Distinct dystrophin mRNA species are expressed in embryonic and adult mouse skeletal muscle

G. Dickson, J.A. Pizzey, V.E. Elsom, D. Love*, K.E. Davies* and F.S. Walsh

Department of Neurochemistry, Institute of Neurology, Queen Square, London WC1 3BG and *Nuffield Department of Clinical Medicine, University of Oxford, John Radcliffe Hospital, Oxford OX3 9DU, England

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We have examined dystrophin mRNA in embryonic, newborn and adult mouse skeletal muscle. A discrete nerve-independent increase in mRNA size was observed between embryonic and adult stages, indicating that a developmentally regulated mRNA isoform switch occurs in the expression of the Duchenne muscular dystrophy (DMD) gene in skeletal muscle. These distinct mRNAs are most likely generated via selection of alternative transcriptional start sites or RNA processing pathways. In addition, denervation of adult muscle was without effect on the expression pattern.

Duchenne muscular dystrophy; Dystrophin; mRNA; Isoform (Skeletal muscle)

1. INTRODUCTION

Duchenne and Becker muscular dystrophies (DMD and BMD) are allelic, X-chromosome linked, degenerative diseases of skeletal muscle, and represent one of the most common potentially lethal genetic disorders in man [1]. The DMD/BMD gene is composed of some 60 exons spread across an unusually large genomic locus of over 2 megabase pairs which encodes an mRNA(s) of approximately 14 kb in both humans and mice [2-4]. Primary structure analyses [5] and immunolocalization studies [6-8] have suggested that the protein product of the DMD gene, called dystrophin [9], is a 125 nm rod-shaped molecule associated in a periodic manner with the subsarcolemmal cytoskeleton of skeletal muscle.

Dystrophin has been characterized as a single or doublet band of approximately 400 kDa by Western immunoblotting expressed at quantitatively similar levels in all physiological types of embryonic and adult muscle, including skeletal, smooth and cardiac tissues [10]. At the RNA level, however, significant quantitative discrepancies exist in the levels of DMD gene expression during muscle development and between muscle types. Thus, relative to adult skeletal and cardiac muscle, embryonic and newborn skeletal and adult smooth muscle tissues contain very little dystrophin mRNA [3,11]. Furthermore, in a fashion analogous to skeletal muscle tissue, an apparent developmental activation of dystrophin mRNA expression occurs during myoblast to myotube transition in cultures of human and mouse skeletal muscle [11-13].

These apparent discrepancies in DMD gene activity between protein and mRNA levels may reflect the existence of distinct mRNA isoforms with differing intrinsic translational efficiencies in immature compared to adult skeletal muscle, and also in mature smooth versus skeletal tissues. Based on minor size differences in Western immunoblot analyses, Hoffman et al. [10] have indeed postulated the existence of such a distinct smooth muscle dystrophin isoform. However, at present, the paradox of dystrophin polypeptide and mRNA levels during development of skeletal muscle remains unresolved. Thus, in this report,
we have examined the level and size of dystrophin mRNA in embryonic, newborn and adult mouse skeletal muscle, and also following sciatic nerve section. Nerve-independent increases in both mRNA abundance and size were observed during myogenesis, clearly demonstrating the existence of distinct embryonic and adult dystrophin mRNA isoforms.

2. MATERIALS AND METHODS

Skeletal muscle tissue samples were dissected from hind limbs of embryonic, newborn and adult mice, immediately frozen in liquid N₂ and stored at −80°C. For denervation studies, mice were anaesthetized and a segment removed from the sciatic nerve as described [18].

mRNA was prepared from skeletal muscle tissues by extraction in 6 M urea and selective precipitation with 3 M LiCl as described [14], and poly(A)⁺ RNA isolated by oligo(dT)-cellulose affinity chromatography. For Northern blot analyses, poly(A)⁺ RNA samples (2–10 μg) were suspended in water, an aliquot (0.2–2 μg) taken for examination by non-denaturing ethidium-agarose electrophoresis, and the remainder denatured by glyoxylation, resolved on 0.8% agarose gels and blotted to Genescreen transfer membrane (NEP [14]). Northern blot filters were probed sequentially by hybridization to 3²P-labelled cDNAs for dystrophin (4.3 kb insert from C23 [15]), glyceraldehyde 3 phosphate dehydrogenase (GPDH [16]) and the neural cell adhesion molecule (N-CAM, λ 9.5 [17]). Hybridizations were performed at 42°C in the presence of 50% (v/v) formamide and 10% (w/v) dextran sulphate, and washed and autoradiographed as described [14]. Bound probe was removed from filters prior to subsequent hybridization by washing at 70°C for 60 min in 50% formamide, 1 × SSC.

Fig.1. Dystrophin mRNA levels increase during skeletal muscle development. Poly(A)⁺ RNA was prepared from 16 day embryonic (lane 1), newborn (lane 2) and adult (lane 3) mouse skeletal muscle. Aliquots were examined by neutral gel electrophoresis (panel A, 2 μg) or glyoxal-gel electrophoresis (8 μg) and sequential Northern blot hybridization with dystrophin (B), GPDH (C) and N-CAM (D) cDNA probes. Autoradiographic exposures were for 4 days (B,D) and 4 h (C). The positions of 28 S and 18 S RNAs (arrows) and hybridization signals (*) are marked.
3. RESULTS

In order to examine dystrophin mRNA expression during skeletal muscle development, poly(A)+ RNA was prepared using mouse hind limb skeletal muscle from 16 day embryos and from newborn and adult (9 weeks) animals. These samples were analysed by Northern blotting and filter hybridization with human cDNA probes for dystrophin [15], GPDH [16] and N-CAM mRNAs [17]. N-CAM and GPDH mRNA levels are known to be repressed and activated respectively during skeletal muscle development [16,18,19]. In addition, characteristic N-CAM mRNA isoform switching occurs around the perinatal stages (fig.1, panels C and D) which correlates with myotube formation [19,20]. Subsequent down-regulation of N-CAM gene expression occurs in an innervation-dependent fashion in adult muscle [21]. The N-CAM and GPDH probes thus control the quality and developmental source of the muscle RNAs. As shown in fig.1 (panel B), while a 14 kb dystrophin transcript is clearly present in adult muscle, only very low levels were present in embryonic and newborn tissue samples. In addition, an apparent increase in dystrophin mRNA size was observed in embryonic compared to adult tissue samples. Increased loading of embryonic RNA samples clearly confirmed a reproducible switch in dystrophin mRNA size occurring between embryonic and newborn stages of skeletal myogenesis (fig.2).

During development, qualitative and quantitative changes in a wide range of contractile apparatus and other cytoplasmic and membrane-associated muscle proteins can be broadly classified as activity (nerve)-dependent or independent [22-24]. To examine further the observed developmental changes in dystrophin mRNA, groups of mice were subjected to sciatic nerve section and RNA prepared from denervated lower hind-limb muscle groups after various periods. As shown in fig.3, denervation periods of up to 7 days were without major effects on dystrophin (panel B) or GPDH (panel D) mRNA levels. However, marked and reproducible reactivation of 5.2 and 2.9 kb N-CAM mRNA species occurred by 3 days, clearly indicating the denervated status and appropriate molecular responses of the tissues examined. Expression of the DMD/BMD gene in skeletal muscle thus appears to be nerve-independent.

4. DISCUSSION

The occurrence of distinct embryonic and adult isoforms is a common feature of many developmentally regulated proteins and has been documented for cytosolic, plasma membrane and in particular contractile and cytoskeletal proteins [23,25,26]. At the nucleic acid level isoform diversity results from the differential activation of members of multigene families, or by the use of alternative transcriptional start sites or RNA splicing and polyadenylation pathways within a single but complex gene transcriptional unit [27]. The present study provides evidence that dystrophin mRNAs occur as embryonic and adult isoforms in mouse skeletal muscle and that isoform switching occurs during the perinatal period in an innervation-independent manner.

The existence of embryonic and adult mRNA isoforms suggests an explanation for observed discrepancies in the relative levels of dystrophin
Fig. 3. Dystrophin mRNA expression is unaffected by denervation. Poly(A)⁺ RNA was prepared from control skeletal muscle (lane 1) and following 1 day (lane 2), 3 days (lane 3) or 7 days (lane 4) of denervation. Aliquots were examined by neutral gel electrophoresis (panel A, 1 µg) or by glyoxal-gel electrophoresis (8 µg) and sequential Northern blot hybridization with dystrophin (B), GPDH (C) and N-CAM (D) cDNA probes. Autoradiographic exposures were for 4 days (B,D) or 4 h (C). The positions of 28 S and 18 S mRNA are arrowed.

protein detected by Western immunoblotting and dystrophin mRNA on Northern blots of embryonic, newborn and adult mouse skeletal muscle [3,10]. Thus, the smaller embryonic dystrophin mRNA, while present in lower abundance than its larger adult counterpart may exhibit higher translational efficiency. In this way, comparable levels of indistinguishable embryonic and adult dystrophin polypeptides might be observed in Western immunoblot analyses of the respective tissues. Furthermore, the developmental increase in skeletal muscle mRNA levels seen here correlates with the observation that well-differentiated cultures of human and mouse myotubes express relatively high dystrophin mRNA levels compared with undifferentiated myoblast progenitor cells [11–13].

At the nucleic acid level, it is generally accepted that, despite its unusually large size, the DMD/BMD gene represents a single genetic locus [2,28]. The present study thus implies that different mRNA isoforms must arise in a developmentally regulated fashion either via selection of alternative promoters of different strengths, or via differential RNA processing pathways which promote stabilization of the adult mRNA isoform. The use of S1-nuclease protection analyses across dystrophin cDNA and genomic fragments to examine the nucleotide sequences of embryonic compared to adult mRNAs will allow these possibilities to be resolved.

The relationship between the mRNA isoforms observed here and dystrophin polypeptide structure remains as yet undefined. The existence of a distinct smooth muscle isoform has been suggested from minor size variations around 400 kDa in
Western immunoblot studies [10]. In another recent study, using fractionated extracts of skeletal muscle a 210 kDa protein reactive with a dystrophin polypeptide antibody, but absent in dystrophic muscle, was reported [7]. Dystrophin polypeptides are also expressed in cardiac and neural tissues. Resolution of protein isoforms and correlation with mRNA sub-types may require, however, detailed information on differential protein coding sequences coupled to subsequent Northern and Western blot analyses with specific oligonucleotide and anti peptide antibody probes.

From primary sequence analyses, dystrophin has been suggested to be a rod-shaped molecule of 125 nm [5] associated in a periodic manner with the membrane cytoskeleton [6,29] and involved in the maintenance of membrane structural integrity in the face of mechanical stress [5,30]. Thus, differential isoform expression may reflect changing requirements in relation to myofibre structure, or differential association with sarcolemmal components, during myofibre development. Indeed, a number of major developmental events occur in the early postnatal period of myofibre formation in the mouse including changes in t-tubule organisation and establishment of monosynaptic innervation and fibre type distribution [31-33]. Furthermore, in the MDX mouse, pathological change is first observed during this period [34]. It remained to be seen, however, whether dystrophin isoform shifts are causally or temporally associated with these events.

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REFERENCES