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Short communication

Rgs4 mRNA expression is decreased in the brain of *Fmr1* knockout mouse

Topi Tervonen[a], Karl Åkerman[b], Ben A. Oostra[c], Maija Castrén[a,*]

[a]*Neuroscience Center, University of Helsinki, P.O. Box 56, Viikinkaari 4, FIN-00014 Helsinki, Finland*
[b]*A.I. Virtanen Institute, University of Kuopio, P.O. Box 1627, FIN-70211 Finland*
[c]*Department of Clinical Genetics, Erasmus University, P.O. Box 1738, 3000 DR, Rotterdam, The Netherlands*

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Abstract

In the *Fmr1* knockout mice, a model for fragile X mental retardation syndrome, the levels of regulator of G-protein signaling (Rgs) 4 but not Rgs2 mRNA were considerably reduced (65% from control) in the cerebral cortex and hippocampal CA1 region. The expression of Rgs4 was normal in animals lacking a related protein, FXR2P, indicating that the decrease in Rgs4 expression was specific for the absence of FMRP, and suggests a role for FMRP in G-protein signaling.

Theme: Development and regeneration
Topic: Process outgrowth, growth cones, and sprouting

Keywords: Regulator for G-protein signaling 4; Regulator for G-protein signaling 2; FMRP; *Fmr1* knockout mouse; In situ hybridization; Gene expression

Fragile X syndrome, caused by the absence or dysfunction of FMR1 protein (FMRP), is a common form of inherited mental retardation [7]. It is associated with behavioral problems and psychiatric morbidity in human [3,8]. FMRP and the fragile-X-related protein (FXR2P) are members of a small family of fragile-X-related (FXR) proteins which share similar structure of RNA binding proteins [18]. *Fmr1*-knockout (*Fmr1*-KO) mice have been shown to serve as a model for human fragile X syndrome [15]. FMRP associates with polysomes and can modify translation of specific mRNAs, including mRNAs for many proteins involved in signal transduction [9–11,17]. The absence of FMRP results in abnormalities of dendritic spines and enhanced LTD, suggesting that FMRP is necessary for normal maturation and function of synapses [5,13,19]. Regulators of G-protein signaling (Rgs) proteins are GTPase-activating proteins for several G-protein subunits and thereby signaling modulators of G-protein-coupled receptors [4]. The major neuronal Rgs transcripts Rgs2 and Rgs4 demonstrate regional specificity, and the dynamic regulation of their mRNA expression has been postulated to correlate with the onset and principal sites of certain synapse establishment [6]. The putative connection of the Rgs2 and Rgs4 proteins to synaptogenesis and mental disorders prompted us to investigate the expression of these proteins in fragile X syndrome by analyzing their mRNA expression in the brain of the *Fmr1*-KO mouse and the knockout mouse for the *Fxr2* gene [2].

Coronal sections (14 μm) of freshly frozen brains of inbred FVB *Fmr1*-KO (n=5) [15], *Fxr2*-KO (n=5) [2] and wild-type (n=5) P10 mice were fixed in 4% paraformaldehyde solution, and in situ hybridization was performed as described previously [16] with oligonucleotide probes for mouse Rgs4 (5′-GCTGGAAGGATTGGTCAGGTCAAGATAGAATCGAG-3′) and Rgs2 (5′-GGGCTCCGTGGTGATCTGTGGCTTTTTACATAAG-3′) 3′end-labeled with [α-^{33}P] dATP (2000 Ci/mmol, New England

* Corresponding author. Tel.: +358 9 191 57627; fax: +358 9 191 57620.
E-mail address: Maija.Castren@helsinki.fi (M. Castrén).

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Nuclear, Boston, MA). Oligonucleotides consisting sense and scramble sequences were used as negative controls. Hybridized sections were exposed to Kodak Biomax MR films (Kodak, France). Quantification of autoradiograms was performed with MCID image analysis system (Imaging Research, St. Catharines, Ontario, Canada), and optical densities were converted to specific radioactivity (nCi/g) using ^{14}C microscales (ARC, St.Louis, MO). Two measurements were obtained per region (the CA1 area from the hippocampus, and the retrosplenial and the piriform cortex) per rat from 2–5 slides containing three sections each and then averaged. The experiments were repeated twice. Statistical analysis was performed in Excel, and Student's *t*-test was performed to confirm statistical significance.

We examined expression of Rgs mRNAs in the mouse brain at P10 when dendritic abnormalities have been found in the *Fmr1*-KO mice. The expression of Rgs4 mRNA was abundant in the discrete neuronal layers of the cerebral cortex and the hippocampus of the wild-type P10 mouse brain, as previously reported (Fig. 1A) [6]. No significant hybridization signal was detected with respective control probes (Fig. 1C). In the neocortex, the high Rgs4 expression was seen in layers II–III and V–VI as in the adult brain. Rgs4 was expressed abundantly in the pyramidal cell layer of the hippocampus but was not detectable in the dentate gyrus. The Rgs4 mRNA levels were significantly lower in the CA1 region of the hippocampus in the *Fmr1*-KO brain than in the control brain (65% of control, $p<0.05$; Fig. 1A). The Rgs4 mRNA levels were also significantly decreased in the retrosplenial cortex of the *Fmr1*-KO mice (65% of control, $p<0.005$; Fig. 1A), suggesting a delayed maturation of the Rgs4 expression pattern during postnatal development. In the piriform cortex, where the Rgs4 expression has been shown to be constant during postnatal development, no change in the Rgs4 mRNA levels was found in the *Fmr1*-KO mice when compared to the wild-type mice (Fig. 1B). We also examined the Rgs4 expression in the mature brain, but we did not find statistically significant differences in the Rgs4 mRNA levels when we compared the Rgs4 expression in the *Fmr1*-KO brain to the expression in the wild-type brain (in the retrosplenial cortex 72±28% from control, $p=0.39$; in the CA1 region of the hippocampus 75±24% from control, $p=0.26$). However, a tendency

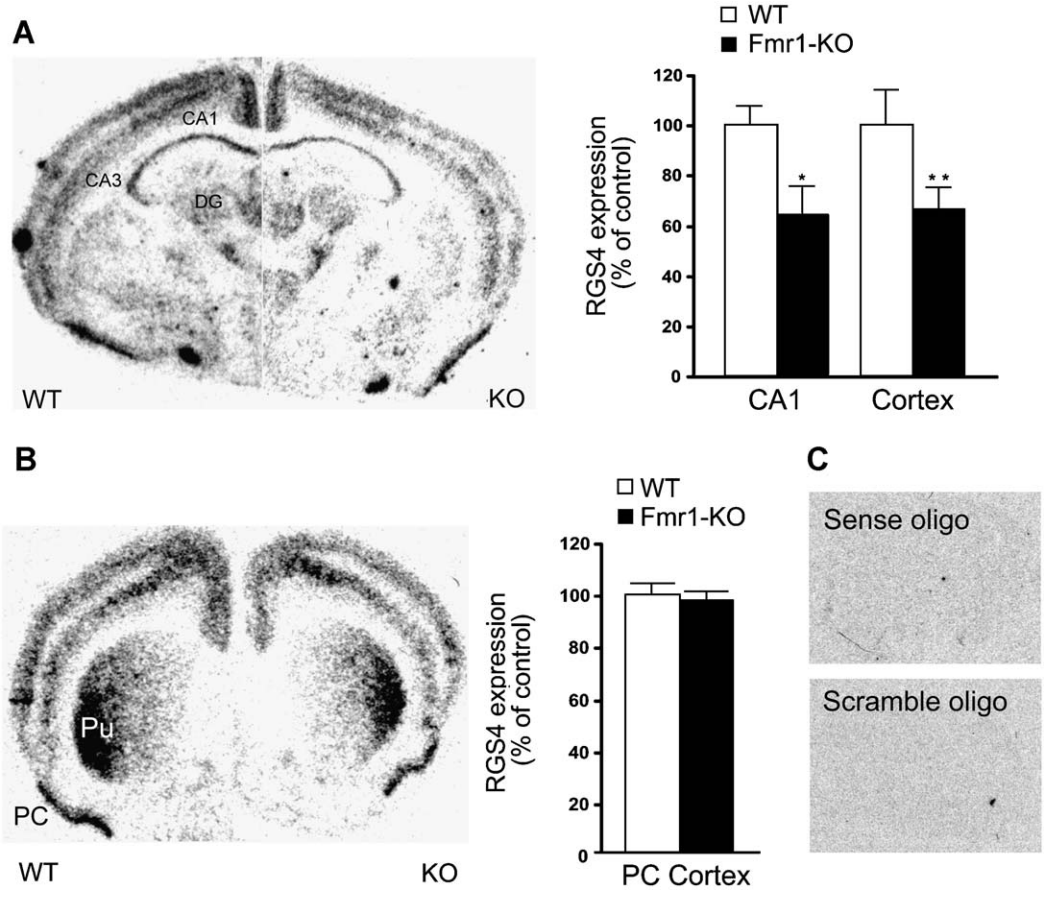

Fig. 1. Expression levels of Rgs4 mRNA were assessed by radioactive in situ hybridization in coronal brain sections of P10 Fmr1-KO mice. (A) Rgs4 mRNA expression was significantly decreased in the hippocampal CA1 region and in the retrosplenial cortex of *Fmr1*-KO mice compared to controls ($n=8$). (B) No difference was found between the Rgs4 expression levels in the piriform cortex of wild-type and *Fmr1*-KO mice. (C) Hybridization signal was not detectable in the negative controls hybridized with the sense or scramble oligonucleotide probes. Error bars indicate±S.E.M. Asterisks (*) and (**) indicate statistically significant differences ($p<0.05$) and ($p<0.005$) versus respective controls, respectively. DG, dentate gyrus; Pu, caudate putamen; PC, piriform cortex.