## Aberrant splicing of ryanodine receptor in myotonic dystrophy

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*(Introduced by A.F. Dulhunty)*

It was reported that mRNAs for chloride channel (Mankodi *et al*., 2002), cardiac troponin T, insulin receptor and myotubularin-related 1 were aberrantly spliced in muscles from myotonic dystrophy (DM). In all cases, the developmentally regulated splice switch that involves a choice between two or more alternative isoforms is skewed, resulting in preferential expression of the isoform that is usually expressed in immature or non-skeletal muscle tissues. We previously reported the induction of the mRNA of small-conductance $Ca^{2+}$-actvated $K^{+}$ (SK3) channel that is usually expressed in immature fibers (Kimura *et al.*, 2000). Taken together, we postulate that there is a maturation-related abnormality in DM that explains the abnormal splicing and transcription. Based on this hypothesis, we investigated the splicing of two candidate mRNAs, which are developmentally regulated.

We used 28 muscle specimens; 10 from myotonic dystrophy type 1 (DM1), 5 from Amyotrophic Lateral Sclerosis, 5 from Polymyositis, 2 from limb-girdle muscular dystrophy and 6 from normal control. Myotubes cultured from 2 muscle specimens; 1 from DM1 and 1 from normal control, were also used. We examined splicing pattern of insulin receptor, ryanodine receptor of skeletal muscle type (RyR1) and β-tropomyosin, using RT-PCR. The total amount of RyR3 mRNA, which is expressed in immature muscles, was quantified. We also employed transgenic mice model of DM1, in which expanded CUG repeat expression in skeletal muscle leads to a DM-like phenotype, for the splicing pattern of RyR1.

We found a significant increase of an alternatively spliced isoform of RyR1 in DM1 patients. The alternative splicing results in the deletion of 5 amino acids at a modulatory region of the receptor and the isoform is normally expressed in undifferentiated muscles. The other splicing isoform of RyR1 was not significantly altered. The splicing of β-tropomyosin and the total amount of mRNA for RyR1 and RyR3 did not differ significantly. In mice, we found a significant increase of the same spliced isoform in long-repeat transgenic mice compared with short-repeat transgenic mice or wild type mice.

The same splicing pattern was found in DM1 patients and DM1 model mice, suggesting that expanded CUG repeat is sufficient for the abnormal splicing. The increase of an immature isoform of RyR1 supports our hypothesis of maturation-related abnormality in DM. Furthermore, RyR1 channel is a major calcium release pathway from sarcoplasmic reticulum and regulate contraction of skeletal muscle. It is possible that the aberrant isoform may be responsible for muscular degeneration of DM, although functional studies for this isoform are needed.

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