Human and murine dystrophin mRNA transcripts are differentially expressed during skeletal muscle, heart, and brain development

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ABSTRACT

Dystrophin transcripts were shown to be alternatively spliced in a pattern characteristic of both tissue type and developmental stage. Multiple novel spliced forms of dystrophin mRNA were identified in murine brain tissue, skeletal and cardiac muscle, diaphragm, and human cardiac Purkinje fibers. The transcript diversity was greatest in adult, non-skeletal muscle tissues. Sequence analysis revealed that four tandem exons of the murine gene are differentially spliced in at least 11 separate patterns to generate distinct isoforms. Two of these forms were observed in all tissues examined, while several others were uniquely observed in cardiac muscle and brain. Cardiac Purkinje fibers express an isoform primarily observed in brain tissue. Several spliced transcripts were observed only in postnatal development. Differential utilization of a fifth exon results in two mRNA splice forms that encode separate embryonic and adult C-termini of dystrophin. Comparison of murine with human dystrophin mRNAs showed that similar isoform expression patterns exist across species. These observations suggest that functionally distinct isoforms of the dystrophin protein are expressed in separate tissues and at different stages of development. These isoforms may be of significance in understanding the various tissue-specific effects produced by dystrophin gene mutations in Duchenne and Becker muscular dystrophy patients.

INTRODUCTION

Defective expression of the protein dystrophin results in the X-linked recessive disease Duchenne/Becker muscular dystrophy (DMD/BMD) (1). Phenotypic effects of dystrophin gene mutations are primarily manifested in limb skeletal muscle, although patient death usually results from respiratory failure, cardiac conduction abnormalities, or cardiomyopathy (2—5). Dystrophin is also expressed in brain tissue, and one-third of all patients display varying degrees of mental retardation (2,6,7). Symptoms of DMD generally do not become apparent until several years after birth, and the severity of cardiac and brain dysfunction do not correlate well with the degree of skeletal muscle impairment (2—4). A limited number of dystrophin mRNA isoforms have been described by analysis of human embryonic tissues, and transcription is known to occur from two alternate promoters in muscle and brain (8,9). Aside from a smooth muscle isoform, little qualitative differences in the 3' mRNA splicing patterns have been observed in different human embryonic tissues (9).

We have examined both mouse and human dystrophin mRNAs for alternative splicing and have compared the pattern of splicing not only in different tissues but also at separate stages of development. Two tissues with phenotypic disease (cardiac conduction system and diaphragm) that have not previously been tested for dystrophin expression were also examined to explore the potential clinical importance of dystrophin isoforms in these tissues (3—5). Our results demonstrate that alternative splicing of dystrophin transcripts generates a variety of tissue and developmental-stage specific mRNAs that may encode functionally distinct protein isoforms.

MATERIALS AND METHODS

Dystrophin transcripts were analyzed by PCR amplification of reverse transcribed RNA isolated from a variety of embryonic and adult tissues (Fig. 1). Isolation of RNA and reverse transcription were performed as described (7, 10). PCR primers derived from the murine (11) and human (12) cDNA sequences were 25 bases in length. Table 1 lists the sequences of the relevant PCR primers used in this study. With one exception identical primers were used to amplify both mouse and human cDNAs. The reverse primer for segment 1 corresponded to a region with three mismatches between species, so separate human and mouse primers were used.

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The forward primer for segment 2 contained a single internal mismatch which did not affect amplification, and the mouse primer was used for both species. PCR reactions (50 μl) utilized 40 ng of cDNA, 2.5 units Taq polymerase, 0.2 mM each primer, 200 μM each dNTP, in 10 mM Tris-HCl, pH 8.3, 50 mM KCl, and 1.5 mM MgCl₂. Amplification conditions were 94°C denaturation (30 sec), 62°C annealing (30 sec), and 72°C extension (2 min), for 30—35 cycles. 10 μl of the reactions were analyzed on 3% NuSieve agarose gels (FMC Bioproducts) containing 0.2 μg/ml of ethidium bromide prior to photography. Similar PCR conditions have been shown previously to produce efficient multiplex amplification of at least nine separate DNA fragments within a single reaction (13,14). Radioactive PCR's were performed as described above but included 25 μCi of α-32P-dCTP (Pharmacia). Transformed colonies were screened for the appropriate insert via PCR by transferring a portion of each colony (with a toothpick) into a 25 μl aliquot of PCR reaction mix that contained primers specific for vector sequences flanking the cloning site. PCR conditions for colonies were 94°C (6 min) followed by 30 seconds each at 62°C, 72°C, and 94°C (25 cycles). Clones were sequenced either from single-stranded phagemid DNA or following asymmetric PCR amplification from 5 μl of an overnight growth of a bacterial colony. Asymmetric reactions (35 cycles) contained 0.2 μM of one PCR primer, and 2 nM of the other, and the single-stranded DNAs were directly sequenced with end-labelled primers using a Sequenase version 2.0 kit (US Biochemicals). Radioactive amplification products from individual subclones were obtained following 15 cycles of PCR from a bacterial colony. These latter reactions utilized dystrophin PCR primers rather than vector primers.

RESULTS

We have explored whether dystrophin mRNA splicing displays tissue-specific or developmentally regulated patterns of expression, and whether these patterns are conserved in mice and humans. Dystrophin mRNA splicing was analyzed via polymerase chain reaction (PCR) amplification of reverse transcribed RNA (Fig. 1). Alternative splicing of the murine transcript was analyzed in the same regions reported to be differentially spliced in human embryonic tissues (9). These studies have identified a variety of previously unreported mRNA isoforms. Some of the spliced forms were only detected in postnatal tissues, and some minor species differences were observed between mouse and human.

Alternate dystrophin carboxy-termini are encoded in fetal and adult tissues

PCR amplification of reverse transcribed RNA isolated from tissues at different stages of development was performed using primers specific for the C-terminal region of the dystrophin mRNA (region 3, Fig. 1). Two distinct spliced forms in this region of the mRNA had previously been identified in embryonic human tissues (9). Figure 2 shows that early in embryonic development a smaller spliced product is expressed in all tissues and appears to be the predominant transcript in mouse skeletal muscle and mouse and human brain tissue. The relative amount of the smaller compared to the larger transcript decreases during development, and all the adult tissues examined express predominantly the larger form. The magnitude of this isoform switch varies among separate mouse and human tissues, and is most dramatic in mouse skeletal muscle and human brain. 20

Figure 1. Schematic illustration of regions of the mouse dystrophin transcript that display differential processing and the location of the PCR primers used in this study. Region 1 is transcribed from two promoters each linked to unique exons. Regions 2 and 3 are described in the text. Horizontal arrows indicate the location of PCR primers used for analysis of the splicing. Numbers below the vertical arrows indicate the location within the murine cDNA sequence of the PCR primer binding sites (see also the legends to Figs. 2 and 4). The sequences of the PCR primers are listed in Table 1. The number of alternatively spliced exons identified in regions 2 and 3 are indicated by breaks in the solid horizontal line. Also shown are the locations of the stop codons in the embryonic or adult mRNA.

![Figure 1](image1.png)

Figure 2. Developmentally regulated alternative splicing of dystrophin mRNA region 3 (see Fig. 1; human bases 10,945—11,345 or mouse bases 10,936—11,336). Shown are the results of PCR amplification from the indicated mouse and human reverse transcribed RNAs. 10 μl of each PCR reaction was electrophoresed through a 3% NuSieve agarose gel and photographed. PCR primers were as indicated in Table 1. The results demonstrate that a 32 bp segment is spliced from embryonic transcripts in mouse (bases 11,214—11,245) and human (bases 11,223—11,254) tissues. Embry. refers to embryonic tissues isolated at the indicated developmental stage (d=day, wk=week). PCR conditions were as described in methods (30 cycles). φX174: HaeIII-digested φX174 DNA.

![Figure 2](image2.png)
week human embryonic skeletal muscle expresses a greater ratio of the larger spliced transcript than does 14 day mouse skeletal muscle, which may reflect the relatively greater developmental stage of the human tissue sample analyzed. Similar results have been obtained with multiple RNA preparations from a variety of human and mouse fetal and adult tissue samples.

Sequence analysis of the subcloned PCR reactions demonstrated that these two amplification products arose via differential utilization of a 32 bp sequence. This sequence represents the penultimate exon of the human and mouse dystrophin genes (JSC, unpublished). The 32 bases spliced from the murine transcript correspond to the 32 bases deleted from this portion of the human mRNA (9). These are bases 11,222–11,253 of the murine sequence (11). The mouse and human mRNA sequences are identical for 30 of these 32 nucleotides. Removal of these 32 bases from the human and mouse transcripts alters the translational reading frame to encode an alternate C-terminus in which the final 14 amino-acids are replaced with an unrelated 32 amino acid sequence. These 32 amino acids are identical in the embryonic mouse and human transcripts, while 13 of the 14 amino acids encoded by the adult transcripts are identical (11,12).

The alternate carboxy-terminus uses a stop codon 86 bps downstream from the stop codon used in the longer (adult) mRNA. A Kyte & Doolittle hydropathicity calculation reveals that these alternate mRNAs encode a hydrophobic carboxy-terminus in embryonic tissues that is switched to a hydrophilic terminus in adult tissues (Fig. 3; [15]).

**Tissue specific expression patterns of alternatively spliced dystrophin mRNAs**

Amplification of region 2 (Fig. 1) of the murine dystrophin mRNA produced a complex pattern of DNA fragments displaying clear tissue differences (Fig. 4A). These results identified 10 separate spliced forms of the mRNA in mouse tissues (Figs. 4C and 5). This contrasts with previous studies of this region of the human dystrophin transcript which identified four spliced forms of the mRNA in embryonic tissues that arose from differential utilization of three segments of the mRNA (9). To enable a direct species comparison we amplified adult human transcripts with the identical conditions and primers used in Fig. 4A. These experiments also produced a variety of products that displayed qualitative differences when the separate tissues were compared (Fig. 4B). Hybridization of Southern blotted PCR products with oligonucleotides specific for each alternatively spliced exon

![Figure 3](image)

**Figure 3.** A Kyte-Doolittle hydropathicity calculation and plot of the adult and embryonic C-terminal dystrophin peptide sequences (15). The amino acid designation for the two sequences is displayed at the bottom of the graph, starting with the last common residue (glycine) located at the 5' end of the splice junction. Positive values denote hydrophobic residues and negative values denote hydrophilic residues. The shorter adult sequence is hydrophilic while the extended embryonic reading frame encodes a hydrophobic C-terminus.

![Figure 4](image)

**Figure 4.** PCR amplification of dystrophin mRNA region 2 (see Fig. 1; mouse cDNA sequences 10,278–10,960, human sequences 10,287–10,969). Shown are the PCR products obtained with the indicated adult mouse (A), or human (B) reverse transcribed RNAs (35 cycles). PCR was performed with multiplex PCR amplification conditions that enable efficient co-amplification of multiple separate fragments (13). Arrows denote the migration of the major isoforms. △ refers to the deletion size (Fig. 5). Sk. Mus. = limb skeletal muscle; diaph. = diaphragm. (C) Denaturing polyacrylamide gel electrophoresis of radiolabelled dystrophin PCR products (30 cycles) obtained from adult mouse RNAs. The tissue distribution of 9 splice forms in the mouse is demonstrated. A 419 bp fragment (Δ255) is visible upon longer exposure of the gel. To the right is shown the amplification products obtained from 8 subclones of the region 2 PCR products (15 cycles). Clones 17 and 11 correspond to each of the two separate 225 deletion isoforms (see Fig. 5). This gel system eliminated heteroduplex formation, but produced an aberrantly migrating hazy band below the Δ66 fragment. These aberrant bands are often observed even when the cloned dystrophin cDNAs are PCR amplified and analyzed on acrylamide gels, and appear to result from overloading of the 0.4mm sequencing-type gels (no corresponding bands are observed when the same reactions are analyzed on agarose gels). (D) Analysis of the dystrophin mRNA splicing pattern in mouse tissues at separate developmental stages. Several of the spliced transcripts are present only postnatal tissues. El4 = embryonic day 14, 1D = newborn.
demonstrated that each amplification product was transcribed from the dystrophin gene (data not shown).

The data in Figure 4 display the dystrophin mRNA splicing patterns in five separate tissues. Region 2 of the mRNA is detected primarily as the ‘full length’ (683bp) form in both mouse and human skeletal muscle and mouse diaphragm, while brain, heart, and Purkinje fiber-mRNAs display a more complex splicing pattern giving rise to several smaller transcripts (Figs. 4A and 4B). Attempts to subclone each species indicated that at least some of the fragments observed on agarose gels arose from heteroduplex formation between separate amplification products (unpublished observations). To eliminate heteroduplexes we analyzed radiolabelled PCR products on denaturing polyacrylamide sequencing gels (Fig. 4C). The major transcripts produced in brain differ from the ‘full-length’ product by deletions of 39 or 330 bps (Figs. 4C and 5). The 330 bp deletion transcript is also observed in human heart tissue but not in skeletal muscle (Fig. 4B). Some of this transcript in heart may arise from Purkinje fibers which appear to express primarily the 330 bp deletion transcript (Fig. 4B). In contrast, mouse heart tissue does not express a significant amount of this isoform. The 66 bp and 291 bp deleted isoforms were observed almost exclusively in heart (Fig. 4C).

Sequence and size analysis revealed that the murine mRNA isoforms arose from differential utilization of four regions of the mouse transcript that likely delineate individual exons of the gene (Figs. 4C and 5). One of the two 225 deletions is an example where splicing of two non-contiguous exons was proven by sequence analysis. The faint bands representing the 198bp and 264bp deleted forms, detected in radioactive PCR reactions and PCR southern blots, arise via the same mechanism (Fig. 5). Many of the smaller spliced transcripts are primarily observed in neonatal and adult mouse tissues (Fig. 4D), which likely explains why several of these isoforms were not detected in previous studies of human embryonic tissues (9). For example, a mouse isoform 159bp smaller than the full-length product is observed in adult tissues, but not in mouse embryos (Fig. 4D, arrow). Several of the other intermediate sized isoforms visible in heart and brain also appear to be primarily expressed in the adult tissue specimens. At least one species differences is apparent, as a spliced human transcript missing 105 bps of sequence (9) is not expressed in mice (Figs. 4B, 4C, and 5).

A segment of the human transcript immediately 5' of region 2 (Fig. 1) has been reported to be alternatively spliced in human smooth muscle (9), but this isoform does not appear to be expressed to a significant degree in the mouse tissues examined. Only trace amounts of this transcript were detected in murine skeletal and cardiac muscle, brain, or smooth muscle from the aorta (unpublished observations). Alternative splicing was not observed between exon 2 and mRNA base 10,000 in mouse skeletal muscle (the region just 3' of region 2). This region was not tested for alternative splicing in other tissues.

Specificity of alternate promoter utilization

PCR analysis demonstrates that the murine and rat genes, contains two promoters each linked to unique first

![Figure 5. Diagram of 11 spliced forms of dystrophin mRNA identified in mouse (m; Fig. 4), human (h; 9. 12), or chicken tissues (c; 15). Ten of these forms have been detected in mouse tissues, each of which has been subcloned and sequenced. The structure of the ‘full-length’ (683bp PCR product) spliced form is shown at the top, while the splicing pattern of the smaller products is illustrated below. Also shown are the nucleotide sequence numbers of the murine (11) and human (12) cDNA sequences immediately flanking the four differentially spliced exons, the sizes of which are indicated (in base pairs) within the ‘full-length’ fragment diagram. The first spliced exon thus begins at base 10,424 of the mouse transcript that likely delineate individual exons of the gene (Figs. 4C and 5). One of the two 225 deletions is an example where splicing of two non-contiguous exons was proven by sequence analysis. The faint bands representing the 198bp and 264bp deleted forms, detected in radioactive PCR reactions and PCR southern blots, arise via the same mechanism (Fig. 5). Many of the smaller spliced transcripts are primarily observed in neonatal and adult mouse tissues (Fig. 4D), which likely explains why several of these isoforms were not detected in previous studies of human embryonic tissues (9). For example, a mouse isoform 159bp smaller than the full-length product is observed in adult tissues, but not in mouse embryos (Fig. 4D, arrow). Several of the other intermediate sized isoforms visible in heart and brain also appear to be primarily expressed in the adult tissue specimens. At least one species differences is apparent, as a spliced human transcript missing 105 bps of sequence (9) is not expressed in mice (Figs. 4B, 4C, and 5).]

![Figure 6. Analysis of the dystrophin promoter utilized in mouse and human tissues. PCR amplification utilized specific oligonucleotides from muscle and brain exon 1. The muscle exon 1 transcript was amplified as a 591 bp fragment (human bases 216 – 806 (12) and murine bases 230 – 820 (11)). The brain type segment was amplified as a 587bp fragment using a 5' primer from the published rat brain exon 1 sequence (8). Both reactions utilized a 3' primer derived from exon 7 of the murine and human genes; the 5' end of this primer was base 806 (human) and 820 (mouse). The fragment containing brain exon 1 was amplified from all tissues except human cardiac Purkinje fibers and mouse heart. The muscle first exon was amplified from all tissues, although the product from the fetal brain samples is faint. The lane displaying the muscle promoter analysis from mouse skeletal muscle is from a separate gel.]

- Human
  - 'Brain' Prom.
  - 'Muscle' Prom.
- Mouse
  - 'Brain' Prom.
  - 'Muscle' Prom.
exons (Fig. 6; [8,9]). Both promoters are active in muscle, brain and heart but display profound tissue differences in their pattern of expression (Fig. 6). These experiments utilized RNA prepared from whole tissues, so it cannot be determined whether the brain promoter expression in muscle tissues arises within myofibers or within non-muscle cells. Purkinje conduction fibers exclusively utilize the muscle type promoter despite the observation of a brain-like pattern of splicing in region 2 of the mRNA (Figs. 4B and 6). Expression of the brain exon 1 in heart tissue must therefore be from non-Purkinje cell types (Fig. 6). Expression of muscle exon 1 in brain tissue also illustrates the point that both promoters may be active in a tissue. It is unclear whether the low level of ‘muscle’ promoter expression in adult brain derives from neuronal origin, from astro-glial cells previously shown to express muscle exon 1 (17), or from capillary smooth muscle. These experiments demonstrate that the ‘muscle’ and ‘brain’ promoters do not display strict tissue-specific expression patterns.

**DISCUSSION**

This report expands upon the number of dystrophin mRNA isoforms and defines their developmental pattern of expression in brain, heart muscle, cardiac Purkinje fibers, skeletal muscle and diaphragm. Dystrophin expression in these tissues is regulated by developmental and tissue specific alternative splicing patterns which have been observed in a number of other proteins expressed in muscle (18–21). The dystrophin gene may provide an interesting model to study tissue specific and developmental factors which influence alternative splice site selection. Cis and trans acting mechanisms have been proposed for regulation of specific splicing formats (18). In this case, splicing events observed at the 3’ end of the dystrophin transcript do not appear to be specified in cis by the promoter that is utilized. For example such a correlation is absent in the analysis of Purkinje fibers, where the active ‘muscle’ promoter is coupled with a ‘brain’ type splicing pattern in region 2 of the transcript (Figs. 4B and 6).

In addition, the choice of promoter usage during development can not be directly correlated with the varying pattern of splicing observed at the 3’ end of the transcript (Figs. 2, 4D, and 6). These observations suggest that dystrophin mRNA isoform generation is primarily regulated by different trans-acting splicing factors produced in brain, heart, and skeletal muscle during development. Furthermore, it is of interest that differential splicing of the penultimate exon of the gene is greater in embryonic tissues (Fig. 2), whereas alternative splicing of exons further 5’ is greater in adult tissues (Fig. 4D). The cis elements that bind the various trans-acting factors remain to be determined.

Bar et al have recently described an additional isoform of dystrophin that is expressed in non-muscle tissues (22). This isoform appears to be transcribed from a third promoter internal to the dystrophin gene and is detected on Northern blots as an approximately 6.5 kb mRNA. Partial sequence analysis and RNase A cleavage experiments suggest that this transcript shares the same exons as the muscle dystrophin transcript except for a separate, truncated 5’ end and the lack of the penultimate exon (22). This non-muscle dystrophin includes regions two and three of the dystrophin transcript (Fig. 1). It is therefore possible that some of the alternatively spliced dystrophin isoforms are transcribed from this internal promoter rather than from the previously described muscle and brain promoters. However PCR analysis of dystrophin mRNA isolated from liver and spleen produces a pattern indistinguishable from that observed in skeletal muscle, suggesting that most of the alternatively spliced isoforms (Fig. 5) are not produced in these two non-muscle tissues (JSC, unpublished observations). Regardless of the exact promoter used, each of the alternatively spliced transcripts detected in figures 2 and 4 are transcribed from the dystrophin gene and represent distinct isoforms of dystrophin mRNA.

Although the dystrophin gene is composed of more than 75 exons (23), alternative splicing was observed with a limited number of exons that encode portions of the C-terminus of the protein. The spectrin-like repeat region was not tested for splicing in non-muscle tissues, and it is possible that additional splice forms are present in this region of the gene. As noted by Feener et al (9), splice forms involving alternate exons of the same size would not have been detected with our methods. A variety of different types of data suggest that the alternatively spliced C-terminus is critical for functional activity. Frameshift mutations that lead to production of a truncated protein lacking the C-terminus almost invariably cause a severe disease phenotype in humans (23,24). Such observations imply that the carboxy-terminus could mediate attachment of dystrophin to integral sarcolemmal glycoproteins (25,26). It has been postulated that deletion of the C-terminus might generate a non-functional protein (23,24,27), and the available data indicate that strong selective pressure has preserved these sequences and their pattern of splicing during evolution (11,12,16). The carboxy-terminal portion of the mRNA and protein sequences represent the most highly conserved region of the dystrophin molecule. The murine (11) and human (12) dystrophin mRNA sequences in region 2 (Fig. 1) display 97% identity at the nucleotide level, and the predicted amino-acid sequences are identical. The 330 base pairs that are alternatively spliced within region 2 (see Fig. 5) differ by only a single nucleotide substitution between the mouse and human sequences. Region 3 of the mRNAs display 93.5% sequence identity at the nucleotide level (375/401 bases) and greater than 98% amino-acid identity (105 of 107 amino-acids for the adult isoform, 126/127 for the embryonic isoform). In contrast, the overall mouse and human dystrophin sequences display 91.6% amino acid identity (11,12).

The observation that alternative splicing is observed in the same regions of the mouse and human dystrophin genes also argues in favor of a functional role for these mRNA isoforms. The pattern of splicing is extremely similar, although two minor species differences were noted in region 2 (Figure 5). In most cases the exact role that individual isoforms play in different muscle types is not clear. Most alternatively spliced muscle genes encode contractile proteins and it is generally assumed that the separate isoforms provide a genetically efficient way to modulate the function of contractile components in response to physiological differences between or within individual muscle types (18,28). Alternative splicing of the genes for troponin T and /3-tropomyosin has been particularly well characterized. The /3-tropomyosin gene has been isolated from chickens and rats and displays an identical pattern of alternative splicing when compared between the two species (20,21). A number of troponin T exons are alternatively spliced such that 64 separate mRNAs could be produced, although only about 10 appear to expressed to a significant degree (19,29). The concentration of calcium required for half-maximal tension development as well as the degree of cooperativity between calcium activated muscle contraction and the degree to which troponymosin moves along a-actin filaments
are both directly correlated with the major troponin T isoforms expressed in a particular muscle type (18).

Most of the dystrophin mRNAs produced via alternative splicing of region 2 are not detected in skeletal muscle or diaphragm (Figs. 4A-C). Shorter spliced forms of dystrophin mRNA are predominantly observed in brain, heart, and cardiac Purkinje fibers, tissues which are also clinically affected in DMD/BMD. The pathological evidence of Purkinje fiber necrosis (5) and clinical heart block in BMD (4) combined with the association of mental retardation (2) suggest that dystrophin isoforms may be important in these specialized tissues. The identification of integral membrane glycoprotein(s) bound to dystrophin in skeletal muscle is intriguing in terms of the function of the isoforms (25,26). Increased calcium channel activity has been correlated with elevated intracellular calcium levels and increased proteolytic activity in mdx and DMD myofibers (30,31). These observations suggest that calcium leak channels may be directly modulated via an interaction with dystrophin, and raise the possibility that dystrophin associated glycoproteins in skeletal muscle could play a role in calcium transport or binding. The potential for dystrophin isoforms to display functional differences in separate tissues during development raises further questions regarding its role in normal muscle and brain physiology.

Several groups have observed that dystrophin is predominantly localized in the sarcoplasms of early embryonic human skeletal muscle, whereas later in embryonic development and in adult muscle virtually all of the dystrophin is localized to the inner face of the sarcolemma (32—35). It is tempting to speculate that the differentially spliced carboxy-terminal isotypes of the mRNA encode functionally distinct dystrophins that differ in their ability to interact with sarcolemmal proteins. The isoform switch observed during muscle development (Fig. 2) completely alters the polarity of the carboxy-terminus and roughly parallels the observed transition from sarcoplasmic to sarcolemmal dystrophin (Figs. 2 and 3; [32]). Interestingly, embryonic heart tissues express relatively lower amounts of the transcript lacking the penultimate exon (Fig. 2). Cardiac muscle is the first tissue to experience mechanical stress during embryonic development, and is also the first to show membrane localization of dystrophin (36,37). While the function of dystrophin isoforms may be affected by the developmental expression of dystrophin associated membrane glycoproteins (25,26,32), these results raise the possibility that the embryonic isoform of dystrophin may play a unique role in pre-contractile muscle.

Few patients have been reported who display deletions limited to the C-terminal region of the dystrophin gene. Hoffman et al have recently described a patient with an unusual phenotype that is more severe than typical DMD, and who has a deletion of the entire C-terminus (38). Despite this patient’s severe phenotype, the skeletal muscle dystrophin was found to be localized to the sarcolemma (38). It is possible that dystrophin is localized to the membrane via a variety of contact points, including the presumptive N-terminal actin binding domain and/or the triple helical spectrin-like repeat domain. Dystrophin deleted for the C-terminal domain might still be able to associate with the sarcolemma, but might not be attached to the integral membrane glycoprotein complex described by Ervasti et al (26). The functional importance of regions 2 and 3 (Figure 1) is supported by the severe phenotype in the patient lacking this region of the gene (38). A second C-terminal deletion was reported by Towbin et al in patient CM (39). This patient has a large C-terminal deletion that begins near region 2 and extends a considerable distance to include the glycerol kinase and adrenal hypoplasia genes (39). Patient CM displays a mild BMD and presumably has membrane-bound dystrophin. However the precise deletion and splicing pattern in this patient remains uncharacterized at the mRNA and protein levels.

Carboxy-terminal isoforms of dystrophin might mediate interactions with a variety of membrane proteins. Cardiac myocytes are known to display a calcium leak activity similar to that observed in skeletal muscle (40), and cardiac muscle expresses several spliced forms of dystrophin mRNA including the ‘full-length’ isoform that predominates in skeletal muscle (Fig. 4). In brain tissue dystrophin immunoreactivity displays a discrete staining pattern localized to cortical postsynaptic neuronal junctions, clearly distinct from the uniform sarcolemmal staining observed in muscle (6). It will be of interest to determine whether distinct dystrophin isoforms are differentially expressed in separate types of neurons. The variety of additional isoforms expressed in heart and brain suggests that dystrophin may function via interaction with a number of tissue-specific and developmentally regulated membrane proteins in these latter tissue types. These proteins could be isoforms of those proteins already identified in skeletal muscle, or might be other ion channels or receptor proteins unique to each tissue. In an analogous manner spectrin, which is structurally related to dystrophin (12), is linked to specific ion channels and integral membrane proteins in a variety of tissues via attachment to ankyrin (41—43). The nature of the proteins bound by the spectrin-ankyrin complex appears to be specified by isoforms of ankyrin, some of which arise via alternative splicing of the C-terminus (41—43). The expression of functionally distinct dystrophin isoforms during ontogeny may complicate strategies for gene therapy of DMD that are based upon delivery of cDNA containing mini-gene vectors to diseased tissues (3, 44).

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