Response to Zhang et al

Amit Anand & Toshie Kai

Replying to: Zhang et al 2014 EMBO J 33: 536–539

Two recent reports by Zhang et al published in Nov (2011) and our group (Anand & Kai, 2012; published online in Dec 2011) have suggested a role for the conserved Tudor domain protein encoded by qin as a component of piRNA pathway. Both studies agree that qin plays a role in the piRNA pathway and is required to repress retroelements but differ in some aspects of their conclusions about qin function. We reported that kumo

\[ \text{kumo}^{A41-13} \]

hereafter designated qinkumo, homozygous females exhibited mislocalization of many piRNA pathway components to the nuage. The key issue is whether the localization of these proteins in perinuclear structure where piRNA pathway components are concentrated in the ovary. We also reported a reduction in piRNAs targeting transposons (Supplementary Table 3 in Anand & Kai, 2012). In contrast, Zhang et al found no change in localization of piRNA pathway components to the nuage and observed an increase in Aubergine(Aub) perinuclear foci. By contrast, in qinkumo/Df(3R)Exel6180 females and Fig 1 shows the result of an independently repeated experiment. In stage 5-6 egg chambers of the heterozygous control, Tudor domain proteins, Tejas (Tej) and Krimper (Krimp), and the Piwi-family proteins, Aub and Ago3, are observed as perinuclear foci. In contrast, in qinkumo/Df(3R)Exel6180 ovaries, the prominent localization of these proteins in perinuclear foci was perturbed. Krimp and Tej foci made larger aggregates in the cytoplasm (with more than 80% of egg chambers showing clear mislocalization), while Aub and Ago3 showed larger aggregates slightly away from nuage with a similar penetrance (>70%). We observed the same defects in later stage egg chambers of qinkumo/Df(3R)Exel6180 ovaries (data not shown). These results confirm our original finding that qin is required for robust localization of these piRNA pathway components to perinuclear nuage (Anand & Kai, 2012). We also note that our earlier report showed that expression of a full-length qin transgene under control of the germline driver, nosGal4, rescued the sterility of the mutant (65% hatching rate compared to sibling control) and the defects in nuage localization of several piRNA pathway components in qinkumo homozygous female (Fig 2 and Supplementary Table 1 in Anand & Kai, 2012), further supporting that the observed defects were mainly caused by the loss of qin function.

In their correspondence, Zhang et al also claimed that piRNA levels between qinkumo homozygous and qinkumo/TM3 ovaries were similar in the small RNA libraries published by both groups (GSE34728, Anand & Kai, 2012; Zhang et al, 2014). Our libraries contain a large number of non-coding RNAs derived from ribosomal RNAs and internally transcribed spacers and our mutant library contained almost three times more endo-siRNAs and flamenco-derived piRNAs than control libraries (Supplementary Table 1, this response). In addition to our previous normalization with noncoding RNA (reads mapping to rfam database), we now compared piRNA levels between the mutant and heterozygote after normalizing them separately with endo-siRNA (Malone et al, 2009; Handler et al, 2011), snoRNA-derived non-coding RNA (Taft et al, 2009), and repeat- derived 21-nt small RNAs. All normalization methods bring flamenco-derived piRNAs to equivalent levels between mutants and controls: flamenco-derived piRNAs are not much affected by germline piRNA pathway mutants (Malone et al, 2009), thus making them a good parameter to examine the raw data normalization. The new analysis confirmed approximately 2.5-fold reduction in cluster-mapping piRNAs and 1.5–1.9-fold reduction in transposon-matching piRNAs in qinkumo homozygous ovaries (Supplementary Table 1, this response). This reduction is comparable to what we reported...
previously (1.9-fold reduction in transposon-mapping piRNAs, Supplementary Table 4, Anand & Kai, 2012). In addition, small RNA libraries of qinkumo and qinkumo/TM3 published by Zamore’s group indicate approximately 2.23-fold reduction in transposon-mapping piRNAs in qinkumo upon normalization with ncRNAs (Zhang et al, this correspondence).

The subtle decrease in transposon-mapping piRNAs reported by our group and the piRNA analysis of qin mutants performed by Zhang et al placed qin in a separate category from other piRNA components such as aub, krimp, armi and spn-E whose loss-of-function leads to a severe loss of piRNAs (Malone et al, 2009). Though we did not focus on Aub- and Ago3- bound piRNA populations in our original study, and hence could not appreciate the homotypic ping-pong, we did report an increase in sense piRNAs mapping to many transposon families (Supplementary Fig 9 and Table 4 in Anand & Kai, 2012; Supplementary Table 1, this response). The piRNA analysis conducted here also shows the abovementioned trend (Supplementary Table 1, this response), which is likely explained by the observations by Zamore and the colleagues that loss of qin reduces antisense piRNAs and increases the level of sense piRNAs as a result of Aub-Aub homotypic ping-pong (Fig 4 and 5 in Zhang et al, 2011).

Supplementary information for this article is available online: http://emboj.embopress.org

References


