**Detection of endogenous DUX4 protein in FSHD muscles:**

**could the spreading of a rare protein cause FSHD?**

Alexandra Tassin, Dalila Laoudj-Chenivesse1, Céline Vanderplanck, SébastienCharron, Eugénie Ansseau, Yi-Wen Chen2, Jacques Mercier1, Frédérique Coppée and Alexandra Belayew.

Laboratory of Molecular Biology, University of Mons, 7000 Mons, Belgium ; 1.INSERM U1046 Physiologie et Médecine expérimentale Cœur et Muscle, CHU A. de Villeneuve, 34295 Montpellier France ;  2.Children’s National Medical Center, Center for Genetic Medicine Research ; Washington DC 20010-2970, USA.

Facioscapulohumeral muscular dystrophy (FSHD) is linked to contractions of the D4Z4 repeat array in 4q35. We identified the double homeobox 4 (*DUX4)* gene in D4Z4 and found that the only stable DUX4 mRNAs were derived from the distal unit and extended to a polyadenylation signal within the flanking *pLAM* region. The DUX4 protein is expressed in FSHD but not in control primary myoblasts. DUX4 is a transcription factor that initiates a deregulation cascade, which leads to muscle atrophy and oxidative stress, both key features of FSHD. The only knowntarget gene of DUX4 is *PITX1*, which encodes a transcription factor involved in hindlimb specification during embryogenesis.

In this study, we focused on the expression kinetics of DUX4 and PITX1 during the differentiation of primary FSHD myoblasts. DUX4 was detected by immunofluorescence in a few scattered proliferating cells and in a larger number of nuclei during their differentiation into myotubes. Intriguingly, DUX4 and PITX1 staining revealed an intensity gradient between consecutive myonuclei, suggesting a protein dispersion from one nucleus to its neighbors. DUX4 and PITX1 were detected either in the same nucleus with a different subnuclear staining pattern or in different nuclei. We further demonstrated that both proteins were regulated by the ubiquitin-proteasome pathway. Finally, we could immuno-detect the DUX4 protein in FSHD muscle extracts.

In conclusion, we propose a new dynamic dispersion model of DUX4 and PITX1 proteins from one nucleus, where the gene is activated, to the adjacent nuclei within the same myotube. This model, together with the transcriptional amplification cascade initiated by DUX4, could explain how a protein that is expressed in scattered myonuclei causes the muscle defects observed in FSHD.

We thank the AFM (France), the ABMM, FRIA, and FNRS (Belgium) for funding.