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ORIGINAL RESEARCH

Drosophila Homolog of FMRP Maintains Genome Integrity by Interacting with Piwi

Fangfang Jiang [a], Falong Lu [b], Peixue Li [c], Wei Liu [a,d,e,*], Lu Zhao [a], Qifu Wang [a], Xiaofeng Cao [b], Lei Zhang [c], Yong Q. Zhang [a,*]

[a] *State Key Laboratory for Molecular and Developmental Biology, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing 100101, China*

[b] *State Key Laboratory of Plant Genomics and National Center for Plant Gene Research, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing 100101, China*

[c] *State Key Laboratory of Molecular Biology, Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 200031, China*

[d] *Science and Technology Center, Fenyang College, Shanxi Medical University, Shanxi 032200, China*

[e] *Department of Medical Laboratory Science, Fenyang College, Shanxi Medical University, Shanxi 032200, China*

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ABSTRACT

Fragile X syndrome (FraX), the most common form of inherited mental retardation, is caused by the absence of the evolutionally conserved fragile X mental retardation protein (FMRP). While neuronal functions of FMRP have been intensively studied for the last two decades, its role in non-neuronal cells remains poorly understood. Piwi, a key component of the Piwi-interacting RNA (piRNA) pathway, plays an essential role in germline development. In the present study, we report that similar to *piwi*, *dfmr1*, the *Drosophila* homolog of human *FMR1*, is required for transposon suppression in the germlines. Genetic analyses showed that *dfmr1* and *piwi* act synergistically in heterochromatic silencing, and in inhibiting the differentiation of primordial germline cells and transposon expression. Northern analyses showed that *roo* piRNA expression levels are reduced in *dfmr1* mutant ovaries, suggesting a role of *dfmr1* in piRNA biogenesis. Biochemical analysis demonstrated a physical interaction between dFMRP and Piwi *via* their N-termini. Taken together, we propose that dFMRP cooperates with Piwi in maintaining genome integrity by regulating heterochromatic silencing in somatic cells and suppressing transposon activity *via* the piRNA pathway in germlines.

KEYWORDS: Fragile X syndrome; FMRP; *piwi*; piRNA; Germline

INTRODUCTION

Fragile X syndrome (FraX), the most common form of inherited mental retardation, is caused by the absence of fragile X mental retardation protein (FMRP), which contains nuclear localization (NLS) and export (NES) signals, and functions as a nucleocytoplasmic shuttling protein. FMRP harbors a tandem Tudor domain mediating protein–protein

Abbreviations: co-IP, co-immunoprecipitation; *dfmr1*, *Drosophila* fragile X mental retardation 1; dFMRP, *Drosophila* fragile X mental retardation protein; *FMR1*, fragile X mental retardation 1; FMRP, fragile X mental retardation protein; FraX, Fragile X syndrome; GFP, green fluorescent protein; GSC, germline stem cell; HP1, heterochromatin protein 1; LTR, long terminal repeat; miRNA, microRNA; PBS, phosphate buffered saline; PBST, phosphate buffered saline with Triton X-100; PcG, Polycomb group; PEV, position effect variegation; PGC, primordial germ cell; piRNA, Piwi-interacting RNA; RT-PCR, reverse transcription-polymerase chain reaction; SEM, standard error of the mean.

* Corresponding authors. Tel/fax: +86 10 6480 7611 (Y.Q. Zhang); Tel/fax: +86 358 7235 034 (W. Liu).
E-mail addresses: weil998@sina.com (W. Liu); yqzhang@genetics.ac.cn (Y.Q. Zhang).

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interactions and three RNA binding domains: two K homology (KH) domains and one Arg-Gly-Gly (RGG) box (Ramos et al., 2006; Santoro et al., 2012). The *Drosophila* homolog of FMRP (dFMRP) is structurally and functionally conserved with the mammalian FMRP. Thus, extensive studies using the *Drosophila* model system over the previous decade have contributed substantially to our current understanding of the molecular pathways implicated in FraX (Zhang and Broadie, 2005; Callan et al., 2010; Tessier and Broadie, 2011; Liu et al., 2012).

Earlier studies have reported that dFMRP is associated with Argonaute2 (Ago2), a core component of the RNA-induced silencing complex (RISC) (Caudy et al., 2002; Ishizuka et al., 2002). However, the role of dFMRP in RNA interference has not yet been ascertained. Mounting evidence has established that FMRP is involved in microRNA (miRNA) pathways in neuronal and non-neuronal cells (Jin et al., 2004; Xu et al., 2008; Yang et al., 2009; Liu et al., 2015). miRNAs are single-stranded and composed of 21−24 nt that are processed from longer (~70 nt) stem-loop precursors by Dicer, a dsRNA-specific RNase III. FMRP interacts with components of the miRNA pathway, including Dicer1 and Ago1 in mammals (Jin et al., 2004) and altered expression profiles of miRNA have been reported in the brain of *Fmr1* knockout mice (Liu et al., 2015). Consistently, Ago1 is critical for dFMRP function in neural development and synaptogenesis in *Drosophila* (Jin et al., 2004). Additionally, a nervous system-specific miRNA (miR-124a) is associated with dFMRP and the steady-state level of miR-124a is regulated by dFMRP in the nervous system (Xu et al., 2008). In the non-neuronal ovary, dFMRP acts together with Ago1 and the miRNA *bantam* to modulate the fate of germline stem cells (GSCs) (Yang et al., 2009).

The Piwi-interacting RNA (piRNA) pathway is a newly discovered small RNA pathway, which is distinct from the miRNA pathway. Compared with miRNAs, piRNAs are larger in size (26−31 nt rather than 21−24 nt), and derived from repetitive genetic elements and single-stranded RNA precursors (Ross et al., 2014; Iwasaki et al., 2015). Piwi, the founding member of the Piwi clade of the Argonaute family, is a critical player in the piRNA pathway (Vagin et al., 2006). Piwi is essential in silencing selfish genetic elements including transposons and repetitive sequences in *Drosophila* germlines (Vagin et al., 2006). In addition, *piwi* mutations lead to severe oogenesis defects including fewer GSCs (Lin and Spradling, 1997; Zamparini et al., 2011). However, how Piwi executes these distinct functions remains unclear.

While neuronal functions of FMRP have been under intensive studies for the last two decades, its role in non-neuronal cells remains poorly understood, despite recent findings that FMRP is involved in DNA damage response across species from *D. melanogaster* to mice (Liu et al., 2012; Alpatov et al., 2014; Zhang et al., 2014). In the present study, we report that dFMRP and Piwi act synergistically in heterochromatic gene silencing in somatic cells and transposon repressing in germline cells. In addition, the expression of *roo* piRNAs was reduced in *dfmr1* mutant ovaries, suggesting that dFMRP participates in piRNA biogenesis. Furthermore, we found that dFMRP and Piwi are present in the same protein complex by co-immunoprecipitation (co-IP) analysis of ovarian lysates. Co-IP assays of S2 cells transfected with various truncated variants of the two proteins further revealed that the interaction is mediated by their N-termini. Taken together, we demonstrated for the first time that dFMRP co-operates with Piwi in maintaining genome integrity by silencing heterochromatic genes and suppressing transposon expression. These findings offer novel insight into the physiological function of FMRP and FraX pathogenesis.

RESULTS

dFMRP and Piwi synergistically suppress heterochromatic silencing

Many genes involved in heterochromatin silencing have been identified by screens for dominant suppressors of position effect variegation (PEV), a phenomenon of gene silencing that occurs when a euchromatic gene is placed in a heterochromatic domain (Schulze and Wallrath, 2007). Insertion of a *white*$^{+}$ transgene in a euchromatic region results in a uniform red eye, whereas insertion of the transgene in the pericentric heterochromatin or much of the small fourth chromosome results in a variegating red eye phenotype (Fig. 1A). A specific *white*$^{+}$ insertion line (*118E-10*) showed loss of silencing by dominant PEV suppressors such as *Su(var)205* (Wallrath and Elgin, 1995). We examined the effect of different *dfmr1* alleles on the PEV line and found that both null alleles (*dfmr1*^{3} and *dfmr1*^{50M}) dominantly suppressed PEV (Fig. 1C and D), consistent with a previous report (Deshpande et al., 2006).

piwi mutations also suppressed PEV [Fig. 1E and (Pal-Bhadra et al., 2004)]. Quantitatively, heterozygous *dfmr1*^{50M} and *piwi*^{2} mutations resulted in 1.5- and 2-fold increase in the production of eye pigment, respectively, compared to the control line *118E-10* (Fig. 1H). To understand a possible genetic interaction between *dfmr1* and *piwi*, we examined PEV suppression in *piwi*^{2}/+;*dfmr1*^{50M}/+ trans-heterozygous mutants. Quantitative analysis showed that the trans-heterozygous mutants produced significantly more eye pigment than the *piwi* or *dfmr1* single heterozygous mutants (Fig. 1H). The enhanced suppression of PEV by the trans-heterozygous mutants suggests that *dfmr1* and *piwi* function in a similar pathway that regulates heterochromatic silencing.

dFMRP is required for PcG-mediated silencing

Different from heterochromatic silencing, Polycomb-group (PcG) proteins participate in the transcriptional silencing of various euchromatic genes such as homeotic genes crucial for proper development. PcG proteins are recruited to DNA sequence elements termed PcG response elements (PREs) located at or near the promoters of the genes and repress their expressions. In *Drosophila*, the *Fab-7* DNA sequence harbors a PRE and regulates the expression of the homeobox gene *Abdominal-B*, located in the *bithorax* complex, a locus subject