

## Muscle Specific Fragile X Related Protein 1 Isoforms are Sequestered in the Nucleus of Undifferentiated Myoblast

### ABSTRACT

The pattern of subcellular partitioning of FXR1P isoforms during myogenesis is unique among the family of the FXR proteins. The model system described here should be considered as a powerful tool for ongoing attempts to unravel structure-function relationships of the different FMR family members since the potential role(s) of FXR1P as a compensatory factor in Fragile X syndrome is still elusive.

### INTRODUCTION

Background The Fragile X Mental Retardation (FMR) protein family is composed of three highly homologous members. The Fragile X Mental Retardation Protein (FMRP) is coded by the X-linked FMR1 gene and its absence is directly associated with human hereditary mental retardation [reviewed in ]. Two other members of this family are the Fragile X Related 1 (FXR1P) and Fragile X Related 2 (FXR2P) proteins that are coded by the FXR1 and FXR2 genes located at 3q28 and 17p13.1, respectively, in human. These genes are highly conserved in vertebrate evolution and contain two KH domains and a RGG box that are functional characteristic motifs in RNA-binding proteins. In addition, they also contain a nuclear localization signal (NLS) as well as a nuclear export signal (NES) making them putative nucleocytoplasmic shuttling proteins [reviewed in ]. Finally, FMRP as well as the other members of the family have been shown to be associated with messenger RiboNucleoParticles (mRNP) within actively translating ribosomes. This association suggests that their roles might be linked to RNA transport and/or translation. Whereas absence of FMRP is the cause of Fragile X Mental Retardation in human, it is not known whether FXR1P and FXR2P are associated to any pathology or phenotype. Also it is not known whether these homologous proteins can compensate for the absence of FMRP in the case of the Fragile X syndrome. In vitro studies showed that all three members interact with themselves and with each other. However, their distribution in certain mouse and human tissues showed individual pattern of expression indicating that each protein also may function autonomously. FXR1P has been shown to have a complex expression pattern in different mammalian cell lines since six distinct isoforms were observed and their respective levels were shown to be cell type specific. In particular, it was observed that 4 distinct FXR1P isoforms of MW 70 and 74 kDa (previously referred to as short) and 78 and 80 kDa (long) are widely expressed in diverse cell lines as well as in different organs in mouse. However, in muscle, these isoforms are replaced by novel super long isoforms of MW 82 and 84 kDa. The replacement of the short and long isoforms by the super long isoforms is clearly apparent during myogenesis of myoblastic cell lines that can differentiate in vitro into myotubes. This model system which mimics, although imperfectly, muscle differentiation has permitted us to show in the present report that transition of the short and long isoforms to the super long is an early event that takes place concomitantly to the expression of muscle-specific genes. In addition, we also show that low levels of the super long isoforms are constitutively expressed in undifferentiated myoblasts and that they are sequestered in the nuclei, while in differentiated myotubes P82,84 are transferred to the cytoplasm where they are incorporated in mRNPs present in actively translating ribosomes.

## CONCLUSION

Conclusions Previous studies on the FMR proteins have shown that although the FXR1 protein is predominantly cytoplasmic, in rare occasions a nuclear localization has been observed in undifferentiated cells in several tissues of human fetuses and mouse embryo. The model system of C2C4 myoblasts that can be manipulated in vitro to differentiate into myotubes provides strong evidence that specific isoforms of FXR1P are indeed sequestered in the nucleus in undifferentiated myoblasts. As a working hypothesis we propose that the pattern of nucleo-cytoplasmic partitioning of FXR1P isoforms is under the control of factors regulating cell differentiation. By extension, we also speculate that isoforms of FMRP, that for the moment have escaped detection due to the very restricted number of available antibodies, might play a nuclear role in mRNA maturation at specific stages of neuronal differentiation and plasticity. In conclusion, the model system described here should be considered as a powerful tool for ongoing attempts to unravel structure-function relationships of the different FMR family members since the potential role(s) of FXR1P and FXR2P as a compensatory factor(s) in Fragile X Mental Retardation is still elusive.