**Uri Nudel**
**David Yaffe**

Rachel Sarig
Ora Fuchs
Sara Neuman
Tzadok Baruchi
Shlomo Goren
Moran Kovalio
Yael Rozenvald

# The Duchenne Muscular Dystrophy Gene: Structure, Evolution, Expression And Function Of Products

## Department of Molecular Cell Biology

**Tel.** 972 8 934 **2815** **Fax.** 972 8 934 **4125**
**E-mail:** uri.nudel@weizmann.ac.il david.yaffe@weizmann.ac.il
**Web page:** www.weizmann.ac.il/

### INTRODUCTION AND OBJECTIVES

Duchenne muscular dystrophy (DMD) is an X-linked recessive disorder of progressive muscle degeneration 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). Dystrophin, the product of the gene in the normal muscle, is a 427 kDa rod-shaped protein. It consists of an actin binding N-terminal domain, a large domain of spectrin-like repeats, a cystein-rich domain, and a C-terminal domain. 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 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 1989; Barnea et al. Neuron 1990). A third isoform is expressed in brain Purkinje cells. 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. 1990). Following these observations, three additional truncated DMD gene products have been identified by others (Fig. 1A). Dp71 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 spectrin-like repeats (Fig. 1A). It is the major product of the DMD gene in brain and many other non-muscle tissues. 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 and the function of the various products. 2) The involvement of Dp71 in brain function and in embryonic development. 3) Applications of some of our findings for prenatal diagnosis of DMD. 4) Myogenic stem cells, their nature and possible application for cell therapy.

Some of our recent investigations are briefly described below.

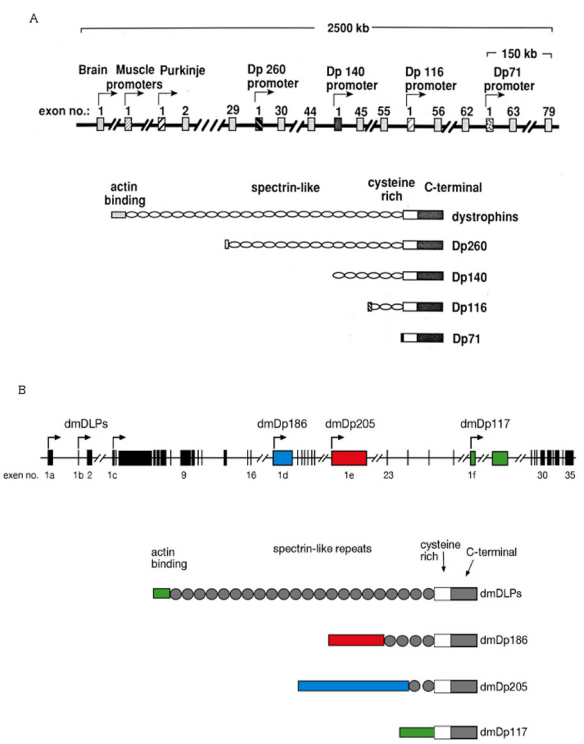


**Fig. 1** *Promoters and products of the DMD gene and its drosophila homologue. The human DMD gene (A) and its drosophila homologue (B), are presented schematically (upper parts). Bent arrows indicate the location of promoters. The products are presented schematically in the lower parts of A and B. dmDLPs = drosophila dystrophin-like proteins*

## RECENT FINDINGS

### Dp71 – The major non-muscle product 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 beta-gal. The insertion of the beta-gal gene enabled us a very sensitive monitoring of the activity of the promoter of Dp71 by X-gal staining. These studies revealed a very specific pattern of Dp71 promoter activity during embryonic development. High activity of Dp71 promoter was often associated with major morphogenic events and with terminal differentiation (Sarig et al. 1999). In spite of this, we did not detect conspicuous abnormality in the Dp71 null mice. Immunological and biochemical analysis indicated partial compensation for the lack of Dp71 by other products of the DMD gene.

However, recently we have found that in the retina, lack of Dp71 is associated with significant changes in the pattern of water and potassium channels and in the regeneration capacity of the retina (Dalloz et al. 2003) We are currently searching for similar effects in other parts of the nervous system and are testing the behaviour and learning capacity of Dp71 null mice.

### 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.

As shown in Fig. 1B, these studies revealed that 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 (ca. 600 million years ago). This conservation, in such distantly related organisms, points to important distinct functions of the multiple products. The characterization of the sea urchin and Drosophila genes and their products allowed us to construct an evolutionary tree connecting the dystrophin gene family in the vertebrates and related genes in invertebrates (Wang et al., 1998; Neuman et al., 2001). The Drosophila dystrophin gene homologue 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 this study (in collaboration with A. Sapir and T. Volk).

### Conservation of intron size in the DMD gene family.

Large introns are of disadvantage (a large target for mutations and energy investment during replication and transcription). Yet, the vertebrate dystrophin gene and its Drosophila homologue are characterized by relatively huge introns, (Ms. in preparation). This indicates the existence of yet unknown functions of the large introns. Relevant to this, we have found that both in mammals and in Drosophila most internal promoters are located in the largest introns. This suggests structural and regulatory functions of the introns.

### *Selected Publications*


Yaffe, D, Prigojin, H., Fuchs, O., Gvirtzer, N., Jean-Pierre, M., Kaplan, J-C, Shomrat, R., Legum, C, Nudel O. (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.

Sarig, R., Mezger-Lallemand, V., Gitelman, I., Davis, C., Fuchs, O., Yaffe, D., Nudel, U. (1999) Targeted inactivation of Dp71, the major non muscle product of the DMD gene – differential activity of Dp71 promoter during development. Hum. Mol. Genet. 8, 1-10.

Neuman, S., Kaban, A., Volk, T., Yaffe, D., Nudel, U. (2001) The dystrophin/ utropin homologues in Drosophila and in sea urchin. Gene 263, 17-29.

Leibovitz, S., Meshorer, A., Fridman, Y., Wieneke, S., Jockusch, H., Yaffe, D., Nudel, U. (2002) Exogenopus Dp71 is a dominant negative competitor of dystrophin in skeletal muscle. Neuromuscul Disord. 12, 836-844.

Dalloz, C., Sarig, R., Fort, P., Yaffe, D., Bordais, A., Pannicke, T., Grosche, J., Mornet, D., Reichenbach, A., Sahel, J., Nudel, U., Rendon, A. (2003). Targeted inactivation of dystrophin gene product Dp71: phenotypic impact in mouse retina. Hum. Mol. Genet. 12, 1543-1554.



***Acknowledgements:***

U.N. - Elias Sourasky Professorial Chair

Supported by grants from MDA, AFM, GIF, ISF, Center for Scientific Excellence (WIS)