**Tex19** paralogs are new members of the piRNA pathway controlling retrotransposon suppression

Yara Tarabay¹,⁵,⁶, Mayada Achour¹,⁶, Marius Teletin¹,², Tao Ye¹, Aurélie Teissandier³, Manuel Mark¹,², Déborah Bourc’his³, Stéphane Viville¹,⁴,*°

¹Institut de Génétique et de Biologie Moléculaire et Cellulaire (IGBMC), Institut National de Santé et de Recherche Médicale (INSERM) U964/ Centre National de Recherche Scientifique (CNRS) UMR 1704/Université de Strasbourg, 67404 Illkirch, France
²Service de Biologie de la Reproduction, Centre Hospitalier Universitaire, 67000 Strasbourg, France
³Institut Curie, department of Genetics and Developmental Biology, CNRS UMR3215, INSERM U934, Paris, France
⁴Centre Hospitalier Universitaire, 67000 Strasbourg, France
⁵present address: Institut de génétique humaine (IGH), 141 rue de la Cardonille, 34396 Montpellier, France
⁶Co-first authors
*To whom correspondence should be addressed
°present addresses: Laboratoire de diagnostic génétique, UF3472 – Infertilité, Nouvel Hôpital Civil, 1 place de l’Hôpital, 67091 Strasbourg cedex and IPPTS, 3, rue Koeberlé, 67000 Strasbourg
Contact information: stephane.viville@unistra.fr
Tel : +33 3 68 85 37 00
Fax : + 33 3 68 85 38 09

Journal of Cell Science • Advance article
JCS Advance Online Article. Posted on 2 March 2017
Summary statement

Through *in vitro* and *in vivo* experiments, the present study demonstrates that the two TEX19 paralogs belong to the family of piRNA-interacting proteins.

Abstract

*Tex19* genes are mammalian specific and duplicated in *Tex19.1* and *Tex19.2* in some species, such as the mouse and rat. It has been demonstrated that mutant *Tex19.1* males display a variable degree of infertility whereas they all upregulate MMERVK10C transposons in their germ line. In order to study the function of both paralogs in the mouse, we generated and studied double knockout (*Tex19DKO*) mutant mice. Adult *Tex19DKO* males exhibited a fully penetrant phenotype, similar to the most severe phenotype observed in single *Tex19.1KO* mice, with small testes and impaired spermatogenesis, defects in meiotic chromosome synapsis, persistence of DNA double-strand breaks during meiosis, lack of post-meiotic germ cells and upregulation of MMERVK10C expression. The phenotypic similarities with *Piwi* KO mice prompted us to check and then demonstrate, by immunoprecipitation and GST pulldown followed by mass spectrometry analyses, that TEX19 paralogs interact with PIWI proteins and their VPTEL domain directly binds piRNAs in adult testes. We therefore identified two new members of the postnatal piRNA pathway.

Key words:

Meiosis, piRNA pathway, Infertility, Transposable elements
Introduction

In all sexually reproducing species, germ cells are the cell lineage that is specialized in transferring genetic information from one generation to the next (Hajkova, 2011). The genome of germ cells needs to be maintained stably and protected in all individuals throughout development. In particular, the genome must be protected against mobile transposable elements (TEs). These TEs can move, amplify themselves and transpose into new genomic loci prompting a serious threat to the genome. Although most TEs are inert, some elements have retained activity and become typically expressed in the germ line where they can transpose into new copies and pass onto the next generation. TEs account for almost half of the human and mouse genomes. They are classified into two categories, class I, which represents the majority of the TE families and class II, which accounts for approximately 3% of the mammalian genome (Zamudio and Bourc'his, 2010, Goodier and Kazazian, 2008). Class I TEs are believed to be ancient traces of viral infections; they transpose themselves using a “copy and paste” mode involving an RNA intermediate, which is converted into cDNA before its integration. Class II TEs are DNA transposons, which transpose using a “cut and paste” mode without any transcription needed (Goodier and Kazazian, 2008, Lander et al., 2001, Zamudio and Bourc'his, 2010).

Despite an undeniable role in evolution (Furano et al., 2004, Ostertag and Kazazian, 2001, Zamudio and Bourc'his, 2010), TEs can be responsible for drastic mutagenic effects illustrated by the growing number of human diseases caused by their transposition and expression (Cowley and Oakey, 2013). Germ cells have developed sophisticated strategies to control TE activities at the transcriptional level by DNA methylation and the post-transcriptional level by RNA degradation or by blocking their integration for example (Gasior et al., 2006, Obbard et al., 2009, Rollins et al., 2006, Yoder et al., 1997, Zamudio and Bourc'his, 2010). For reasons that remain unclear, the control of TEs in the mammalian germ line is gender-specific. Indeed, it has been shown that the protection of male germ line genomes relies on the PIWI family of ARGONAUTE proteins, which produce and bind small RNAs around 24-30 nucleotides long (named piRNAs) (Ghildiyal and Zamore, 2009). In mice, the PIWI clade contains three members, namely MIWI (PIWIL1), MILI (PIWIL2) and MIWI2 (PIWIL4) (Kuramochi-Miyagawa et al., 2004), which interact with germ line-specific members of the TUDOR family proteins such as TDRD1, TDRD5, TDRD6, TDRD7 and TDRD9 (Chuma et al., 2003, Hosokawa et al., 2007, Vasileva et al., 2009, Zheng and Wang, 2012). They also interact with other factors such as MOV10L1, MVH (DDX4), MAEL or...
GASZ (Costa et al., 2006, Ma et al., 2009, Soper et al., 2008, Zheng et al., 2010, Kotaja et al., 2006, Kuramochi-Miyagawa et al., 2004). Mutation of these piRNA pathway members give rise to a similar phenotype consisting of male infertility due to meiosis defects that can take place at different stages of meiosis and lead to an up-regulation of most TEs (Zamudio and Bourc'his, 2010). Despite a large number of studies, particularly involving loss-of-function mice, the exact mechanism whereby piRNAs control TE expression and the link between the loss of TE control and meiosis defects are far from fully understood. Currently, only the male process is beginning to be deciphered while the female one remains to be discovered.

In 2001, Wang et al described a set of testis expressed (Tex) genes, including Tex19 (Wang et al., 2001). In a previous study, we showed that Tex19 is a mammal-specific gene, which is duplicated in the mouse genome into two paralogs, Tex19.1 and Tex19.2. By multiple sequence alignment of mammalian TEX19 proteins, two highly conserved domains, named MCP and VPTEL respectively, were identified. However, none of them shares homology with known proteins, preventing functional prediction (Kuntz et al., 2008). Both genes are co-expressed in the ectoderm, then in primordial germ cells (PGCs). Later on, they are co-expressed from embryonic day 13.5 until adulthood in testes. However, only Tex19.1 transcripts are present in developing and adult ovaries as well as in the placenta (Celebi et al., 2012). Tex19.1 Knockout (KO) mice show a variable degree of male infertility due to impaired meiosis associated with an up-regulation of MMERVK10C retrotransposon expression (Ollinger et al., 2008, Tarabay et al., 2013). More recently, it was shown that Tex19.1 plays an important role in placenta function, as Tex19.1KO mouse embryos exhibit intra-uterine growth retardation and have small placentas due to reduced number of spongiotrophoblast, glycogen trophoblast and sinusoidal trophoblast giant cells (Reichmann et al., 2013, Tarabay et al., 2013). Tex19.2KO mice are fertile presenting only a subtle phenotype with discrete seminiferous tubule degeneration in adult male testes (unpublished observations Tarabay et al). The co-expression of Tex19.1 and Tex19.2 in PGCs, gonocytes and spermatocytes suggests that these two genes could play redundant functions during spermatogenesis.

By generating a double knockout of both Tex19.1 and Tex19.2 genes (Tex19DKO), we demonstrate here that TEX19 paralogs exhibit redundant functions, which are essential for male fertility. We show that the Tex19DKO phenotype is fully penetrant, mimicking the most severe phenotype observed in single Tex19.1KO mice. All adult DKO males are infertile and display impaired spermatogenesis: meiosis is blocked at the pachytene stage- during which
chromosome synapsis is not correctly formed or incomplete- and this leads to testis degeneration. Furthermore, MMERVK10C retrotransposon expression is up-regulated during meiosis in Tex19DKO testes. Through GST pull-down and immunoprecipitation experiments, we found that TEX19 paralogs interact with PIWI proteins and their accessory partners; they have moreover the ability to bind RNAs of around 30 nucleotides, a diagnostic size for piRNAs, but are not required for their biogenesis. Detailed analysis further showed that TEX19 directly interacts with piRNAs via its VPTEL domain. Altogether, our study provides novel evidence that TEX19 proteins are new members of the PIWI/piRNA pathway acting during post-natal spermatogenesis.

Results

**Tex19 paralogs play an essential role in male fertility**

*Tex19.1*KO mice show a variable level of male infertility. : one third of the KO male mice are almost indistinguishable from wild type (WT) mice, one third display a mild phenotype and the last third a severe phenotype with a complete meiosis arrest at pachytene (Ollinger et al., 2008, Tarabay et al., 2013). Independently of the severity of the phenotype, a two-fold up-regulation of MMERVK10C elements was consistently observed among *Tex19.1*KO animals (Ollinger et al., 2008, Tarabay et al., 2013). In contrast, *Tex19.2*KO mice are fertile and their testes are undistinguishable from controls. However, some seminiferous tubules show epithelium vacuolization (unpublished observations Tarabay et al). Considering the overlapping profile of *Tex19.1* and *Tex19.2* expression (Celebi et al., 2012), we wondered whether there was any functional redundancy between the two genes in testes. We therefore generated conditional double KO mice for *Tex19.1* and *Tex19.2* genes, which exist as tandem duplication (Fig 1A).

The KO of both alleles (so called *Tex19DKO*) was confirmed by PCR (Fig 1B) and the absence of the encoded proteins by western blot using testes extracts from double wild-type (DWT), *Tex19.1*KO, *Tex19.2*KO or *Tex19*DKO samples (Fig 1C and data not shown).

Interbreeding of *Tex19* double heterozygote (DHZ) animals resulted in a statistically significant deviation of the frequency of double homozygous animals from the expected Mendelian 1:2:1 ratio (207 (29.3%) *Tex19*DWT mice, 440 (62.3%) *Tex19* double heterozygote (DHZ) mice and 59 (8.3%) *Tex19*DKO mice, p<0.05, Student t-test). Yang et
al., 2010 previously described a more important female lethality among Tex19.1KO animals, an observation which was not shared by other independent studies including ours (Ollinger et al., 2008, Tarabay et al., 2013, Yang et al., 2010). Nonetheless, we found that the number of Tex19DKO females was significantly reduced when compared to Tex19DKO males (32.2% of females versus 67.8% males, P<0.05), suggesting a gender-specific lethality. Surviving Tex19DKO mice did not present gross somatic abnormalities. Females displayed normal fertility, but males were sterile.

Tex19DKO males displayed a constant and severe reduction in testis size (Fig 2A). The weight of Tex19DKO adult testes (ranking from 19mg up to 35mg, mean 24.4mg ±10.5mg per testis) was three-fold less than that of WT testes (ranking from 109mg up to 135mg, mean 113.5mg ±5.52mg per testis) (Fig 2B). In contrast to WT seminiferous tubules (Fig 2C), Tex19DKO testes histology showed degenerate seminiferous epithelium with tubules containing only early spermatogenic cells, but a complete lack of post-meiotic germ cells, suggesting a meiotic arrest (Fig 2D); consequently, the epididymis was free of spermatozoa (Fig S1A, B). Adult Tex19DKO seminiferous tubules exhibited a drastically increased level of apoptosis when compared to WT (Fig S1C). This phenotype is comparable to the most severe phenotype observed in the single Tex19.1KO (Tarabay et al., 2013). Thus, Tex19 paralogs are required for male meiosis and are essential for male fertility.

To further investigate the meiotic defect of Tex19DKO mutants, we tested the assembly of the synaptonemal complex by co-immunostaining of the central and lateral elements with antibodies against SYCP1 and SYCP3, respectively. In contrast to WT spermatocytes where all autosomes were fully synapsed at pachytene (Fig 2E), synapsis failed to occur properly in Tex19DKO, as evidenced by the complete or partial absence of the SYCP1 signal (Fig 2F). Furthermore, as progression of homologous recombination and chromosome synapsis are interdependent, we checked whether homologous recombination was also affected by studying DNA double strand break (DSB) distribution, by immunostaining against phosphorylated histone γH2AX. γH2AX staining is normally present throughout chromatin during the zygotene stage. As synapsis proceeds, the DSBs are resolved, resulting in γH2AX disappearance from autosomes, but not from the sex chromosomes. In WT pachytene cells, synthesis was complete and only the sex chromosomes were stained for γH2AX (Fig 2G). However, the incompletely synapsed pachytene chromosomes in Tex19DKO nuclear spreads exhibited strong diffuse γH2AX staining, suggesting that the disruption of Tex19 paralogs caused meiotic arrest at the pachytene stage because of the persistence of DSBs (Fig 2H).
Alternatively, γH2AX retention in spermatocytes from Tex19DKO may be a consequence of asynapsis.

In order to date the developmental onset of the Tex19DKO phenotype, we analyzed testis sections at post-natal (p) day 10, 16, 20 and at 16 weeks of age (Fig 3). At p10, Tex19DKO were histologically indistinguishable from WT and Tex19DHZ testes (Fig 3A-C). Tex19DKO showed vacuolization and seminiferous epithelium degeneration as soon as p16 (Fig 3D-F). Thus, the Tex19DKO testes phenotype became histologically visible between p10 and p16, corresponding to the timing when the first spermatocytes reach the pachytene stage during the first post-natal wave of spermatogenesis. This confirmed a spermatogenesis arrest occurring between the zygotene and the early pachytene stages of meiosis prophase I.

Finally, increased TE expression has been proposed to be responsible for impaired chromosome synapsis and meiotic defects during spermatogenesis in Dnmt3L, Miwi2 and Mili mutant mice (Aravin et al., 2007, Bestor and Bourc’his, 2004, Carmell et al., 2007, Kuramochi-Miyagawa et al., 2004, Zamudio et al., 2015). Overexpression of MMERVK10C retrotransposons could similarly be responsible for the meiotic defects seen in Tex19JKO mice (Ollinger et al., 2008, Tarabay et al., 2013). In Tex19JKO mice, we observed a two-fold and four-fold up-regulation of MMERVK10C expression at p10 and p16, respectively; no upregulation was observed at other TEs (LINEs and IAP) (Fig S2), confirming the specific impact of Tex19 deficiency in the control of MMERVK10C.

TEX19 associate with PIWI proteins and pachytene piRNAs

To gain insight into TEX19 function, we went on seeking its protein partners through GST pull-down and immunoprecipitation (IP) experiments. For the GST-pull-down experiment, GST alone, GST-TEX19.1 or GST-Tex19.2 was incubated with total testicular protein extract of adult mice (Fig S3); complexes were then analyzed by mass spectrometry (MS). In GST-TEX19.1 and GST-TEX19.2 samples, we detected bands corresponding to MAEL, MIWI, MILI, TDRD6, RANBP9 and MVH while these bands were absent in GST alone (Fig 4B). These interactions were further confirmed by western blot (WB) in co-immunoprecipitation experiments. Using a specific anti-TEX19.1 monoclonal antibody, we further immuno-purified TEX19.1 complexes in adult testes and identified individual polypeptides by MS. Among the identified proteins, we found MAEL, MIWI, MILI, and MVH which were already
identified by GST pull-down, but also TDRD8, TDRD6, EDC4, PABPC2, PSMC3, RANBP9, ANGEL1, SRPK1, DDX20, DDX46. We confirmed the presence of MAEL, MIWI, MILI, MVH, EDC4, TDRD6 and RANBP9 in TEX19.1 immuno-purified complexes isolated from cytoplasmic adult testes extracts by WB (Fig 4C, D). These interactions were further validated by the reciprocal detection of TEX19.1 in immuno-purified MAEL, MIWI, MILI, MVH, EDC4 and RANBP9 complexes (Fig 4D). Altogether these results suggest that TEX19.1 and TEX19.2 interact with proteins of the post-natal PIWI/piRNA pathway, namely core components such as MIWI, MILI, and accessory proteins such as EDC4, TDRD6, RANBP9 and MVH.

The identification of TEX19 paralogs as partners of the PIWI proteins along with the phenotypic similarities observed between Tex19KO mice and piRNA mutant mice prompted us to check if TEX19s could bind small RNAs, and in particular piRNAs. For this purpose, we immuno-precipitated TEX19.1, TEX19.2 and MILI (as a positive control) in adult mouse (aged 12 weeks) testes extracts and assessed for the presence of small RNAs by $^{32}$P 5’ end labeling and gel electrophoresis (Fig 5). As expected, MILI associated with ~26-30 nucleotide (nt) small RNAs. TEX19.1 purification also revealed an interaction with small RNAs ranging in size between 26 and 30 nt, migrating similarly to MILI associated piRNAs. Using the 6Tex:4D2 antibody that recognized both TEX19 paralogs on an adult Tex19.1 KO testicular extract from a normal size testis from a 12 weeks old male, we showed that TEX19.2 also interacted with small RNAs of the same size range (Fig 5). All bands disappeared upon RNase treatment, confirming the RNA nature of these entities. Furthermore, no such band was detected in the ascitic fluid control sample or in the sample precipitated with a specific anti-TEX19.1 monoclonal antibody in Tex19.1 KO testicular extract, confirming the specificity of the interaction between TEX19.1 and small RNAs. Thus both TEX19 paralogs interact with small RNAs in a size range compatible with piRNAs.

To further identify the small RNA species associated with TEX19 proteins, we generated small RNA-seq libraries by Illumina sequencing after TEX19 and TEX19.1 immunoprecipitation from adult WT testes. To infer the TEX19.2-bound fraction, we analyzed TEX19-complexes in Tex19.1KO testes. Size distribution and genomic content were then compared to available datasets from MILI and MIWI-bound piRNAs from adult testes (Robine et al., 2009). MIWI was formerly shown to preferentially associate with 29-30nt piRNA species, while the main piRNA size in MILI complexes is shorter, around 26-27nt. Interestingly, TEX19-, TEX19.1- and TEX19.2-associated small RNAs were found to
segregate in size with MIWI-bound piRNAs, with a diagnostic 30nt size (Fig 6A). TEX19.2 showed a stronger association to 22-23nt long RNAs, which is a diagnostic size for microRNAs: as small RNA libraries are expressed as relative rather than absolute values, this may reflect a weaker affinity of TEX19.2 towards piRNAs but a genuine preferential association with miRNAs cannot be excluded.

Because the three libraries were very similar in size and content, but the TEX19.1 sample had a better quality, we focused the rest of our analysis on the latter. Out of 1.6 million reads, we noticed a strong preference (94.42%) for a uridine at position one (U1 bias), in agreement with the biogenesis mode of piRNAs (Aravin et al., 2006, Frank et al., 2010, Kawaoka et al., 2011) (Table S2, Fig S4). The genomic distribution appeared identical to both MILI and MIWI IPs (Fig 6B): the majority (70%) of the TEX19.1-derived reads originated from intergenic regions, while less than 20% of the reads mapped to repeated elements. This is in line with the known genomic distribution of pachytene piRNA species, which are produced in adult testes from a discrete number of unique and intergenic piRNA clusters. Accordingly, 90% TEX19.1-associated reads uniquely mapped onto the genome (Fig 6C), and we moreover found the large majority of TEX19.1-associated small RNAs to be derived from the same piRNA loci clusters previously defined as regulated by MILI and MIWI (Fig 6D) (Beyret et al., 2012). We nonetheless examined the content of the repeated fraction of the TEX19.1 library and found that around 50% were derived from LTR retrotransposons, among which around 1% belonged to the MMERVK10C class (Fig 6B). This means that along all 25-30nt-bound RNAs, only 0.1% derived from MMERVK10C elements. Finally, to address the role of TEX19.1 in the biogenesis of piRNAs, we sequenced whole testes small RNA libraries in three adult (12 weeks of age) Tex19.1 KO animals showing normal size testis: the size distribution appeared similar to WT testes, and in particular there was no relative redistribution towards 22-23nt microRNA species, as an indication of impaired piRNA production (Fig 6E). Enrichment in 30nt-centered small RNAs was even higher in the Tex19.1 KO background compared to WT: this likely reflects different cellular composition, and in particular an overrepresentation of pachytene cells in Tex19.1 KO testes, due to the meiotic arrest. Altogether, these results suggest that TEX19 proteins sample the same set of primary, unique piRNA species as MILI and MIWI, at the time of their production at the pachytene stage in the adult testis. However, they are not required for their synthesis, and likely act as simple cargo.
TEX19 paralogs interact directly with piRNAs

In order to assess a direct interaction between TEX19.1, TEX19.2 and piRNAs, we performed electrophoretic mobility shift assays (EMSA) using GST-TEX19.1 and GST-TEX19.2 proteins as well as different domains of TEX19.1 fused to GST (Fig S5 and S6) against the piR-117061 piRNA. We choose this piRNA because it has a very significant absolute count (2361 and 2028) in two biological replicates prepared using anti-TEX19.1 antibody (Table S3) and it has a U at position one “U1”. We found that the piR-117061 was ‘shifted’ up upon electrophoresis in the presence of GST-TEX19.1, GST-TEX19.2, GST-D2Cter (125-351aa) or GST-VPTEL of TEX19.1, but not in the presence of GST-alone or GST-MCP (1-124aa) or GST-Cter (163-351aa) of TEX19.1. This result stresses the specificity of the interaction between piR-117061 and TEX19.2 and TEX19.1 via the VPTEL domain (Fig 7A). The specificity of this interaction was further confirmed by showing that a modified form of piR-117061, in which we replaced U1 and A10 by a C was unable to bind TEX19.1, its VPTEL domain or TEX19.2 (Fig 7B) and was not able to replace piR-117061 in a competition experiment (Fig 7C). By adding an increased concentration of the non-radiolabeled piR-117061 to a fixed concentration of GST-VPTEL (5µM pmole) and radiolabeled piR-117061, we showed that the non-radiolabeled piR-117061 completely displaced the radiolabeled piR-117061 at one hundred fold concentration (Fig 7D). Finally, we showed that the quantity of piR-117061 ‘shift’ up was proportional to the quantity of the TEX19.1 VPTEL domain (Fig 7D).

Discussion

Tex19 genes are specific to mammals and can be found as tandem duplication in the mouse and rat genomes. In the mouse, Tex19.1 and Tex19.2 expression is restricted to the germ line and the placenta (Celebi et al., 2012). Analyses of Tex19.1KO mice have previously highlighted a variable phenotype. In the severest form, Tex19.1 deficient males were infertile and presented an arrest at the pachytene stage of meiosis, in association with a failure to silence MMERVK10C elements (Ollinger et al., 2008, Tarabay et al., 2013). Tex19.2KO males are fertile; however a subtle phenotype of testes degeneration could be observed in adult mice (unpublished observations Tarabay et al, 2013). The present study describes the
functional consequences of deleting both TEX19 paralogs and the involvement of these proteins in the piRNA pathway. Surviving Tex19DKO male and female mice were perfectly healthy, but males exhibited a fully penetrant phenotype similar to the severest Tex19.1KO phenotype (Ollinger et al., 2008, Tarabay et al., 2013). Indeed, Tex19DKO males have highly degenerated testes, with a lack of germ cells beyond the pachytene stage, linked to defects in chromosome synapsis during meiosis. Compared to Tex19.1KO testes (Ollinger et al., 2008, Tarabay et al., 2013), we showed here a stronger up-regulation of MMERVK10C retrotransposon in double Tex19DKO testes, which was two-fold at p10 and four-fold at p16 compared to WT counterparts. This fully penetrant phenotype comforts the hypothesis of a functional redundancy between Tex19.1 and Tex19.2. Moreover, this phenotype bears striking resemblance with the Dnmt3L, Miwi2 and Mili mutant phenotypes (Aravin et al., 2007, Bestor and Bourc'his, 2004, Carmell et al., 2007, Kuramochi-Miyagawa et al., 2004). One explanation was proposed by Zamudio et al, who suggested that improper TE silencing may alter the meiotic process through local remodeling of the chromatin landscape and (Zamudio et al., 2015).

The essential role of TEX19 may extend beyond the context of spermatogenesis as we report here a severe lethality of Tex19DKO animals, with only 8.3%, instead of the expected 25% of mutant animals. Moreover, this lethality was sexually dysmorphic as among the survivors, only one third were females. We did not observe such lethality in our single Tex19.1KO mouse models (Tarabay et al., 2013), but Yang et al., 2010 also reported such female-specific lethality in their Tex19.1KO mice and suggested that the variable severity among different studies could be linked to strain specificities (Yang et al., 2010, Kwon et al., 2003). However, our studies on Tex19.1KO or Tex19DKO mice were conducted onto the same genetic background (C57Bl/6). A strong bias in sexual lethality was also observed in C57Bl/6 mice by Reichmann et al, but only when females were lactating and nursing a pre-existing litter during pregnancy (Reichmann et al., 2013). Yet, none of our studies were conducted under such lactating conditions. Consequently, this difference between Tex19.1KO and Tex19DKO phenotypes is quite surprising since Tex19.2 is not expressed in the placenta, neither in WT nor in Tex19.1KO animals. This early postnatal lethality could be indicative of defects in placenta (Reichmann et al., 2013, Tarabay et al., 2013), but also suggests a functional redundancy between Tex19.1 and Tex19.2 during embryo development. The exact reason for the preferential female lethality of the Tex19DKO mutation remains unclear, but may be linked to X chromosome-specific effects.
The main findings provided by our work relate to the identification of the protein partners of TEX19 in adult testes. Through GST pulldown and IP experiments, we indeed showed for the first time that TEX19.1 exists in a complex with members of the PIWI/piRNA pathway: MAEL, MIWI, MILI and MVH. We also confirmed a significant level of interaction between TEX19.1 and UBR2 by IP, as it had been previously reported (Yang et al., 2010). Most importantly, we found that both TEX19 paralogs bind small RNAs that have all the characteristics of piRNAs: around 30nt in size and with a strong preference for a uridine as the first nucleotide (Frank et al., 2010, Kawaoka et al., 2011, Brennecke et al., 2007). Finally, this interaction seems to be direct and to occur through the well-conserved VPTEL domain of TEX19 proteins. We can therefore conclude that TEX19 paralogs are new members of the family of proteins that bind piRNAs and that the VPTEL domain could be a new small RNA-binding motif. While this study may reveal the piRNA-binding function of the VPTEL domain, we cannot speculate about the function of the MCP domain, which lacks homology with any known proteins. PIWI proteins have, in addition to their ability to bind piRNAs, an RNA-guided nuclease function linked to a catalytic triad DDH localized in the PIWI domain and which is required for piRNA biogenesis (Martinez and Tuschl, 2004). No such triad can be found in TEX19 proteins. Accordingly, the production of 25-30 nt small RNAs is not altered in Tex19-deficient testes. This suggests that while TEX19 proteins bind to piRNAs, they are not involved in the catalysis of piRNA synthesis.

Finally, it is now well established that TEX19 paralogs are required for the control of MMERVK10C transposons in testes (Ollinger et al., 2008, Tarabay et al., 2013). Despite a consistent effect of Tex19 deficiency on these elements, we found that MMERVK10C-derived reads only represent 0.1% of all 25-30nt TEX19-bound small RNAs. In contrast, TEX19 mostly binds unique, non-transposon derived small RNAs in adult testes, which are similar to the MILI and MIWI-associated piRNAs. This is not surprising, as the post-natal PIWI machinery is responsible for producing piRNAs derived from discrete intergenic regions as male germ cells enter the pachytene stage (Aravin et al., 2006, Girard et al., 2006) although the function of these mammalian post-natal piRNAs remains enigmatic (Goh et al., 2015). This poor enrichment of TEX19-bound small RNAs in MMERVK10C fragments raises the question as to whether the reactivation of MMERVK10C in Tex19.1KO and Tex19DKO mice reflects a direct role of TEX19 proteins in silencing these elements-likely via post-transcriptional mechanisms- or an indirect effect of the Tex19 mutation. Further work should be dedicated to resolve this question.
In conclusion, our analysis of the double-mutant mice for the two TEX19 paralogs undoubtedly reveals the crucial role of these proteins for male fertility in mammals, likely via their association with the PIWI/piRNA pathway.
Material and methods

Generation of *Tex19* double knockout mice

The *Tex19* double mutant mouse line was established at the MCI/ICS (Mouse Clinical Institute- Institut Clinique de la Souris-, Illkirch, France; http://www-mci.u-strasbg.fr). For *Tex19.1*, the targeting vector was constructed as follows (Fig 1A): three PCR fragments (from 129S2/SvPas ES cell genomic DNA) one 2.2 Kb fragment corresponding to inter-loxP fragment encompassing exons 2 and 3, a 4.5 kb and a 3.1kb fragment corresponding to the 5’ and 3’ arms respectively were sequentially subcloned in a MCI proprietary vector. This MCI vector contains a LoxP site as well as a floxed and flipped Neomycin resistance cassette. A unique PacI restriction site was inserted downstream to the 3’ loxP site. The linearized construct was electroporated in 129S2/SvPas mouse embryonic stem (ES) cells. After G418 selection, targeted clones were identified by PCR using external primers and further confirmed by Southern blot with 5’ and 3’ external probes.

For *Tex19.2*, the targeting vector was constructed as follows: three PCR fragments (from 129S2/SvPas ES cell genomic DNA) a 2.0 Kb fragment corresponding to inter-lox P fragment encompassing exons 2 and 3, a 4.4 kb and a 3.5kb fragment corresponding to the 5’ and the 3’ arms respectively were sequentially subcloned in a MCI proprietary vector. This MCI vector contains a Lox5171 site as well as a F3 surrounded Hygromycin resistance cassette. A unique PacI restriction site was inserted upstream to the 5’ loxP site. The linearized construct was electroporated in one fully valid *Tex19.1* KO clone. After hygromycin selection, targeted clones were identified by PCR using external primers and further confirmed by Southern blot with 5’ and 3’ external probes. Restriction digestion by PacI followed by Southern blot allowed selection of a clone showing targeting of both constructs on the same allele. This clone was injected into C57BL/6J blastocysts, and male chimeras tested for germline transmission. The NeoR and HygroR cassettes were deleted by breeding the F1 mice with Flp mice (Rodriguez et al., 2000). Excision of both *Tex19.1* and *Tex19.2* inter-Lox fragments was done by breeding the mice with a Cre deleter line (Dupe et al., 1997). The absence of *Tex19.1* and *Tex19.2* mRNA and protein was tested by RT-PCR using oligonucleotides described previously (Tarabay et al., 2013) and by western blotting respectively.

All animal experimental procedures were performed according to the European authority guidelines.
Antibody production, Western blotting, preparation of cytoplasmic and nuclear fractions

Injecting the entire protein or the peptide DLGPEDAEWTQALPWRC into mice allowed the generation of anti-TEX19 monoclonal antibodies. The specificity of both antibodies was tested as described elsewhere (Tarabay et al., 2013). The 7Tex:1F11 antibody is TEX19.1 specific, whereas 6Tex:4D2 antibody detects both TEX19.1 and TEX19.2.

Cytoplasmic and nuclear fraction were prepared as described http://openwetware.org/wiki/Cytoplasm_and_nuclear_protein_extraction

RT-PCR and quantitative RT-PCR

RNA was prepared using the RNeasy mini or micro kit (Qiagen) following the manufacturer’s instructions. After DNase I digestion (Roche, Mississauga, ON, Canada), 1µg RNA was reverse-transcribed by random priming using Superscript II (Invitrogen/Life Technologies, Burlington, ON, Canada). QPCR reaction was performed using SYBR® green JumpStartTM Taq ReadyMixTM (Sigma-Aldrich, Oakville, ON, Canada) and LightCycler 480 (Roche, Mississauga, ON, Canada). The efficiency and specificity of each primer pair was checked using a cDNA standard curve. All samples were normalized to β-actin and Rrm2 expression (Tarabay et al., 2013).

Histology

Testes were collected and fixed in Bouin’s fluid for 48 hours and then embedded in paraffin. Adult male testis were dissected at 16 weeks of age. For histological analyses, 5 µm thick sections were stained with hematoxylin/eosin. All slides were examined using a DMLA microscope (Leica) with 10X, 20X, 40X and 100X objectives with apertures of 0.3, 0.5, 0.7 and 1.3, respectively. Images were taken with digital camera (CoolSnap; Photometrix) using CoolSnap v.1.2 software and then processed with Photoshop CS2 v.9.0.2 (Adobe).

Immunostaining of meiotic chromosome spreads

Meiotic chromosome spreads were prepared as described previously (Mark et al., 2008).

TEX19 protein production and purification

Tex19.1 cDNA or its domains were cloned, by RT-PCR starting from a testis cDNA library, into pCR®4-TOPO (Invitrogen/Life Technologies, Burlington, ON, Canada) then into pENTR
1A (Life Technologies) then by LR recombinase into pHGGWA or pGGWA. The recombinant proteins GST-TEX19, MCP, VPTEL-Cter, VPTEL, Cter, and protein GST-alone were individually expressed in E. coli BL21 cells. The induction by 0.1mM of IPTG was performed at a cell density of A600 = 0.8 at 20°C overnight and a portion of the proteins examined by 10% SDS-PAGE. Then, sonication was performed 3s ON and 3s OFF for 5 min at amplitude 33%. Cleared lysates were prepared in lysis buffer (Tris HCl 50mM pH9, NaCl 400mM, DTT 2mM, triton 1% and protease inhibiting cocktail) and centrifuged for 1 hour at 20000 rpm. The soluble fusion proteins were purified by batch procedure incubating the lysate with 500µL of Glutathione Sepharose (Amersham Biosciences) for 1 hour at 4°C with gentle shaking. Then the Glutathione Sepharose was collected by centrifugation and washed five times with lysis buffer. After boiling, the bound proteins were analyzed by 10% SDS-PAGE followed by Western blotting using anti-TEX19 monoclonal antibodies 7Tex:1F11 or 6Tex:4D2.

For GST pulldown assays, total testicular extracts of adult mice (aged 12 weeks) were added to recombinant protein fixed to Gluthatione Sepharose beads for 2h00 at 4°C. After fives washes, proteins were separated on 10% SDS–PAGE and subsequently stained with Bio-Safe Coomassie (Bio-Rad, Hercules, CA, USA). The gel was then sent to Taplin Biological Mass Spectrometry Facility to be analyzed by LC/MS/MS (Taplin, Boston, MA, USA).

Purified recombinant proteins were used in EMSA assays as described hereafter.

**Co-Immunoprecipitation**

Antibodies were chemically cross-linked to protein G–Sepharose beads using dimethyl pimelimidate (DMP; Sigma-Aldrich D-8388) and used to purify TEX19.1, TEX19.2, MAEL, MIWI, MILI, MVH complexes from adult mouse testes (aged 12 weeks) cytoplasmic fractions. 30mg of clarified DWT testes cytoplasmic extracts were incubated with 300µl protein G-Sepharose beads coupled to antibodies; anti-TEX19.1 (7Tex:1F11,100µl), anti-TEX19.1/TEX19.2 (6Tex:4D2, 10ml of hybridoma supernatant), anti-MAEL (18µg, Abcam, Toronto, ON, Canada), anti-MIWI (20µg, Aviva System Biology, San Diego, CA, USA), anti-MILI (40µg, Abcam), anti-MVH (12µg, Abcam), anti-TDRD66 (50µg, Millipore/Upstate, Etobicoke, ON, Canada), anti-EDC4 (12µg, Abcam), anti-RANBP9 (8µg, Proteintech, Rosemonte, IL, USA) or an ascitic fluid control (negative control, 100µl) in Co-IP buffer (50 mM Tris, pH 8, 150 mM NaCl, 1Mm EDTA, complete protease inhibitor) overnight at 4°C. After five washes with Co-IP buffer, the retained proteins were denatured.
by Laemmli buffer with 5% β-Mercaptoethanol and boiled to 100°C, then separated by SDS-PAGE as described hereafter. If required, gels were stained with Coomassie blue and sent to Taplin Biological Mass Spectrometry Facility to be analyzed by LC/MS/MS (Taplin, Boston, MA, USA).

**Small RNA immunoprecipitation and purification**

TEX19 libraries were prepared as described elsewhere (Reuter et al., 2009).

**Electrophoretic Mobility Shift Assay (EMSA)**

For 32P-labeling, piR-117061 (5’UAGGACCUGGAACUUAACCUUGUUAUGGG 3’) and piR-nonspecific (5’CAGGACCUGCGAACUUAACCUUGUUAUGGG 3’) were incubated for 60min at 37°C in 20µl kinase buffer containing 5µl RNA (0.5 nmol), 2µl buffer B, 4µl PEG, 10U T4 PNK kinase (10U/µl, Fermentas), 40U RNAsine (40U/µl), 2 µl [γ-32P]ATP (6000 Ci/mmol) (Perkin Elmer), 5µl ddH2O.

EMSA assays were performed with 5 µM GST-alone, GST-TEX19.1, GST-MCP, GST-VPTEL/Cter, GST-VPTEL, GST-Cter and 125 femtomoles (8000cpm) denatured radiolabeled piRNA in the binding buffer (250µg Herpain, 10Mm MOPS pH 7, 50mM MgCl2, 1 mM DTT,10% glycerol, 40U RNAsine, 10ng/µl tRNA of E.coli) as described (Bendak et al., 2012).

**Small RNA sequencing and analysis**

Small RNA-seq of whole adult testes and TEX19-IP were done using Illumina HiSeq 2000 on libraries (18-45nt RNAs size-fractionated on acrylamide gel) prepared with the Illumina TruSeq small RNA protocol. For the analysis, adapters were removed using Cutadapt (Martin, 2011). The trimmed sequencing reads of each library were then mapped and annotated with ncPRO-seq (Chen et al., 2012) onto the mouse reference genome (mm9). Briefly, read alignment was done using Bowtie v0.12.8, allowing a sum of qualities of mismatching bases lower than 50 (-e 50). A number of 5,000 hits per aligned read were allowed. Aligned reads were then annotated using RepeatMasker and refGene databases, and the positional read coverage was weighted by mapping site numbers.

The piRNA cluster analysis was performed as described (Beyret et al., 2012). A cluster was defined as a group of piRNAs where the reads were less than 1,500bp away from each other.
Clusters with at least 1,000 normalized read count were reported. The UCSC genome tracks were generated using the HOMER software (v4.3) using unique mapped reads.

**Funding**

The study was funded by Agence Nationale de la Recherche (ANR-11-BSV2-002 « TranspoFertil ») and l’Agence de BioMédecine (« AMP, diagnostic prénatal et diagnostic génétique »).

**Acknowledgment**

Y.T., M.A., M.T., T.Y., A.T., M.M., D.B., S.V.: Data analysis and interpretation, manuscript writing, final approval of manuscript. We would also like to thank Robert Drillien for his critical reading of the manuscript. We are grateful to the Institute of Genetics and Molecular and Cellular Biology (IGBMC) platforms. The study was funded by Agence Nationale de la Recherche (ANR-11-BSV2-002 « TranspoFertil ») and l’Agence de BioMédecine (« AMP, diagnostic prénatal et diagnostic génétique »). This work was supported by the French Centre National de la Recherche Scientifique (CNRS), Institut National de la Santé et de la Recherche Médicale (INSERM), the Ministère de l’Education Nationale et de l’Enseignement Supérieur et de la Recherche, the University of Strasbourg, the University Hospital of Strasbourg.
References


STRA8-deficient spermatocytes initiate, but fail to complete, meiosis and undergo premature chromosome condensation. *J Cell Sci*, 121, 3233-42.


Fig 1: Genetic targeting of Tex19 paralogs in mice. (A) The loci of the Tex19 paralogs are shown before and after homologous recombination upon removal of the neomycin (Neo) gene by crossing the mice with the Flp-recombinase strain. LoxP sites surrounding Tex19.1, Lox5171 sites surrounding Tex19.2 and PacI restriction sites (PacI RS) are depicted; (B) Examples of PCR genotyping; one example of each genotype, Tex19.1KO (+/+ and -/-), Tex19.2KO (+/+ and -/-) and Tex19DKO is shown. Wild type alleles are indicated by +/+ whereas mutant alleles are indicated by -/-, (C) Western blot using the 6Tex:4D2 antibody recognizing both paralogs on adult testes extracts from WT, Tex19.1KO and Tex19DKO showing the absence of TEX19 proteins in the Tex19DKO. β-Tubulin is used as a loading control. M: molecular size marker.
Fig 2: *Tex19DKO testicular defects.* (A) Testes from *Tex19DKO* (DKO) mice are smaller than testes from double wild type (DWT) littermates (8-week old); (B) Adult testes weight in mg. Mean testes weight is significantly reduced in *Tex19DKO* mice (Student t-test *p*<0.01) (C, D) Histological sections stained with hematoxylin and eosin through the testes of adult
mice. (E-H) Double immunostaining of spread nuclei using antibodies against SYCP1 and either SYCP3 or γH2AX, as indicated. In E, F, the synaptonemal complexes (SC) appear yellow from overlapping of SYCP3 (green) and SYCP1 (red) signals. In both DWT and Tex19DKO spermatocytes at the zygotene stage, short fragments of SC begin to form. In DWT spermatocytes at the pachytene stage, all nineteen bivalents are fully synapsed. In about 20% of the Tex19DKO spermatocytes at the pachytene stage, only few chromosomes are fully (arrowheads) or partially (arrow) synapsed, while the majority is unsynapsed (thin green strands). (I, K) DWT and Tex19DKO spermatocytes at the zygotene stage express γH2AX (green signal) throughout the nucleus. (J) In DWT spermatocytes at the pachytene stage, γH2AX expression becomes restricted to the (almost) unsynapsed XY body. (L) In about 80% of the Tex19DKO spermatocytes at the pachytene stage, γH2AX remains widely distributed thought the nucleus, even in regions that are apparently synapsed as assessed from the presence of thick SYCP3-positive strands (in red). Bar (in D): 100 μm (in L): 10 μm.
Fig 3: Testes histology in double wild type (DWT), double heterozygous (DHZ) and double knockout (DKO) animals. In DWT (A, D, G, J) and DHZ (B, E, H, K) testis histology is normal. The seminiferous tubules contain Sertoli cells and germ line cells are found at all studied ages. (L) Tex19DKO testes exhibit a severe phenotype with a diminished tubule diameter, vacuolization of the seminiferous epithelium and a reduction of the germ cell number at 16 weeks. (C) An abnormal testicular phenotype starts to be visible at 16 days of age (p16) (F) and increase at 20 days of age (p20) days (I, L) but is absent at 10 days of age.
Thus, the histological phenotype starts to be visible between p10 and p16. Bar (in L): 100 μm.
Fig 4: GST pull-down and immunoprecipitation of TEX19 paralogs. (A) Western blot (WB) showing GST-TEX19.1 and GST-TEX19.2. (B) Western blot analysis of the GST pulldown revealing that MAEL, MIWI, MILI, MVH, TDRD6 and RANBP9 are present in the analyzed pull down samples. (C) Reciprocal immunoprecipitation experiment confirming the association of MAEL, MILI, MIWI, MVH, RANBP9, EDC4 with TEX19.1 by WB. (D) Co-immunoprecipitation of TEX19.1 by anti-TEX19.1 (7Tex: 1F11) and the detection by WB of either MAEL, MILI, MIWI, MVH, TDRD6, RANBP9 or EDC4. IBIAB (Immuno blot with IP antibody) is indicated with the corresponding IP antibody. Ascitic fluid from an unimmunized animal is used as a negative control.
Fig 5: Interaction of TEX19.1 and TEX19.2 with ~ 30 nt RNAs. MILI, TEX19.1 or TEX19.2 were immunoprecipitated (IP) from two month-old mouse testis protein extract by specific antibodies and bound RNA were then $^{32}$P-end-labeled and separated on a 15% denaturing urea-polyacrylamide gel. Immunoprecipitated RNAs are predominantly ~ 30 nt long. The size and mobility of the oligoribonucleotide markers is indicated on the right. The asterisk indicates RNAase treatment. Ctrl- indicates negative control; Ascitic fluid from an unimmunized animal is used as a negative control.
Fig 6: PiRNA populations isolated from TEX19 paralogs and MILI complexes were cloned, sequenced and annotated. A) Size distribution of Small RNA-seq libraries immunoprecipitated with TEX19 and TEX19.1 from WT adult testes, and with TEX19 from Tex19.1-ko. These libraries exhibit a peak around 29-30nt, which is reminiscent of MIWI-associated piRNAs; in contrast, MILI-associated piRNAs range in size between 26-27nt. (B) Genomic annotation of TEX19.1-associated reads compared to MILI and MIWI-associated reads. In agreement with piRNA size, the analysis was restricted to 25-32nt long reads. (C) The vast majority of TEX19.1 reads have unique hits on the genome, similarly to MILI and MIWI-associated Small RNAs. (D) Venn diagram distribution of piRNA cluster defined by the piRNA population associated with TEX19.1 compared to MILI and MIWI. (E) Size distribution of whole adult testes Small RNA-seq libraries obtained from a WT and three Tex19.1-KO animals. The 22-23nt peak corresponds to microRNAs, the piRNAs are clustered in two peaks, at 26-27nt and at 29-30nt.
Fig 7: Tex19 paralogs interact directly with ~26-30 nt piRNA: (A) and (B) Electrophoretic Mobility Shift Assay “EMSA” was performed in the presence of 5µM of GST-alone, GST-TEX19.1, GST-D1 (1-124aa), GST-D2Cter (125-351aa), GST-D2 (125-163aa “VPTEL”) or GST-Cter (164-351aa) of TEX19.1 or GST-TEX19.2. All proteins were incubated with 125 femtomoles (8000cpm) of radiolabeled piR-117061 or modified piR-117061. (C) An
increased concentration of the modified non-radiolabeled piR-117061(2C) was used with a fixed concentration (5µM) of TEX19.1 VPTEL domain and fixed quantity of radiolabeled piR-117061 (8000 cpm). (D) An increased concentration of the non-radiolabeled piR-117061 was used with a fixed concentration of the VPTEL domain of TEX19.1 (5 µM) and a fixed quantity of radiolabeled piR-117061 (8000 cpm).
Fig S1: (A) and (B) Histological sections stained with hematoxylin and eosin through the caudal epididymis of adult mice, note absence of sperm in the mutant epididymis (C) TUNEL assays: the positive signal was converted to a red false color and superimposed with the DAPI nuclear stain (blue false color); Note a normal, low, proportion of apoptotic germ cells in DWT testes but an increase in the proportion of TUNEL- positive germ cells in Tex19DKO testes (black arrows).
**Fig S2:** RT-qPCR of MMERVK10C, L1ORF2, IAPΔ1 and MuERV1 retrotransposon expression in Tex19DKO (DKO) p10 and p16 mice testes compared to double wildtype (DWT). Expression levels were normalized to the β-actin or Rrm2 housekeeping gene, n values indicate the number of biological replicates, DWT: double wild type; DKO: double knockout,*p<0.03 (Student t-test).
Fig S3: (A) Silver staining of GST-Tex19.1 pull down using G-sepharose beads. G-sepharose beads and GST combined to G-sepharose beads are deposited as control. (B) Production and purification of GST, GST-TEX19.1, GST-TEX19.2, GST-MCP (1-124aa), GST-VPTEL Cter (125-351aa) or GST-VPTEL (125-163aa) of TEX19. Gel 10% and blue Coomassie coloration were used.
TEX19.1 piRNA library

Fig S4: A: Percentage of the distribution of TEX19.1 piRNA. TEX19.1
Table S1: Adapter trimming and alignment

<table>
<thead>
<tr>
<th>Sample</th>
<th>Out percent</th>
<th>Input</th>
<th>Too-short Adapter</th>
<th>Non-clipped</th>
<th>Output</th>
<th>Aligned reads</th>
<th>Failed</th>
<th>Suppressed -m</th>
<th>Output reads</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tex19.1 in WT (1)</td>
<td>27.68%</td>
<td>6985974</td>
<td>4980436</td>
<td>3241</td>
<td>68727</td>
<td>1933570</td>
<td>1647203 (85.19%)</td>
<td>283557 (14.66%)</td>
<td>2810 (0.15%)</td>
</tr>
<tr>
<td>Tex19.1 in WT (2)</td>
<td>22.64%</td>
<td>6368438</td>
<td>4826897</td>
<td>2150</td>
<td>97851</td>
<td>1441540</td>
<td>1214051 (84.22%)</td>
<td>225055 (15.61%)</td>
<td>2343 (0.17%)</td>
</tr>
<tr>
<td>Tex19.2 in Tex19.1-/-</td>
<td>78.52%</td>
<td>15596146</td>
<td>3035682</td>
<td>176061</td>
<td>139045</td>
<td>1224358</td>
<td>9700101 (79.21%)</td>
<td>2524130 (20.61%)</td>
<td>21127 (0.17%)</td>
</tr>
<tr>
<td>Tex19.1 &amp; 2 in WT</td>
<td>72.35%</td>
<td>13615661</td>
<td>3590834</td>
<td>23129</td>
<td>150175</td>
<td>9851523</td>
<td>8202474 (83.26%)</td>
<td>1637355 (16.62%)</td>
<td>11694 (0.12%)</td>
</tr>
</tbody>
</table>

Table S2: Tex19.1 reads display a strong preference for a uridine at position one (U1-bias)

<table>
<thead>
<tr>
<th>piRNA name</th>
<th>Absolute count Tex19.1 in WT (1)</th>
<th>Absolute count Tex19.1 in WT (2)</th>
<th>piRNA sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>gi</td>
<td>109016873</td>
<td>gb</td>
<td>DQ727168.1</td>
</tr>
<tr>
<td>gi</td>
<td>108994920</td>
<td>gb</td>
<td>DQ710717.1</td>
</tr>
<tr>
<td>gi</td>
<td>108994167</td>
<td>gb</td>
<td>DQ710038.1</td>
</tr>
<tr>
<td>gi</td>
<td>108955533</td>
<td>gb</td>
<td>DQ687738.1</td>
</tr>
<tr>
<td>gi</td>
<td>109001017</td>
<td>gb</td>
<td>DQ715342.1</td>
</tr>
<tr>
<td>gi</td>
<td>108984009</td>
<td>gb</td>
<td>DQ700204.1</td>
</tr>
<tr>
<td>gi</td>
<td>108983531</td>
<td>gb</td>
<td>DQ701739.1</td>
</tr>
<tr>
<td>gi</td>
<td>109013632</td>
<td>gb</td>
<td>DQ724840.1</td>
</tr>
<tr>
<td>gi</td>
<td>108036260</td>
<td>gb</td>
<td>DQ564985.1</td>
</tr>
<tr>
<td>gi</td>
<td>108950508</td>
<td>gb</td>
<td>DQ710862.1</td>
</tr>
<tr>
<td>gi</td>
<td>108958333</td>
<td>gb</td>
<td>DQ688999.1</td>
</tr>
<tr>
<td>gi</td>
<td>108954985</td>
<td>gb</td>
<td>DQ687488.1</td>
</tr>
<tr>
<td>gi</td>
<td>109012695</td>
<td>gb</td>
<td>DQ724202.1</td>
</tr>
<tr>
<td>gi</td>
<td>108951359</td>
<td>gb</td>
<td>DQ684704.1</td>
</tr>
<tr>
<td>gi</td>
<td>108969977</td>
<td>gb</td>
<td>DQ694365.1</td>
</tr>
</tbody>
</table>