactions, possibly fulfilling a role similar to the Src homology (SH2, SH3) or pleckstrin homology domains. The WW/WWP domain appears to be inside the region in dystrophin required for β-dystroglycan binding [27,36] and as such may be involved in that interaction, further analyses are required to substantiate these suggestions.

Structural and functional analyses are beginning to shed light on the roles of dystrophin and utrophin in the cytoskeleton. It is clear however, that there is still a long way to go before we can confidently fill in the missing link.

Acknowledgements: This work was funded in part by the Muscular Dystrophy Group of Great Britain and Northern Ireland.

References

[1] Emery, A.E.H., Duchenne Muscular Dystrophy, 1993, Oxford University Press, Oxford, pp. 392.
[2] Tinsley, J.M., Blake, D.J., Roche, A., Fairbrother, U., Riss, J., Byth, B.C., Knight, A.E., Kendrick-Jones, J., Suthers, G.K., Love, D.R., Edwards, Y.H. and Davies, K.E. (1992) Nature 360, 591–593.
[3] Tinsley, J.M. and Davies, K.E. (1993) Neuromusc. Disord. 3, 537–539.
[4] Way, M., Pope, B., Cross, R.A., Kendrick-Jones, J. and Weeds, A.G. (1992) FEBS Lett. 301, 243–245.
[5] Winder, S.J., Hemmings, L., Maciver, S.K., Bolton, S.J., Tinsley, J.M., Davies, K.E., Critchley, D.R. and Kendrick-Jones, J., (1995) J. Cell Sci. 108, 63–71.
[6] Hemmings, L., Kuhlman, P.A. and Critchley, D.R. (1992) J. Cell Biol. 116, 1369–1380.
[7] Ervasti, J.M. and Campbell, K.P. (1993) J. Cell Biol. 122, 809–823.
[8] Fabbrizio, E., Bonet-Kerrache, A., Leger, J.J. and Mornet, D. (1993) Biochemistry. 32, 10457–10463.
[9] Senter, L., Luise, M., Presotto, C., Betto, R., Teresi, A., Ceoldo, S. and Salviati, G. (1993) Biochem. Biophys. Res. Commun. 192, 899–904.
[10] Corrado, K., Mills, P.L. and Chamberlain, J.S. (1994) FEBS Lett. 344, 255–260.
[11] Levine, B.A., Moir, A.J.G., Patchell, V.B. and Perry, S.V. (1990) FEBS Lett. 263, 159–162.
[12] Levine, B.A., Moir, A.J.G., Patchell, V.B. and Perry, S.V. (1992) FEBS Lett. 298, 44–48.
[13] Bonet-Kerrache, A., Fabbrizio, E. and Mornet, D. (1994) FEBS Lett. 355, 49–53.
[14] Winder, S.J. and Kendrick-Jones, J. (1995) FEBS Lett. 357, 125–128.
[15] Davison, M.D. and Critchley, D.R. (1988) Cell 52, 159–160.
[16] Koenig, M., Monaco, A.P. and Kunkel, L.M. (1988) Cell 53, 219–228.
[17] Koenig, M. and Kunkel, L.M. (1990) J. Biol. Chem. 265, 4560–4566.
[18] Cross, R.A., Stewart, M. and Kendrick-Jones, J. (1990) FEBS Lett. 262, 87–92.
[19] Kahana, E., Marsh, P.J., Henry, A.J., Way, M. and Gratzer, W.B. (1994) J. Mol. Biol. 235, 1271–1277.
[20] Yan, Y., Winograd, E., Viel, A., Cronin, T., Harrison, S.C. and Branton, D. (1993) Science 262, 2027–2030.
[21] Pons, F., Augier, N., Heilig, R. and Leger, J.J. (1990) Proc. Natl. Acad. Sci. USA 87, 7851–7855.

[22] Sato, O., Nonomura, Y., Kimura, S. and Maruyama, K., (1992) J. Biochem. 112, 631–636.
[23] Kake, T., Spissinger, T. and Maruyama, K. (1994) Muscle Nerve S1, S37.
[24] Ervasti, J.M., Kahl, S.D. and Campbell, K.P. (1991) J. Biol. Chem. 266, 9161–9165.
[25] Blake, D.J., Tinsley, J.M., Knight, A.E., Winder, S.J., Kendrick-Jones, J. and Davies, K.E. (1995) Trends Biochem. Sci. 20, 133–135.
[26] Ahn, A.H. and Kunkel, L.M. (1995) J. Cell Biol. 128, 363–371.
[27] Suzuki, A., Yoshida, M. and Ozawa, E. (1995) J. Cell Biol. 128, 373–381.
[28] Bork, P. and Sudol, M. (1994) Trends Biochem. Sci. 19, 531–533.
[29] Andre, B. and Springael, J.Y. (1994) Biochem. Biophys. Res. Commun. 205, 1201–1205.
[30] Tinsley, J.M., Blake, D.J., Zuellig, R.A. and Davies, K.E. (1994) Proc. Natl. Acad. Sci. USA 91, 8307–8313.
[31] Ibraghimov-Beskrovnaya, O., Ervasti, J.M., Leveille, C.J., Slaughter, C.A., Sernett, S.W. and Campbell, K.P. (1992) Nature 355, 696–702.
[32] Ahn, A.H., Yoshida, M., Anderson, M.S., Feener, C.A., Selig, S., Hagiwara, Y., Ozawa, E. and Kunkel, L.M. (1994) Proc. Natl. Acad. Sci. USA 91, 4446–4450.
[33] Yang, B., Ibraghimov-Beskrovnaya, O., Moomaw, C.R., Slaughter, C.A. and Campbell, K.P. (1994) J. Biol. Chem. 269, 6040–6044.
[34] Adams, M.E., Butler, M.H., Dwyer, T.M., Peters, M.F., Murnane, A.A. and Froehner, S.C. (1993) Neuron 11, 531–540.
[35] Suzuki, A., Yoshida, M., Yamamoto, H. and Ozawa, E., (1992) FEBS Lett. 308, 154–160.
[36] Suzuki, A., Yoshida, M., Hayashi, K., Mizuno, Y., Hagiwara, Y. and Ozawa, E. (1994) Eur. J. Biochem. 220, 283–292.
[37] Pearlman, J.A., Powaser, P.A., Elledge, S.J. and Caskey, C.T. (1994) FEBS Lett. 354, 183–186.
[38] Lupas, A., Dyke, M.V. and Stock, J. (1991) Science 252, 1162–1164.
[39] Xie, X., Harrison, D.H., Schlichting, I., Sweet, R.M., Kalabokis, V.N., Szent-Gyorgi, A.G. and Cohen, C. (1994) Nature 368, 306–312.
[40] Milner, R.E., Bussan, J.L. and Michalak, M. (1992) Biochem. J. 288, 1037–1044.