MINI-REVIEW

PIWI, piRNAs, and Germline Stem Cells: What’s the link?

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Germline stem cells undergo asymmetric divisions. When they divide, they produce a stem cell as well as a more differentiated cell. Little is known about what allows germline stem cells to undergo these self-renewing divisions. Work in Drosophila melanogaster has given us insight into the proteins and small non-coding RNAs involved in maintaining these cells. One of the most interesting findings is that the asymmetric division of these stem cells is controlled by a protein called PIWI (P-element induced wimpy testis), which belongs to the Argonaute (AGO) family, and this protein is only expressed in gonadal cells [1].

The AGO family of proteins is known for its role in gene silencing and its association with small non-coding RNAs, such as small interfering RNAs (siRNAs) and microRNAs (miRNAs) [2,3]. Given this link, several researchers found a new subset of small non-coding RNAs associated with PIWI that were aptly named PIWI-associated RNAs (piRNAs). It is becoming apparent that the expression of piRNAs are required to maintain the integrity of the germline stem cell genome. This review will give a brief overview of the PIWI proteins, piRNAs, and their roles in maintaining germline stem cells.

THE ARGONAUATE FAMILY

The AGO family proteins are ~100 kDa highly basic proteins [2]. All members of the AGO family contain three domains: PAZ, MID, and PIWI. The PAZ domain binds to the 3’ end of the non-coding RNA; whereas, the MID domain binds to the 5’ end of the small non-coding RNA [4]. The PIWI domain has an RNase H-like fold, which has been shown to have slicer activity in some proteins. Interestingly, the catalytic motif for each family differs. The RNase H family of proteins tends to contain a DDE motif in the catalytic domain; the AGO family of proteins contains a DDH motif [5]; and the PIWI family of proteins contains a DDL motif. Some of the AGO proteins (e.g., AGO2) and the Drosophila melanogaster PIWI have slicer activity [6]. Initially, PIWI and AGO were
discovered as the same family of proteins, and later this family was divided into two subfamilies: PIWI and AGO [7].

**THE PIWI SUBFAMILY AND THEIR KNOWN FUNCTIONS**

PIWI originally was identified in a P-element enhancer trap screen searching for P-element insertion that affected the asymmetric division of germline stem cells in *Drosophila melanogaster*. The P-element insertions generated from this screen lead to sterility in male flies because of severe defects in spermatogenesis as well as a depletion of germline stem cells in fly ovarioles [1].

Further study into *Drosophila* PIWI revealed that these proteins existed in other organisms, such as worms [7] and humans [8,9], and all the proteins appear to have similar roles in the asymmetric division of germline stem cells, as well as being involved in the piRNA biogenesis pathway.

Much study has gone into understanding the role of PIWI proteins in the germline. *Drosophila melanogaster* has three PIWI proteins: PIWI, AUBERGINE (AUB), and ARGONAUTE3 (AGO3). It has been shown that *Drosophila* PIWI is mostly a nuclear protein [10] and is involved in the asymmetric division of germline stem cells [7,11]. Male and female PIWI mutant flies begin with the normal number of germline stem cells, but later on, during oogenesis or spermatogenesis, respectively, they are unable to maintain germline stem cell asymmetric division, which results in a loss of germline stem cells and ultimately a reduced number of gametes [11]. As discussed later, the fly PIWI proteins are all involved in piRNA biogenesis, which ultimately leads to the silencing of deleterious retrotransposons. Through heterochromatic gene silencing, possibly by an interaction with Heterochromatin protein 1a or by methylation of specific regions of DNA, PIWI may be involved in epigenetic regulation [12,13]. Finally, during oogenesis, PIWI is associated with the some of the cytoplasmic components of the miRNA machinery (namely, DICER-1 and dFMRP) in the cytoplasm, and the expression of these components has been shown to be important in the formation of pole cells at the posterior end of the embryo [10]. Pole cells are the precursors to the germline stem cells and, thus, are extremely important.

Mice express three PIWI proteins: MILI, MIWI, and MIWI2 [14,15]. MILI and MIWI are cytoplasmic proteins, while MIWI2 is found in the nucleus and is expressed only for a short time during development.

Many mutant and knockout mice have been generated in order to understand the function of these proteins. The L1 retrotransposon found in the mouse genome is not methylated in MILI mutant mice, suggesting that MILI may play a role in directing the methylation of DNA in certain regions. Similarly, DNA at certain transposon loci is not methylated in MIWI2 knockout mice. These methylation patterns imply that PIWI proteins may play a role in transcriptional gene silencing through RNA-dependent DNA methylation mechanisms. Interestingly, in MILI knockout mice, MIWI2 is found in the cytoplasm. However, in MIWI2 knockout mice, MILI is still localized in the cytoplasm, suggesting that MILI is able to direct the nuclear localization of MIWI2 [16]. Finally, MILI been shown to play a role in translational up-regulation of some messenger RNAs [17]. It should be noted that while mutations of PIWI proteins in flies leads to a reduced number of gametes and mutant gonads, mutations of PIWI proteins in mice only affects the male gonads.

**piRNAs AND THE piRNA BIOGENESIS PATHWAY**

Six groups independently discovered piRNAs [4]. Several methods and the latest technologies, such as deep sequencing, were used to study the RNAs expressed from different organisms to come to the same conclusion [6,18-22]. They found that piRNAs are much longer than currently known small non-coding RNAs and vary in length from 26 to 32 nucleotides. They appear to have a strand bias, in that they can be sense or an-
tisense to the transcript they are targeting, while siRNAs and miRNAs are always anti-sense to their targets. The 3’ terminus of the piRNA is 2’O-methylated by DmHen1 [23]. PiRNAs initially were found in flies, mice, and rats. Interestingly, the mouse homologue, MILI, was found to bind to a subset of prepachytenic piRNAs, which are less abundant than other piRNAs and are only expressed during a short window during spermatogenesis [18].

Further investigation into this phenomenon and the mapping of where piRNAs are generated within the genome revealed that they clustered mainly in unannotated regions of the genome that tended to contain transposons and repetitive elements and in heterochromatic regions [3]. Surprisingly, it was found that PIWI and AUB tended to bind to antisense transcripts, while AGO3 tended to bind to sense transcripts. This information led to the suggestion that piRNAs were produced from a long, single-stranded precursor. Further, the AUB-bound piRNAs were complementary to the first 10 nucleotides of the complementary AGO3-bound piRNAs, and the AGO3-bound piRNAs tended to have the nucleotide Adenine at position 10 of the piRNA specified from the 5’ end. These data ultimately led to the formation of the ping-pong piRNA biogenesis pathway [24].

In flies, this pathway hypothesises that PIWI and AUB bind to maternally deposited piRNAs (primary piRNAs). This complex, in turn, binds to the transcripts produced by retrotransposons and cleaves a transcript generating a sense piRNA (secondary piRNAs) that binds to AGO3. The cycle continues as the AGO3-piRNA complex binds to the retrotransposon transcript, generating another set of anti-sense piRNAs and leading to the silencing of retrotransposons within the germline, thus preventing them from inserting elsewhere in the genome. This pathway appears to fit the data found in mice [24,25]. The model is still incomplete, as it only indicates that one cleavage of the retrotransposon transcript is occurring, but, in fact, two cleavages of the transcript are required to generate the fully functional piRNA. Research is ongoing to fully understand this pathway.

CONCLUSIONS

To date, the research conducted on PIWI indicates its importance in the asymmetric division of germline stem cells. It also points to a potential pole in epigenetic regulation and the initial formation of pole cells in flies through the interaction of the cytoplasmic components of the miRNA machinery.

Much of the research conducted on piRNAs indicates that they serve to preserve the integrity of the germline stem cell genomes. It is interesting that they are only a couple of nucleotides longer than the much better studied miRNAs and siRNAs and that their role appears to be to silence retrotransposons, repetitive elements, and heterochromatic regions, while the role of siRNAs and miRNAs is to reduce the expression of genes. All the small non-coding RNAs appear to be generated from longer transcripts and use the AGO family of proteins.

Although we have discovered much about the miRNA and siRNA processing pathways and their mechanisms of action, we are still left in the dark as to how piRNAs are generated and how they function. What is the second enzyme that cleaves the piRNA from the long transcript? Is the control of methylation of the retrotransposons the only mechanism by which they function? Do the piRNA-PIWI complexes function in both the cytoplasm and the nucleus? Does their location define their function? What is the importance of the prepachytenic piRNAs?

This is a new field with many questions waiting to be answered. Further research into the PIWI proteins, as well as the piRNAs, will help us understand how our germline stem cells undergo self-renewing divisions, and in going through these divisions, how they manage to maintain the integrity of their genomes.

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