

## Minireview

Conservation of components of the dystrophin complex in *Drosophila*<sup>1</sup>

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**Abstract** Defects in the dystrophin complex (DC) underlie several human genetic disorders, but our dissection of its function is complicated by potential redundancy of the multiple vertebrate isoforms of most DC components. We here complete our previous description of *Drosophila* dystrophin, and show that the fly retains all essential components of the DC, but with substantially less diversity. Seventeen known human components (three dystrophin-related proteins, two dystrobrevins, five sarcoglycans, five syntrophins, one dystroglycan and one sarcospan) appear to be reduced to eight in *Drosophila* (one, one, three, two, one and none, respectively). The simplicity of this system recommends it as a model for its human counterpart. © 2000 Federation of European Biochemical Societies. Published by Elsevier Science B.V. All rights reserved.

**Key words:** Dystrophin; Duchenne muscular dystrophy; Dystroglycan; Dystrobrevin; Syntrophin; Sarcoglycan; Limb-girdle muscular dystrophy; *Drosophila melanogaster*

## 1. Introduction

Gene duplication, affording an opportunity for functional divergence, is thought to be of major importance in metazoan evolution. Comparisons of gene families imply two major phases of duplication since vertebrate origins [1]. As a result, a single invertebrate gene is often orthologous to the last common ancestor of a substantial vertebrate gene family. Concordant with this finding, we have previously shown that invertebrates from distinct phyla (nematodes, arthropods, mollusks, echinoderms and protochordates) each have a single protein, orthologous to the last common ancestor of three related vertebrate proteins, dystrophin, utrophin and DRP2 [2].

Duchenne, Becker and the limb-girdle muscular dystrophies are human myopathies caused by disruptions in genes encoding dystrophin [3] or other components of the dystrophin complex (DC; Fig. 1A) expressed in skeletal muscle cells. Together with sarcospan and dystroglycan, the known vertebrate DC comprises three other protein families: the syntrophins ( $\alpha 1$ ,  $\beta 1$ ,  $\beta 2$ ,  $\gamma 1$  and  $\gamma 2$ ), dystrobrevins ( $\alpha$  and  $\beta$ ) and sarcoglycans ( $\alpha$ ,  $\beta$ ,  $\gamma$ ,  $\delta$  and  $\epsilon$ ). In its entirety, the DC is

considered important for maintaining the integrity of skeletal and cardiac muscle cells, with dystrophin serving to increase stability of the sarcolemma during muscular contraction [4,5]. Roles in intracellular signalling have also been proposed, involving neuronal nitric oxide synthase (nNOS) [6–8], voltage-gated sodium channels [9,10] and acetylcholine receptors [11,12], yet it is not clear as to how these might contribute towards normal dystrophin function in vertebrate muscle and other tissues.

The relatively simple and well-characterised musculature and nervous system of *Caenorhabditis elegans* have allowed this invertebrate to be used in recent studies for the investigation of DC function. It has been shown that the *C. elegans* genome contains homologues of vertebrate dystrobrevins and syntrophins, and that their interaction with dystrophin is evolutionarily conserved [13]. Mutations in *dys-1* (dystrophin orthologue) or *dyb-1* (dystrobrevin orthologue) are shown to result in cholinergic signalling defects [14–16]. Unlike *Drosophila melanogaster*, no homologues of vertebrate NOS or voltage-gated sodium channels have been identified in the *C. elegans* genome. The significance of interactions between these proteins and syntrophins is unknown, though it has been proposed that impaired nitric oxide signalling can contribute towards a cardiac and skeletal myopathy [6,7].

As with the nematode, the genome project has now been completed for *D. melanogaster* [17]. Consequently, we have completed our characterisation of the gene encoding the fly dystrophin homologue and further identified expressed sequence homologues of all associated vertebrate proteins. The fly retains the components and presumably the function of the complete DC. With the reduced level of DC complexity shown by phylogenetic analysis (Fig. 2), and its experimental amenability, we propose *D. melanogaster* as a suitable invertebrate model for the study of DC biology.

2. Methods: characterisation of *Drosophila* orthologues

The Berkeley and European *Drosophila* genome databases were searched for sequences homologous to human components of the dystrophin-associated complex (dystrophin, dystrobrevins, syntrophins, sarcoglycans, dystroglycan and sarcospan) using TBLASTN. Fly sequences encoding homologous open reading frames were used to design primers (sequences available on request) for reverse transcription-polymerase chain reaction, rapid amplification of cDNA ends and sequencing. Where possible, expressed-sequence tag contigs were also used for exon characterisation. Protein sequences were aligned using CLUSTALW v1.4 [18], and phylogenetic analysis for each family of proteins was performed using greatest regions of continuous homology between human

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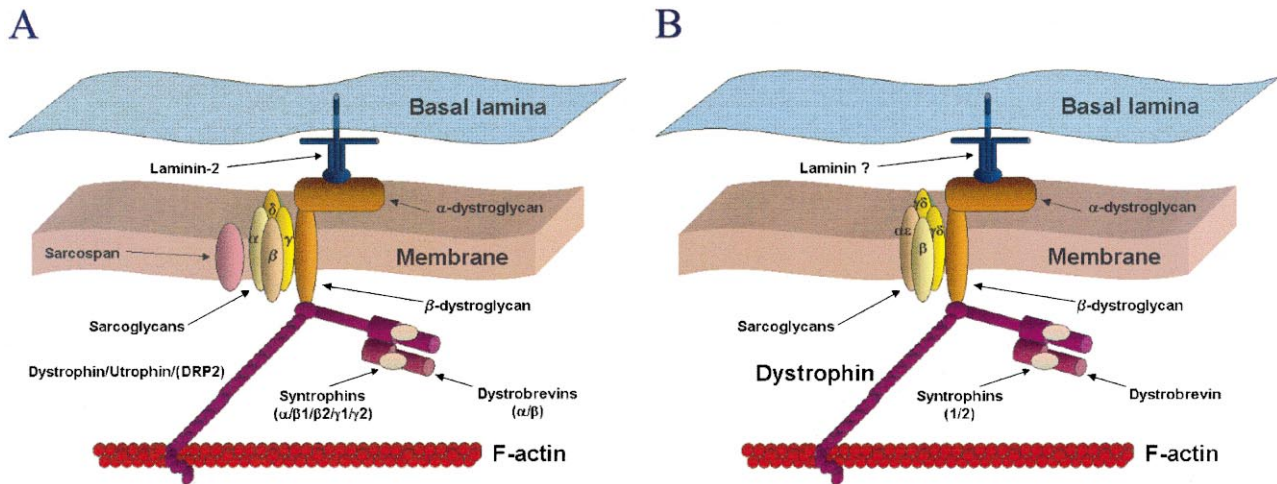


Fig. 1. The main components of the vertebrate DC are conserved in *Drosophila*. (A) Vertebrates: the rod-like dystrophin molecule serves as a link between the actin cytoskeleton (via its N-terminus) and the membrane (via its C-terminal regions). An interaction with  $\beta$ -dystroglycan provides a link with the extracellular matrix, where  $\alpha$ -dystroglycan may associate with laminin, perlecan and agrin. Four sarcoglycans form a heterotetrameric transmembrane complex in close proximity to both sarcospan and  $\beta$ -dystroglycan. The syntrophins interact with equivalent C-terminal regions on dystrophin and the dystrobrevins, which in turn interact with each other. (B) Represented by *D. melanogaster*, the invertebrate DC is expected to maintain the same essential architecture. Flies possess orthologues of all major components, and interactions between dystrophin, dystroglycan, syntrophins and dystrobrevin are likely to be conserved. The fly possesses only three sarcoglycan genes, and it is believed that the  $\gamma/\delta$ -orthologue exists as a dimer in the expected heterotetrameric sarcoglycan structure of flies. We failed to identify a fly orthologue of sarcospan. Flies also possess homologues of other proteins which have been found to interact with the vertebrate DC (e.g. laminins, NOS, voltage-gated sodium channels).

and orthologues in the neighbour-joining option of CLUSTAL. Trees were drawn using TreeView [19] and modified for presentation whilst maintaining branch length and topology.

### 3. The conservation of dystrophin in *Drosophila*

Residing within genomic scaffold clone AE003726, we have found the *Drosophila* orthologue of dystrophin (DmDYS) to be encoded by an unusual gene of at least 130 kb in length (we could not establish the size of one intron), with 31 introns ranging from 61 bp up to 48 kb. No genes were detected within the large introns using NIX to apply a range of bioinformatic gene-detecting algorithms, and the reason for the large size of this gene remains as enigmatic as the human dystrophin gene size. The intron/exon structure is virtually unrelated to that of the human gene (one coding exon is an extraordinary 3.5 kb in length). The 2.7 Mb human dystrophin gene encodes a large protein of 3685 amino acids in length, comprising four main sections: an actin-binding N-terminus, a rod-like spectrin repeat region, a cysteine-rich region (containing a WW domain, four EF-hands and a ZZ domain) and a C-terminal region [3,20]. Using the highly conserved C-terminal end, we previously isolated a similar sequence representative of the sole *Drosophila* dystrophin-like protein [2]. Our recent characterisation of the complete coding sequence for DmDYS reveals a shorter protein (3124 residues) that retains all four sections distinctive of its vertebrate counterpart. The cysteine-rich and C-terminal regions remain the most highly conserved part of DmDYS, with an identity of 54% between human and fly. These regions of the vertebrate protein contain sequences known to interact with  $\beta$ -dystroglycan, the syntrophins and dystrobrevins.

An alignment of the N-terminus (Fig. 3A) reveals a lower degree of conservation in the predicted actin-binding region

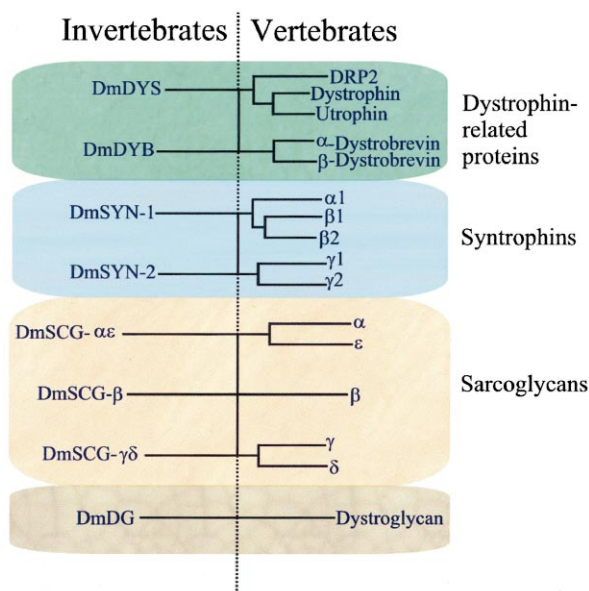


Fig. 2. Dendrograms of vertebrate and invertebrate DC sequences. The dendrograms, drawn to scale with each other, are rotated so as to display the invertebrate DC complement (represented by *D. melanogaster*) on the left, with the corresponding vertebrate complement (represented by *Homo sapiens*) on the right. The midline represents the midpoint root of each tree, and hence the putative last common ancestors of flies and humans. Shaded boxes delineate the main protein families of the complex. The vertebrate system is substantially more complex (16 versus eight proteins). We note that the topologies of the syntrophin and dystrophin/dystrobrevin trees are identical.

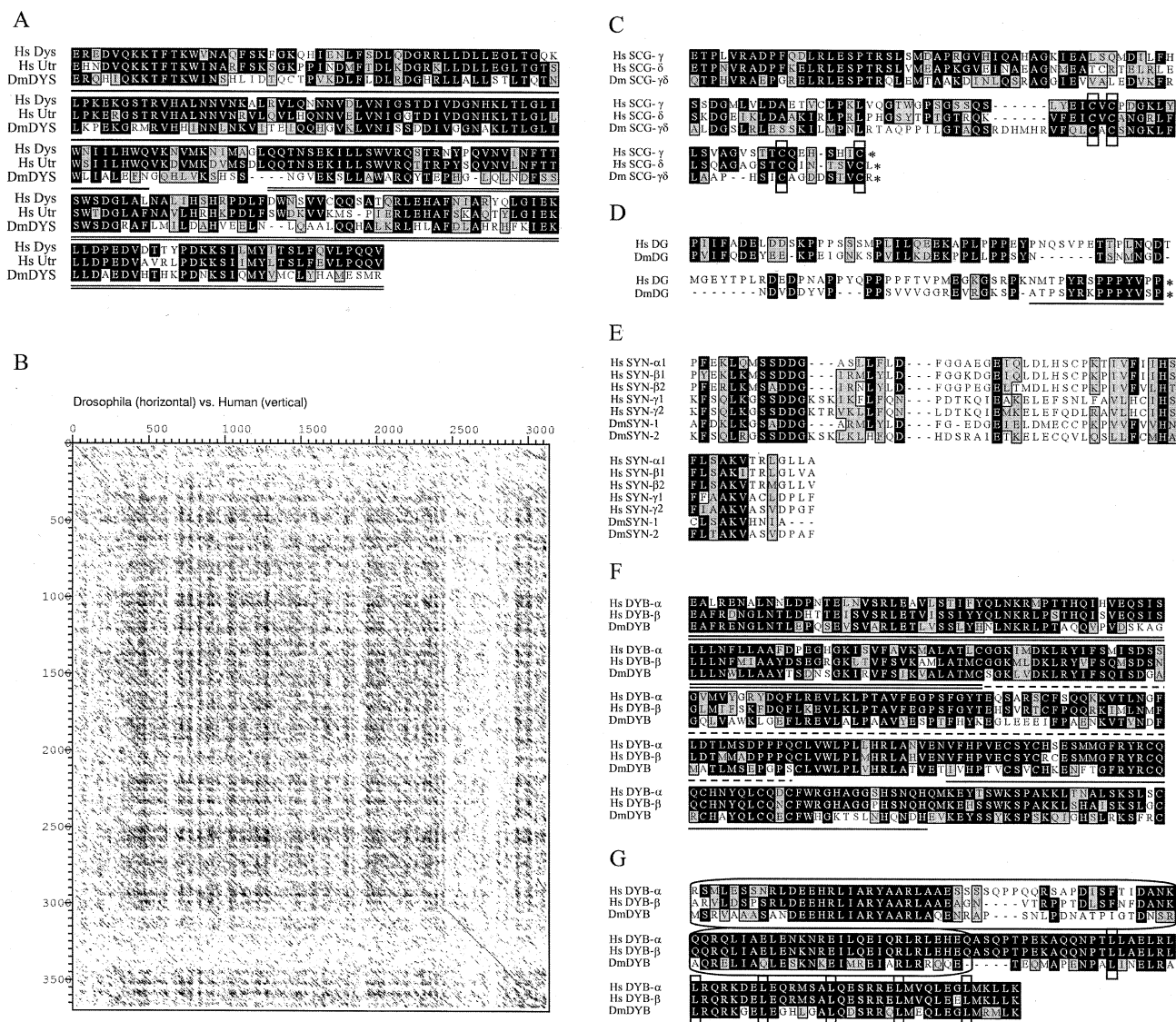


Fig. 3. Selected alignments of DC protein sequences. (A) Alignment of the actin-binding N-terminal domains of human dystrophin (Hs Dys), utrophin (Hs Utr) and DmDYS, highlighting two calponin-homologous (CH) regions (single and double underlines). Identical and similar residues are indicated (black and grey, respectively). (B) Dot matrix plot of direct comparison between full length human (vertical) and fly (horizontal) dystrophin proteins (DOTTER [44]). Top left = N-termini; bottom right = C-termini. Continuous diagonal lines signify areas of specific high homology between the proteins. The central hatched region identifies repeat sequences sharing generic similarity along the rod domains of both dystrophins. (C) C-terminal alignment of human γ- and δ-sarcoglycans against DmSCG-γδ, all proteins sharing four conserved extracellular cysteine residues. (D) C-terminal alignment of human dystroglycan against DmDG. The dystrophin-interacting polyproline motif is underlined. (E) The C-terminal dystrophin-interacting SU regions of human syntrophins against DmSYN-1 and DmSYN-2. (F) Human dystrobrevins against DmDYB, with two EF-regions [20] (double- and dotted-underlined), ZZ motif (single-underlined). (G) The syntrophin-binding regions (boxed) and conserved leucine-heptad repeats of human and *Drosophila* dystrobrevins. More comprehensive alignments are available at the following URL: <http://www.kcl.ac.uk/ip/ebitimiigbaseimokumo/molecularneuroscience.html>.

(43% identity, 62% similarity); the *C. elegans* sequence is correspondingly divergent (22% identity, 43% similarity). Assuming that the N-termini of worm and fly dystrophins bind actin, it is apparent that the mode of interaction is subject to fewer evolutionary constraints than are those of the C-terminal domains. Unlike the human protein, there does not appear to be any recognisably unique basic region within the central rod domain that may represent an alternative means of F-actin-binding [21].

Analysis of the rod domain shows a high level of degeneracy, and unlike the *C. elegans* DYS-1, the fly rod domain is 20% shorter than its human dystrophin counterpart (and 12%

shorter than utrophin). The central region of the DmDYS rod domain departs from the canonical dystrophin-like spectrin repeat motif, with its characteristic tryptophan residues [22]. Dotplot analysis (Fig. 3B) shows that any specific resemblance between human and fly dystrophin (indicated by a strong diagonal) is replaced for much of the rod domain by a more generic adherence to a looser repeat motif (indicated by the hatched box effect). Finally, the first and last few spectrin repeats of both fly and worm dystrophins show a high degree of continuous specific similarity to human dystrophin, suggesting a more critical role for these particular repeats.

#### 4. The invertebrate DC: piece by piece

Vertebrate dystrophin is known to interact via its cysteine-rich and C-terminal regions with  $\alpha$ - and probably  $\beta$ -dystrobrevin [23], as well as with the syntrophins ( $\alpha 1$ ,  $\beta 1$ ,  $\beta 2$ ,  $\gamma 1$  and  $\gamma 2$ ; [24,25]) and the transmembrane  $\beta$ -dystroglycan subunit [26,27]. The sarcoglycans ( $\alpha$ ,  $\beta$ ,  $\gamma$  and  $\delta$ ) are also found spanning the sarcolemma in close proximity to dystroglycan and sarcospan [28]. The high degree of conservation of the dystrophin C-terminus between fly and human suggested the likely presence in fly of homologues of the proteins known to interact with this region. We have subsequently cloned partial cDNAs suggestive of coding sequences for a dystrobrevin (DmDYB), dystroglycan (DmDG) and two syntrophins (DmSYN-1; DmSYN-2). Similarly, putative genes for three sarcoglycans have been found (DmSCG- $\alpha\epsilon$ ; DmSCG- $\beta$ ; DmSCG- $\gamma\delta$ ).

In vertebrate skeletal muscle,  $\alpha$ -,  $\beta$ -,  $\gamma$ - and  $\delta$ -sarcoglycans form a heterotetrameric complex (Fig. 1A), and are of particular interest due to their involvement in human limb-girdle muscular dystrophies [29]. The highly homologous  $\delta$ - and  $\gamma$ -sarcoglycans in turn show significant homology to  $\beta$ -sarcoglycan.  $\alpha$ -Sarcoglycan differs considerably from  $\beta$ ,  $\gamma$  and  $\delta$ , and in smooth muscle,  $\alpha$ -sarcoglycan is substituted by the closely related  $\epsilon$ -sarcoglycan [30]. We have found the sarcoglycan family of proteins to be simplified in *D. melanogaster*. The fly genome encodes a single orthologue of vertebrate  $\alpha$ - and  $\epsilon$ -sarcoglycans (DmSCG- $\alpha\epsilon$ ), a  $\beta$ -sarcoglycan (DmSCG- $\beta$ ) and a single orthologue of  $\gamma$ - and  $\delta$ -sarcoglycans (DmSCG- $\gamma\delta$ ). Given the stoichiometry of the mammalian sarcoglycan complex, we suggest that DmSCG- $\gamma\delta$  exists as a homodimer in association with DmSCG- $\alpha\epsilon$  and DmSCG- $\beta$ . The DmSCG- $\gamma\delta$  protein is the most conserved of *Drosophila* sarcoglycans (35% identical, 56% similar to human  $\gamma$ - and  $\delta$ -sarcoglycans), sharing a short N-terminal region, a highly conserved transmembrane domain (typical of type II membrane topology) and four extracellular cysteine residues (Fig. 3C) with its human orthologues. Similarly, DmSCG- $\beta$  appears to share the same type II characteristics as its human counterpart, although its sequence is less conserved (19% identical, 35% similar). The homology between DmSCG- $\alpha\epsilon$  and human  $\alpha/\epsilon$ -sarcoglycans is also quite low (18% identical, 36% similar), but the N-terminal regions share a predicted signal sequence, typical of type I transmembrane proteins.

Vertebrate dystroglycan is a dystrophin-associated protein expressed as a large propeptide, which is later cleaved into  $\alpha$ - and  $\beta$ -subunits [31].  $\alpha$ -Dystroglycan retains its interactions with the transmembrane  $\beta$ -subunit, and interacts with components of the extracellular matrix. We have identified a single orthologous sequence in *D. melanogaster*, which is moderately conserved throughout the length of the protein (31% identity, 48% similarity), with a predicted transmembrane domain and recognisable polyproline motif at the C-terminus (Fig. 3D). This motif is a characteristic signal for WW domain-binding and has recently been implicated in the phosphotyrosine-regulated interaction with the utrophin WW domain [32]. The region corresponding to the C-terminal (membrane-proximal) one-third of the extracellular  $\alpha$ -dystroglycan subunit is also more highly conserved, and this may provide further evidence for interactions with biglycan protein [33].

Consistent with findings in *C. elegans* [13,25], the fly genome contains two expressed homologues of the five verte-

brate syntrophins (DmSYN-1 and DmSYN-2). These cytoplasmic proteins are known to interact via their PDZ domain with the intracellular signalling molecule nNOS [34] and voltage-gated sodium channels [9,10], as well as with the 'syntrophin-binding' region of the dystrophins and dystrobrevins. Both DmSYN-1 and DmSYN-2 retain the characteristic syntrophin domain structure: a PH domain interrupted by a PDZ domain is followed by a second PH domain, with the proteins ending C-terminal with a 'syntrophin-unique' (SU) region (Fig. 3E). DmSYN-1 is more similar to human  $\alpha 1$ -,  $\beta 1$ - and  $\beta 2$ -syntrophins (40% identical), and DmSYN-2 more similar to the recently characterised human  $\gamma 1$ - and  $\gamma 2$ -syntrophins (35% identical).

The characterisation of a *Drosophila* homologue of dystrobrevin completes the fly complement of known cytoplasmic DC components. Dystrobrevin is thought to have arisen very early in evolution through an initial duplication of the original dystrophin ancestor [2]. The fly orthologue, DmDYB, shows continuous similarity to its two vertebrate counterparts, including four EF-hands and a ZZ domain (Fig. 3F), a syntrophin-binding region (Fig. 3G) and a high degree of conservation towards its N-terminus, a region postulated to interact with the vertebrate sarcoglycans-sarcospan complex [35]. The putative syntrophin-binding region contains a single highly conserved motif shared between vertebrates and invertebrates (DEEHLRIARYAARLA). As with the dystrophins, the second coiled-coil region is more similar between phyla and includes six conserved leucine-heptad repeats.

During this investigation, we were unable to identify a sarcospan homologue, despite its association with sarcoglycans in vertebrate muscle [36]. It may be that sarcospan is a vertebrate-specific protein, or that low selective pressure has allowed the fly and human sequences to diverge beyond recognition (indeed, null mutations of mouse sarcospan cause no observable phenotype [37]).

##### 4.1. Chromosomal gene localisations and cDNA accession numbers for *Drosophila* DC proteins

Localisations were extracted from database entries for genomic clones (Flybase), and have not been experimentally confirmed.

Dystrophin/utrophin/DRP2 orthologue (DmDYS): AF277386, 3R 92A6-92A7;  $\alpha/\beta$ -dystrobrevins orthologue (DmDYB): AF277387, 2R 49A5-49A7;  $\alpha 1/\beta 1/\beta 2$ -syntrophins orthologue (DmSYN-1): AF277388, 3L 79A1;  $\gamma 1/\gamma 2$ -syntrophins orthologue (DmSYN-2): AF277389, 2R 53C7-53C14;  $\alpha/\epsilon$ -sarcoglycans orthologue (DmSCG- $\alpha\epsilon$ ): AF277391, 2L 29A1-29C1;  $\beta$ -sarcoglycan orthologue (DmSCG- $\beta$ ): AF277392, 3R 87B14-87B15;  $\gamma/\delta$ -sarcoglycans orthologue (DmSCG- $\gamma\delta$ ): AF277393, X 2B8-2B9; dystroglycan orthologue (DmDG): AF277390, 2R 52D2-52D15.

#### 5. The simplicity of invertebrates

Our characterisation of the fly DC shows that flies possess essentially the same DC components as vertebrates, implying a role of fundamental importance. Furthermore, regions and domains known to mediate the interactions between members of the complex are highly conserved between human and fly, suggesting that the gross structure of the complex is identical (Fig. 1B). Similarities between the human and fly proteins follow the expected phylogeny (Fig. 2), and the ramified

topology of the vertebrate branches presumably relates to significant adaptations in vertebrate evolution. Divergence of  $\alpha$ - and  $\epsilon$ -sarcoglycan may have coincided with the divergence of vertebrate smooth and striated muscle, and the evolving nervous system may have demanded newly adapted forms of syntrophin, dystrobrevin and dystrophin (DRP2). In this latter regard, it is interesting to note that the tree topologies of the dystrophin/dystrobrevin family and the syntrophin family are identical. It is not known whether this reflects coordinate specialisation of function or merely a need for more elaborately controlled spatial or temporal expression patterns. The sarcoglycans appear to have diverged rather unequally, with DmSCG- $\gamma\delta$  and human  $\gamma\delta$ -sarcoglycans maintaining more similarity. This may suggest a more critical role for  $\gamma\delta$ -sarcoglycans in the functioning of its heteromeric complex. Surprisingly, the fly dystroglycan orthologue is very loosely conserved, despite its essential mammalian role [38]. Serving as a link between the extracellular matrix proteins and the cytoplasmic DC, dystroglycan has only one recognisable binding motif. Its polyproline C-terminus is known to link both utrophin and dystrophin to the cell membrane [32,39]; it is unclear as to what extent the primary sequence is important for direct extracellular interactions.

The smaller number of syntrophins and dystrophin-related proteins represents the most noticeable reduction of complexity in the invertebrate DC. The vertebrate dystrophins and syntrophins appear to have diverse but unclear roles in the central and peripheral nervous systems, and in skeletal, cardiac and smooth muscle. Many of these roles may have been acquired through the elaboration of gene families, and it is not clear which are ancestral and which are derived. Synaptic localisation seems to be a recurring theme, with both dystrophin itself and DRP2 being associated with synapses in the brain [40,41], and utrophin being localised to the neuromuscular junction (NMJ) [42]. Given the possible association between DRP2 and central cholinergic transmission [41], the cholinergic nature of the NMJ (with a suggested role of dystroglycan in localising acetylcholinesterase [43]) and the perturbation of cholinergic signalling in the dystrophin-deficient worm [14,16], it is conceivable that a role in cholinergic transmission is ancestral in the dystrophin family, and that the sarcolemmal association of vertebrate dystrophins is a recent adaptation. The further identification of *Drosophila* proteins orthologous to the muscle-specific sarcoglycans reflects an important aspect of the conservation of the DC that will hopefully shed light on relationships between ancestral and acquired muscular functions.

With completion of the *Drosophila* genome project, the clones reported here are likely to represent the entire *Drosophila* repertoire of proteins orthologous to the known vertebrate DC. Their existence implies that the fly (and presumably most metazoans) has the potential to form a complex almost identical to the well-characterised human skeletal muscle DC. We suggest that the reduced heterogeneity of the DC components in this experimentally amenable organism makes it an ideal model for resolving the fundamental ancestral role of the DC.

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