

Imaging of Dynamic Changes of the Actin Cytoskeleton in Microextensions of Live NIH3T3 Cells with a GFP Fusion of the F-Actin Binding Domain of Moesin

ABSTRACT

Despite the fact that shape alone could not differentiate between retraction fibers and advancing, retracting or stable filopodia, the fluorescence imaging of C-moesin-GFP revealed just as rapid and dynamic changes of the actin cytoskeleton in microextensions. The involvement of regional regulatory control is due to opposite events that occurred in close proximity and presumably independently of each other. This sensitive tool should be useful in examining mechanisms of localized actin dynamics in the cell cortex.

INTRODUCTION

Three homologous members make up the Fragile X Mental Retardation (FMR) protein family, with the FMRP being encoded by the x-linked FMR1 gene and its absence is linked to human hereditary mental retardation [reviewed in]. This family includes two other members: the Fragile X Related 1 (FXR1P) protein, coded in human by the FXR1 gene located at 3q28 and 17p13.1, and another lineage that encodes two KH domains and a RGG box—motifs in functional characteristic motifs of RNA-binding proteins. They also contain a nuclear localization signal (NLS) and NES, which make them putative nucleocytoplasmic shuttling proteins (reviewed in). There are indications that their functions may be related to RNA transport and/or translation. While FMRP is the cause of Fragile X Mental Retardation in human, it is unclear whether FXR1P and FxR2P are associated with any pathology or phenotype. Additionally, there is no evidence that these homologous proteins can counteract this absence. In vivo studies revealed that all three members interact with each other and in the same way. However, their expression patterns showed distinct patterns in certain tissues of mice and humans, suggesting that each protein may also function independently. Six different isoforms were identified and their levels were found to be specific to each cell type, indicating that FXR1P displays a complex expression pattern in various mammalian cell lines. They found four different FXR1P isoforms of MW 70 and 74 (previously called short) and later 78 and 80 kDa (long) are widely expressed in various cell lines as well as in mouse organs. The replacement of isoforms with novel MW 82 and 84 kDa super long isomerisms occurs in muscle. This phenomenon is evident during myogenesis of myocyte cell lines that can differentiate into myotubes. The model system that replicates, albeit imperfectly, muscle differentiation has enabled us to demonstrate, as previously mentioned, in the present report, that the short and long isoforms undergo a transitional event that coincides with the expression of muscle-specific genes, leading to the super long transition. Furthermore, we demonstrate that super long isoforms are expressed in low levels by undifferentiated myoblasts and are stored in the nuclei, whereas in differentiated myotubes, P82,84 are transferred to the cytoplasm and become part of mRNPs found in actively translating ribosomes.

CONCLUSION

Findings These studies suggest that PP5 plays a role in regulating GR nucleocytoplasmic shuttling and that the nuclear accumulation of GR is caused by suppressing PP5 expression without any hormone-mediated response. Hence, the previously reported increase in GR-induced

transcriptional activity following ISIS 15534 induced suppression of PP5 expression may be due to the nuclear accumulation of highly bound gre (a type of genetic material) that is capable of binding DNA, but still requires agonist interaction to induce maximum transcriptionally active synthesis. The specific manner in which PP5 hinders the nuclear accumulation of GRs is still unknown, as it remains unclear whether it acts to prevent the nucleus from being expelled.