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EXON 12 OF *FRAGILE MENTAL RETARDATION 1* RAT ORTHOLOG IS EXPRESSED IN MATURE, CYTOPLASMIC FMRP ISOFORMS IN RAT CEREBRAL NEURONS

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Fragile Mental Retardation Protein (FMRP) displays three characterized RNA-binding domains: two KH domains and an RGG box domain. It is expressed as different isoforms due to alternative splicing of *FMR1* hnRNA. When exon 12 is included in *FMR1* messages, the variable loop of FMRP KH2 domain encoded by exons 11 through 13 is predicted to increase in length. The functional significance of exon 12 inclusion is not yet understood nor is clear the role played by the FMRP KH2 variable loop. In addition, exon 12 has been shown to be preferentially excluded from *FMR1* mRNAs. To gain insight into the biological relevance of *FMR1* exon-12 alternative splicing, we raised polyclonal antibodies that specifically recognized the segment encoded by this exon. Rabbit immune sera, named 3460, identified isoforms containing exon 12-encoded amino-acids as bands migrating between 60 and 95 kDa in SDS-PAGE. The antibodies detected rat, mouse and human FMRP isoforms. In cultured cortical cerebral neurons from rats, we observed that these FMRP isoforms were cytoplasmic, and distributed to the perinuclear region and processes of beta-III tubulin-positive cells. Staining of cells expressing glial fibrillary acidic protein (GFAP) was negative. 3460 immune sera and monoclonal anti-FMRP 1C3 antibody showed overlapping staining in neuronal cells, and replacement of 3460 immune sera by the pre-immune sera abolished co-localized labeling. We conclude that exon-12 inclusion in *FMR1* mRNAs is biologically relevant as they are translated into mature, cytoplasmic FMRP isoforms. The developed antibodies thus constitute tools for investigating the functional relevance of *FMR1* exon-12 expression in the brain.