Endogenous phosphorylation of the dystrophin protein modulates protein function

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Dystrophin is a 427 kDa protein containing an NH_2-terminal actin-binding domain, a rod domain containing 24 spectrin-like repeats, a WW domain, a cysteine-rich domain, and a C-terminal domain. It forms a sarcomeric complex called the dystrophin-glycoprotein complex (DGC) that acts to link the sarcolemma to the actin cytoskeleton and transmit the forces of contraction. In addition dystrophin is hypothesized to have signalling functions *via* interactions with the dystroglycans, sarcoglycans, and dystrobrevins. Post-translational modification, particularly phosphorylation, is a mechanism by which protein function is modulated. *In vitro* studies have demonstrated that dystrophin is phosphorylated by various kinases and that some phosphorylation events may affect dystrophin interactions with actin and the syntrophins (Senter *et al.*, 1995; Madhavan & Jarrett, 1999). Yet whether dystrophin is endogenously phosphorylated *in vivo* and how this affects protein function remains to be investigated. We hypothesized that the structure and function of the endogenous dystrophin protein is modulated by phosphorylation.

All experiments were performed in accordance with the Institutional Animal Care and Use Committee of the University of Washington in Seattle, WA, USA. Full length dystrophin (Dp427) was immunoprecipitated from the hind limb muscle of C56BL/6 mice that were killed by rapid cervical dislocation, or from C2C12 cell lysate and subjected to SDS-PAGE. Dystrophin bands were excised from the gel and subjected to in-gel proteolytic digest, after which the resultant peptides were analysed by Liquid Chromatography-Mass Spectrometry (LC-MS/MS) for the identification of phosphorylated residues. Approximately 50-60% of the Dp427 sequence was analysed by mass spectrometry resulting in the identification of two phosphorylated threonine residues within the rod domain and three phosphorylated serine residues within the C-terminal domain.

Over expression of the dystrophin isoform Dp116 preserves functional muscle mass and prolongs the lifespan of severely dystrophic *mdx/utrn*$^{-/-}$ mice without preventing the characteristic degeneration/regeneration cycles of dystrophic muscle, thereby providing a tool in which to study dystrophin function in a pathologic environment (Judge *et al.*, 2011). Mass spectrometric analysis of Dp116 immunoprecipitated from the hind limb muscles of *mdx/utrn*$^{-/-}$ *Dp116* transgenic mice (that had been killed by rapid cervical dislocation) identified multiple sites of phosphorylation within the cysteine rich and C-terminal domains. *In vitro* mutagenesis studies confirmed that two of these phosphorylation sites, both within the cysteine-rich domain, may modulate the dystrophin-dystroglycan interaction as replacement of these amino acids with a negatively charged amino acid (which acts to mimic a phosphorylation event) restores dystrophin binding to β-dystroglycan which is lost when the amino acid is mutated to a non-phosphorylatable residue.

Taken together, these studies demonstrate that dystrophin is phosphorylated on multiple amino acids in healthy and dystrophic muscle *in vivo*, and that phosphorylation within the WW domain modulates dystrophin protein function.

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