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The Duchenne muscular dystrophy gene: Structure, evolution, expression and function of products

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Introduction and objectives

Duchenne muscular dystrophy (DMD) is an X-linked recessive disorder, which manifests as a progressive degeneration of muscles, and death. A significant proportion of DMD patients also suffer from mental retardation. The gene which is defective in DMD is the largest known gene, consisting of almost 0.1% of the human genome (2,500 Kbp). The product of the DMD gene in normal muscle, dystrophin, is a 427 kDa protein.

In muscle, dystrophin is an essential part of a large complex that links the actin cytoskeleton with the cell membrane and the extracellular matrix and stabilizes the myofibers during contractions. We and others found that a very similar isoform of dystrophin, encoded by the same gene, is expressed in the brain (Nudel et al. Nature 331, 635, 1988) The expression of the two isoforms is regulated by two different promoters. One is active in muscle cells and glia cells, the other is active mainly in neurons (Nudel et al., Nature 337:76, 1989). We also described a 70.8 kDa protein, called Dp71, which is the product of a promoter located between exons 62 and 63 of the DMD gene (Bar et al., Biochem. J. 272:557, 1999). Following these observations, four additional DMD gene products have been identified by others (Fig. 1A). Dp71 is of special interest. It consists of the cysteine-rich and C-terminal domains of dystrophin, which bind to a group of membranal proteins (DAPs), but lacks the actin binding domain and the spectrum-like repeats (Fig. 1A). It is the major product of the DMD gene in brain and many other nonmuscle tissues. However, it is not expressed in skeletal muscle.

The main subjects of our recent studies are: 1) The structure and evolution of the huge and complex DMD gene, in vertebrates and in invertebrates. 2) The regulation of expression and the function of the various products. 3) The possible involvement of Dp71 in brain function and in embryonic development. 4) Possible applications of some of our findings for prenatal diagnosis of DMD and for gene therapy.

Dp71 – The major non-muscle products of the DMD gene

We have previously described the specific inactivation of Dp71 in transgenic-mice by replacing Dp71 first exon with a gene encoding β-Gal and the analysis of these mice. The insertion of the β-Gal gene enabled us a very sensitive monitoring of the activity of the promoter of Dp71. X-gal staining of Dp71 null embryos revealed a very specific and interesting pattern of Dp71 promoter activity. High activity of Dp71 promoter was often associated with major morphogenic events and terminal differentiation. In spite of this, we did not detect, so far, conspicuous pathological effects in the Dp71 null mice. Immunological and biochemical analysis indicate partial compensation for the lack of Dp71 by other products of the DMD gene.

We have produced transgenic mice that overexpress Dp71 in many tissues. The most affected tissue in these mice is skeletal muscle, which normally does not express Dp71.

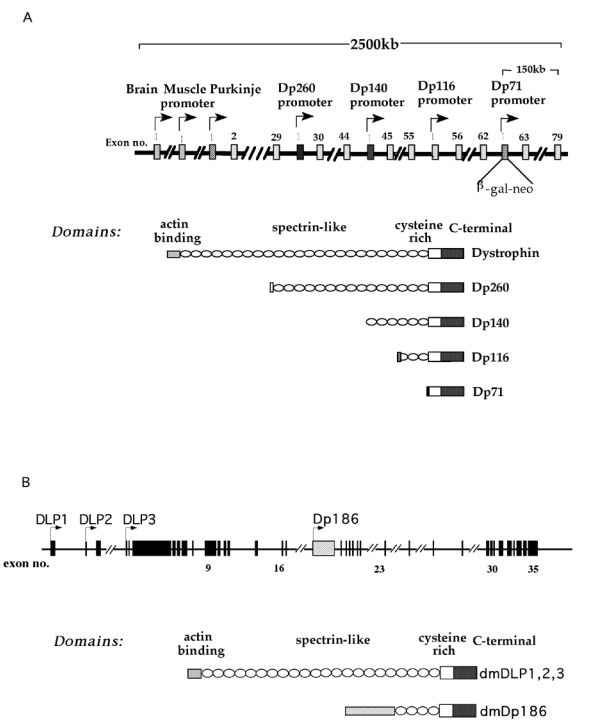

Fig. 1 *Promoters and products of the DMD gene and its Drosophila homologue The human DMD gene (A) and its Drosophila homologue (B) are presented schematically (upper parts). Arrows indicate the location of promoters. The products are presented schematically in the lower parts of A and B.*

Foci of degeneration and regeneration of fiber were detected in the muscle. Immunohistological stainings showed that the exogeneous Dp71 was localized under the sascolemma. Western blot analyses demonstrated a significant decrease in dystrophin level in the muscle. It thus seems that Dp71 acts as a dominant negative competitor of dystrophin by replacing it in the DAPs complex. Since Dp71 can not bind actin filaments, the complex can not function normally as a linkage between the extracellular matrix, the sarcolemma and the actin cytoskeleton. These mice can be used as model animals to study DMD.

The Dystrophin/Homologues in Sea Urchin and *Drosophila* – Evolutional and Functional Implications

To study the evolution of the DMD gene and the significance of its various products, we have searched for genes encoding dystrophin-like proteins in sea urchin and in *drosophila*. We have previously described a sea urchin gene encoding a protein with very strong structural similarity and sequence identity to human dystrophin. The same gene encodes a protein that is an evolutionary homologue of human Dp116, one of the small products of the mammalian DMD gene. We also cloned and characterized a *drosophila* gene closely related to the human dystrophin gene. Like the human gene, the *drosophila* gene encodes at least three isoforms of full-length dystrophin-like proteins (dmDLP1, dmDLP2 and dmDLP3), regulated by three different promoters located at the 5' end of the gene, and a smaller product (dmDp186) regulated by an internal promoter (Fig. 1B). The full-length products and the small product have distinct patterns of expression. Interestingly, like the small products of the human dystrophin gene, the small product of the *drosophila* gene (dmDp186) is expressed mainly in the brain and the central nervous system (Fig. 2). Thus, the complex structure of the dystrophin gene, encoding several large dystrophin-like isoforms and smaller truncated products with different patterns of expression, existed before the divergence between the protostomes and deuterostomes. This conservation, in such distantly related organisms, points to important distinct functions of the multiple products.

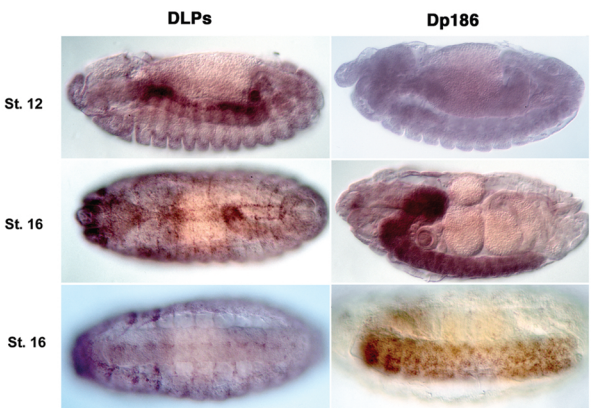

Fig. 2 *Differential expression of dmDLPs and dmPp186 in Drosophila embyos. Transcripts were detected by in-situ hybridization using mRNA-specific DNA probes. DmDp186 mRNA is detected mainly in the brain and central nervous tissue in stage 16 embryos . dmDLP mRNAs are detected mainly in the midgut endoderm of stage 12 embryos, and in pericardial cells, segment borders, and cells along the midline of the CNS of stage 16 embryos.*

The *drosophilla* dystrophin like gene provides an excellent model system for the analysis of the function of the various products of the DMD gene. Gene silencing and genetic manipulations are being employed. (in collaboration with Talila Volk)

Prenatal diagnosis of DMD

We have developed a sensitive method to detect small amounts of dystrophin in most chorionic villus sampling (CVS) and amniotic fluid (AF) cell cultures from normal fetuses but not from DMD affected fetuses. We are using several approaches aimed at developing a reliable test for prenatal diagnosis of DMD based on dystrophin expression in AF and CVS cells.

Selected Publications

Yaffe, D, et al. (1998) Dystrophin expression in chorionic villus sampling and amniotic fluid cell cultures. In: Chorion Villus Sampling, K.A. Rao and G.Allahbadia, eds, vol. 23, pp.110-116.

Wang, J., et al. (1998) A sea urchin gene encoding dystrophin-related proteins. Hum. Mol. Genet. 7,581-588.

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Vilquin, J-T, et al. (1999) Myoblast transplantations lead to the expression of the laminin _2 chain in normal and dystrophic (dy/dy) mouse muscles. Gene Therapy 6, 792-800.

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Sarig, R., et al. (2000) Increased efficiency of homologous recombination in ES cells by cleavage at both ends of homology in the targeting vector. Trans. Res. 9, 79-80.

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Acknowledgements

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