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Small "Non-Coding RNAs" (sncRNAs) and their Role in Gene Repression, Post-Transcriptional Gene Regulation and Therapeutics. Part-III: PIWI Interacting RNA (piRNA); its Biogenesis, Role in Post-Transcriptional Gene Silencing (PTGS) and Therapeutic Applications

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Abstract: This review focuses on the biogenesis and functions of piwi interacting RNAs (piRNAs) which are single stranded and endogenously derived from non-coding RNA precursor. PIWI-interacting RNAs (piRNAs) are single-stranded, 23–36 nucleotides (nt) long that act as guides for an animal-specific class of Argonaute proteins: the PIWI proteins. The biogenesis and processing of piRNAs are strictly regulated. The first piRNA (small non-coding RNA associated with PIWI proteins) was discovered in 2001 and was double stranded, transcribed from genomic repeat of the Suppressor of Stellate [Su(Ste)] locus present on Y chromosome in *Drosophila melanogaster* testes. These were of larger size and called repeat-associated siRNAs (rasiRNAs). Later, a distinct class of small silencing piRNAs was identified in mice; which was derived from single stranded precursors rather than double-stranded RNA (dsRNA) transcript. Since piRNAs associate with PIWI (but not AGO) sub-family of Argonaute protein and also processed by DICER independent pathway; these are different from siRNAs and miRNAs. This review deals with the biogenesis and functional role of piRNAs in post-transcriptional gene silencing (especially transposon silencing) and their possible role in therapeutics. Abnormal expression of piRNA in cells becomes the basis for diverse diseases, particularly in neoplasms and diseases of reproductive system. Now the piRNAs can become a novel biomarker for early diagnosis and therapeutic targets for precise medicine.

Keywords: PIWI Interacting RNA (piRNA), Biogenesis, PIWI Protein, Processing in Yb Body and Nuage, Transposon silencing, Therapeutic applications

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Introduction

Small non-coding RNAs composed of a few to up to 200 nucleotides (nt) include small interfering RNAs (siRNA), microRNAs (miRNAs) and piwi interacting RNAs (piRNAs). This review deals

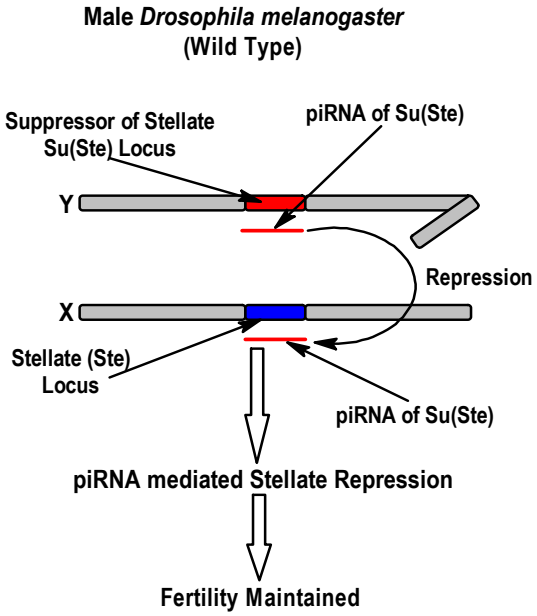

Fig. 1: The Stellate-Su(Ste) genetic system is maintained only in the genome of male *Drosophila melanogaster*. Testes-specific X-linked Stellate genes are repressed in the testes of wild-type flies with the help of piRNAs derived from the homologous Y linked Su(Ste) locus that is essential for male fertility.

primarily with biogenesis, role in post-transcriptional gene silencing and therapeutic applications of piwi interacting RNAs (piRNAs); however, the other two forms like siRNAs and miRNAs are reviewed by Srivastava (2026 a, b).

PIWI-interacting RNAs (piRNAs) are single-stranded, 23–36 nucleotide (nt) long that act as guides for an animal-specific class of Argonaute proteins: the PIWI proteins. The first piRNAs (small non-coding RNA associated with PIWI proteins) were discovered in 2001 and were double stranded, transcribed from genomic repeat of the Suppressor of Stellate [Su(Ste)] locus present on Y chromosome in *Drosophila melanogaster* testes (Aravin *et al*., 2001); they were of larger size and called repeat-associated siRNAs (rasiRNAs). Later, Aravin *et al.* (2006, 2007), Vagin *et al.* (2006) and Lau *et al*. (2006) recognized the piRNAs as a distinct class of small silencing RNAs in mice, derived from single stranded precursors rather than double-stranded RNA (dsRNA) transcript. Since piRNAs associate with PIWI (but not AGO) sub-family of Argonaute protein and also processed by DICER independent pathway; this makes them different from siRNAs and miRNAs (Guzzardo *et al*., 2013). It was observed that piRNAs regulate the transposons [transposition of which causes damage (alterations) of genome intracellularly] within the genome. In male *Drosophila melanogaster* the piRNA-mediated regulation of transposons was found essential for preserving the genetic integrity for normal gonadial development, gametogenesis and reproduction. Owing to their processing mechanisms and different sources of transposons, piRNA sequences are much more diverse than those of any other known class of cellular RNAs and constitute the largest class of noncoding RNAs. Transposon regulation by piRNAs is conceptually similar to that in immune systems, which is capable of recognising "self" and "non-self". Similar to our immune systems, piRNAs use a complex mechanism to effectively select and silence the nonself genes. Further, while siRNAs and miRNAs are expressed ubiquitously, piRNAs are predominantly found in animal gonads and are thought to be indispensable for fertility because it represses transposons (transposable elements), "the molecular parasite" of genome. However, now piRNAs were identified in almost all eukaryotic genome including humans (Grimson *et al*., 2008).

In *Drosophila melanogaster* males lacking a Y

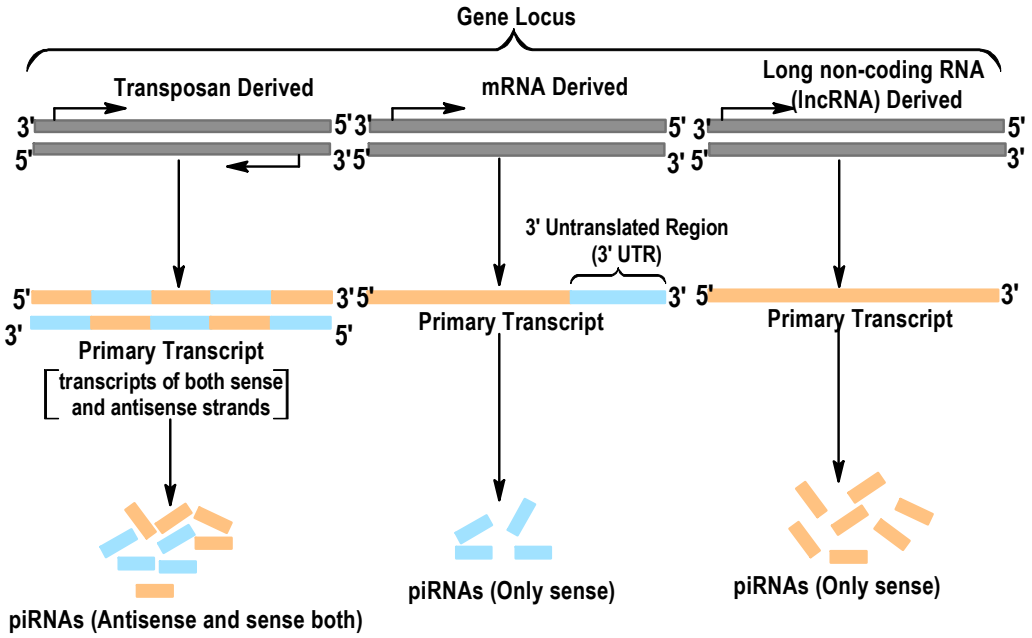

Fig. 2: The primary transcript of transposon derived piRNAs are typically transcribed from both the genomic strands and produce both sense and antisense piRNAs. However, mRNA derived piRNAs are produced from 3' UTR of its primary transcript and always anti-sense to the mRNA from which they are processed. Long non-coding RNAs (lncRNAs) produce piRNAs from the entire transcript. Functions of only transposon derived piRNAs are well understood.

chromosome, primary spermatocytes contain either needle or star-shaped proteinaceous crystals. Accumulation of such crystalline proteins in spermatocytes cause meiotic defects and male sterility. Crystal formation is the result of Stellate (Ste) locus present on X chromosome. The Stellate locus contains a tandemly repeated gene whose transcription has only been detected in testes. Formation of crystals appears to be a direct consequence of over-expression of the tandemly repeated Stellate gene. The presence of a specific homologous Suppressor of Stellate [Su(Ste)] locus on the Y chromosome prevents the appearance of crystals in spermatocytes (Fig. 1). Stellate genes are repressed *via* complementary piRNAs derived from transcripts of the homologous Y-linked Suppressor of Stellate [Su(Ste)] locus in male *Drosophila* and maintain the fertility (Fig. 1). Loss of the Su(Ste) locus leads to Stellate de-repression followed by accumulation of abundant crystalline aggregates in spermatocytes and to meiotic disorders leading to male sterility.

Initially, the function of piRNAs was known to be as transposon silencer and to maintain the gene integrity in germline cells only. However, later the presence of piRNAs have been observed in both: the germline cells and the somatic cells in many other animals including mammals (Grimson *et al.*, 2008) which substantiated the role of piRNAs in regulation of gene expression apart from transposon silencing (Rizzo *et al.*, 2014; Liu *et al.*, 2018). Abnormal expression of piRNA in cells results in diverse diseases, particularly in neoplasms and diseases of reproductive system. piRNAs are now under trial to explore the possibilities of its application as novel biomarker for early diagnosis and finding therapeutic targets for precise medicine (Wu *et al.*, 2020).

piRNA classification and evolution:

piRNAs can be divided into four classes according to their origins and functions within the cells (Fig. 2).

(1) The best-studied class of piRNA is repeat-associated piRNAs, which silence transposons in insects and mice.

(2) The piRNAs originates from the 3' untranslated regions (UTRs) of messenger

Table 1: Different Argonaute Family Proteins in Human, Mouse, and *Drosophila*

Organisms	Argonaute Family Protein	
	AGO* (Argonaute) Sub-family Protein (used for siRNAs and miRNAs)	**PIWI** Sub-family Protein (Used for piRNAs)**
Human	AGO1-4 (siRNA and miRNAs)	PIWIL1 (HIWI) PIWIL2 (HILI), PIWIL3, PIWIL4 (HIWI-2)
Mouse	AGO1-4 (siRNA and miRNAs)	PIWIL1 (MIWI) PIWIL2 (MILI), PIWIL4 (MIWI2)
Drosophila	AGO-1 used for miRNA AGO-2 used for siRNA	Piwi, Aub, Ago-3

*AGO proteins are ubiquitously expressed in all the cells. Functions for post-transcriptional gene regulation.
**PIWI proteins are mainly expressed in germline cells. Functions to repress the transposons and to maintain the genome integrity.

BOX 1

PIWI Genes in *Drosophila melanogaster*

PIWI proteins and piRNAs, though originally discovered mainly in the gonads of *Drosophila melanogaster;* later studies found that these are conserved in a wide range of eukaryotes (from sponges to humans). The prototype of PIWI proteins is encoded by the *Drosophila* piwi gene (P-element-induced wimpy testes gene), which was originally identified as an essential gene for germline development. *Drosophila* contains three distinct PIWI genes (i.e., *ago3*, *aubergine (aub)*, and *piwi*). From among them, *piwi* and *aub* are required for both male and female fertility; whereas *ago3* is essential for female fertility only. Mutation in each of these PIWI genes in ovary causes failure of transposon repression (hence gonadal development) indicating that all three PIWI proteins have conspicuous roles in gonadal development and transposon silencing. *Aub* and *Ago3* cleave their target transposon transcripts in the cytoplasm; whereas, *Piwi* regulates its target transposons at the transcriptional level in the nucleus itself.

RNAs (Fig. 2). Examples of such mRNA derived piRNAs include piRNAs in the ovarian somatic follicle cells of flies and in the pre-pachytene meiotic spermatocytes of mice. How mRNA derived piRNAs are made or what they do is still unknown.

(3) The third piRNAs found in the mouse testis is derived from intergenic long non-coding RNAs (lncRNAs; Fig. 2). Their targets and functions are not clearly known. Though, these three classes of piRNAs originate from different sources in the cell; even then, these three are highly conserved and have been found from Cnidaria (*Nematostella vectensis*), Porifera (*Amphimedon queenslandica*) to the higher animals including mammals.

(4) The fourth class of piRNA is *Caenorhabditis elegans* specific. Worm piRNAs, called 21U RNAs cooperate with 22G siRNAs to repress 'non-self RNA transcription" *via* histone methylation.

Argonaute Proteins Family:

Argonaute proteins lie at the centre of all small non-coding RNA pathways (e.g. siRNA, miRNAs or piRNAs). Argonaute proteins are found in almost all eukaryotes, bacteria and archaea (Tolia and Joshua, 2007). During animal evolution, Argonaute proteins have diverged into two clades: AGO sub-family proteins, which is associated with miRNAs and siRNAs; and PIWI sub-family proteins, which bind piRNAs (Pasquinelli *et al*., 2007; Wu *et al*., 2020). Animals typically encode multiple PIWI-family members (Table 1; BOX 1).

AGO subfamily proteins are ubiquitously expressed and can bind to microRNAs (miRNAs) and small interfering RNAs (siRNAs), both of

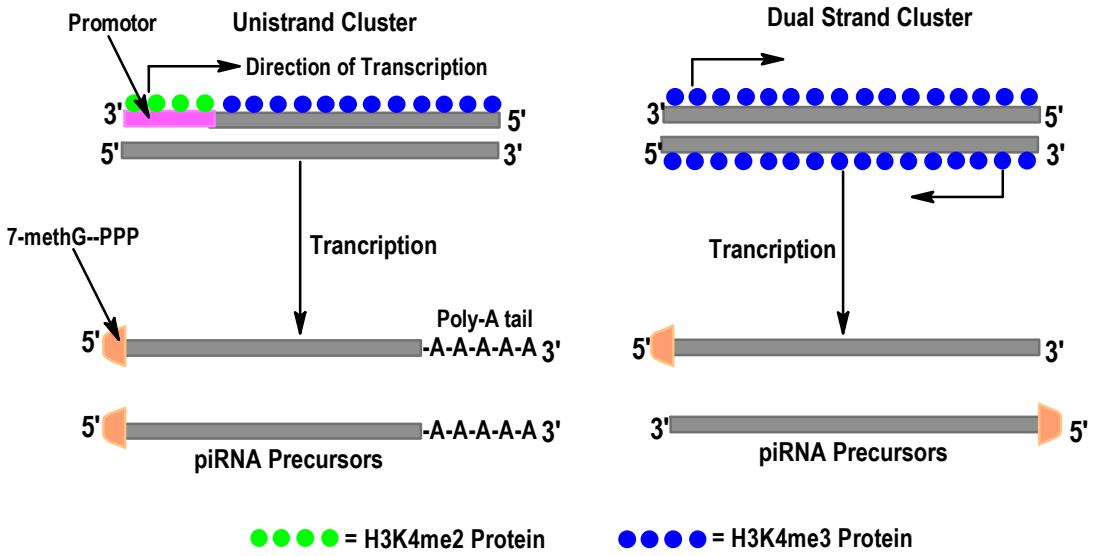

Fig. 3: Difference between uni-strand and dual-strand cluster transcription.

Trimethylation (adding me3 of histone (H3) at lysine 4 (H3K4me3) is a chromatin modification mechanism known to mark the transcription start sites (TSS) of active genes in animals. H3K4me2 and H3K4me3 are enriched at the transcription start site (TSS) and function to regulate gene transcription as epigenetic marks.

which are processed from double-stranded precursors into mature small non-coding RNAs of 20 to 22 nt in length in a Dicer-dependent mechanism (Kim *et al*., 2009).

PIWI subfamily proteins (PIWI proteins) are expressed and matured mainly in germline cells and responsible for processing of piRNAs through Dicer independent mechanism (Siomi *et al*., 2011). These form specific RISC (RNA induced silencing complex) with a small RNA population known as PIWI-interacting RNAs (piRNAs); these RISCs are termed piRISCs (Iwasaki *et al*., 2015).

PIWI proteins have 2 or 3 RNA-binding domains: the N-terminal PAZ domain, MID domain and the C-terminal PIWI domain. The N-terminal PAZ domain binds the 3'-end of the guide RNA; the middle MID domain binds the 5'-phosphate of guide RNA; while C-terminal PIWI domain acts as an RNase-H (an endonuclease) that can cleave RNA (i.e., exhibits slicer activity). Small non-coding RNAs, such as piRNAs are positioned in the PIWI protein and guide to their targets through Watson–Crick base pairing. The PIWI domain cleaves (slices) the phosphodiester bond between the two nucleotides in the target RNA that base-pair with the 10th and 11th nucleotides of the small guide piRNA. The slicer activity of PIWI protein is responsible for processing the precursor piRNA into mature piRNA during its biogenesis. A subset of PIWI proteins, present in *Drosophila piwi* and mouse MIWI2 (officially PIWIL4), can repress transposon transcription by promoting histone or DNA methylation.

Biogenesis and Maturation of piRNA:

piRNA clusters and Transcription:

The loci that give rise to piRNAs are not dispersed throughout the genome, but are concentrated in "specific piRNA clusters" which can be divided into two types i.e. uni-strand and dual-strand clusters (Fig. 3). Uni-strand clusters give rise to only one strand transcript; whereas, dual-strand clusters produce transcripts of two complementary strands corresponding to both the genomic strands. Additionally, some piRNA precursors can be generated from the 3' UTR of protein-coding genes, or from individual transposons. The best-studied piRNA cluster is *flamenco* (*flam*). Flamenco is predominantly (if not exclusively) active in the fly's somatic follicle cells (the somatic cells surrounding the germinal follicle cells).

Box 2

Trimethylation (adding me3) of histone (H3) at Lysine 4 (H3K4me3) is a chromatin modification mechanism known to mark the transcription start sites (TSS) of active genes. In animals, H3K4me2 and H3K4me3 are enriched at the transcription start site (TSS) and function as epigenetic marks that regulate gene transcription.

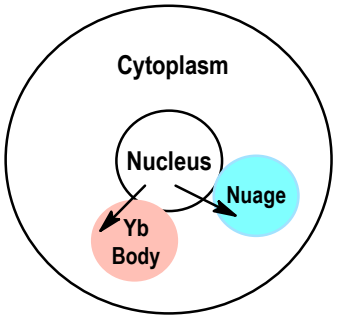

Fig. 4: Cell showing perinuclear (cytoplamic) non-membranous piRNA processing sites; Yb body (in follicle cells) and Nuage (in germ cells) in *Drosophila melanogaster*.

Flamenco generates single stranded piRNAs which are predominately antisense to transposon's sense mRNA. While *flamenco* produces piRNAs only from one strand (a 'uni-strand' cluster), most germline piRNA clusters produce transcripts from both strands ('dual-strand' clusters). Uni-strand clusters contain demethylated histone H3 lysine 4 (H3K4me2) at their promotors and produce piRNA precursors with 7-methyl guanylate caps on the 5' end and 3' polyadenylation. Dual-strand clusters lack those features and harbour the H3K4me3 region (BOX-2) producing piRNA precursors with no poly-A tail (Xi *et. al* 2020) (Fig. 3).

Interestingly, transcription of dual-strand clusters (but not uni-strand clusters) requires RDC complex [Rhino (a paralog of Heterochromatin Protein-1, HP1), Deadlock protein (Del) and Cut off (a yeast Cuff protein) complex], that warrants dual strand clusters to produce piRNAs by suppressing inaccurate splicing, polyadenylation and cleavage of their transcripts.

Uni-strand piRNA cluster transcriptions have been observed in many metazoans including mammals like mice and humans, which seems to have evolutionarily conserved sequences (Lim and Kai, 2015). Although dual-strand piRNA cluster transcriptions have been reported in germinal cells of *Drosophila*; in somatic follicle cells of the fly, transcription takes place in uni-strand cluster sequences.

Once the transcription is finished in nucleus, the precursor piRNAs are transported out (exported) of the nucleus (in perinuclear region) into the cytoplasm for further processing. piRNAs are slightly longer (24–31 nucleotides long) as compared to miRNAs and siRNAs and possess 2'-O-methyl modification sites at its 3' terminus. These are processed from their precursor transcripts *via* a Dicer-independent mechanism (Xi *et al*., 2020). piRNA clusters harbour a large number of and various types of transposons; therefore, piRNAs regulate mainly the activities of transposons (like expression and transposition) within the genome. Later, many other regulatory and pathological roles of piRNAs were recognised.

piRNA Processing and Maturation:

There are two major pathways proposed for the processing and maturation of piRNAs: (1) the primary processing pathway and (2) the secondary processing pathway (also called ping cycle) that amplifies secondary piRNAs (Czech *et al*., 2018). In *Drosophila* ovaries, the primary pathway operates in gonad follicle (somatic) cells; whereas, both primary and secondary pathway operates in germline cells. In follicle (somatic) cells, piRNA processing takes place in a

BOX 3

Yb bodies are perinuclear, non-membranous organelles in *Drosophila* ovarian follicular (somatic) cells that are central to the production of piRNAs, which repress transposons. Different components of Yb bodies, *viz* Yb protein, *Armitage* (Armi), *Vreteno* (Vret), *Shutdown* (Shu), *Zuccini* (Zucc), *Trimmer*, *Papi*, *Methyl transferase* (Hen-1) and *Heat shock protein-90* (Hsp90) which are essential for producing mature piRNAs.

perinuclear structure called Yb bodies (Czech and Hannon, 2018) (Fig. 4). However, by contrast, in germ cells, piRNA is processed at another perinuclear, multiprotein structures called nuage; and the export of the primary cluster transcripts to the nuage (processing sites) requires the help of protein DEAD box helicase (UAP56).

While primary piRNAs have a bias toward uridine (U) at their 5' end (1U bias); secondary piRNAs show 10-nucleotide complementarity with primary piRNAs at their 5' ends and possess a sense bias with adenosine (A) at the tenth nucleotide (10A bias).

(i) *Primary Processing Pathway of piRNAs Maturation in Follicle* (*somatic*) *Cells*:

Primary transcripts assigned to produce the piRNAs are exported from nucleus to the processing sites present in the cytoplasm, side by the nuclear envelope (i.e., perinuclear region).

In *Drosophila* ovarian follicle (somatic) cells, primary pathway operates and only *Piwi* is expressed among PIWI subfamily members; and piRNA precursors are transcribed from piRNA clusters such as the flamenco (*flam*) locus as a long unidirectional single-stranded transcripts; which is exported to cytoplasm in a perinuclear non-membranous Yb body for further processing (Fig. 4). The *flam* locus harbours a large number of truncated (small segments) transposons, most of which are antisense oriented relative to transposon coding strands. Long precursor transcripts from the piRNA clusters, after reaching in Yb body are processed through multiple steps into piRNAs *via* intermediates. Different components of Yb body which participate in piRNA processing are given in BOX 3.

Figure 5 shows that after reaching into the Yb body, piRNA transcripts are subjected to an endonuclease Zucchini (Zuc) and its co-factors which cuts it into pieces with 5' U bias and produce piRNA intermediates with a 5' uracil having phosphate. This processing step requires three other proteins viz. Vreteno (Vret), Minotaur (Mino), and Gasz; however, their molecular mechanisms of actions are not fully known. Subsequently, Armitage (Armi = an RNA helicase) causes to unwind the piRNA intermediates and reshaping the structures of piRNA. Another protein called Yb protein (an eponymous for Yb bodies) with Tudor-domain is needed for piRNA biogenesis in follicle cells and directly binds to piRNA intermediates *via* its N-terminal domain. However, germ cells are devoid of Yb protein where its function is carried out by two homologues called Brother of Yb (BoYb) and Sister of Yb (SoYb). Vreteno, another Tudor-domain protein found in both tissues (follicle and germ line tissues) is essential for piRNA production. Unwind pieces of piRNA with 5' U are subsequently loaded into *Piwi* protein to make piRNA-Piwi complex and undergo for further processing. Loading of piRNA intermediates into Piwi proteins requires Heat shock protein 90 (Hsp90) and its co-chaperone Shutdown. However molecular mechanism of loading process is not fully understood. The 3' end of this intermediate complex is further cleaved either by Zuccini resulting in the formation of phased Piwi-piRNAs complex; or alternatively, piRNA intermediates are trimmed at 3' end to their mature size by a putative exonuclease called trimmer and its co-factor Papi. Simultaneously, the newly formed 3' terminal end is methylated at the 2' oxygen by the small RNA 2'-O-methyltransferase (i.e., Hen1) to

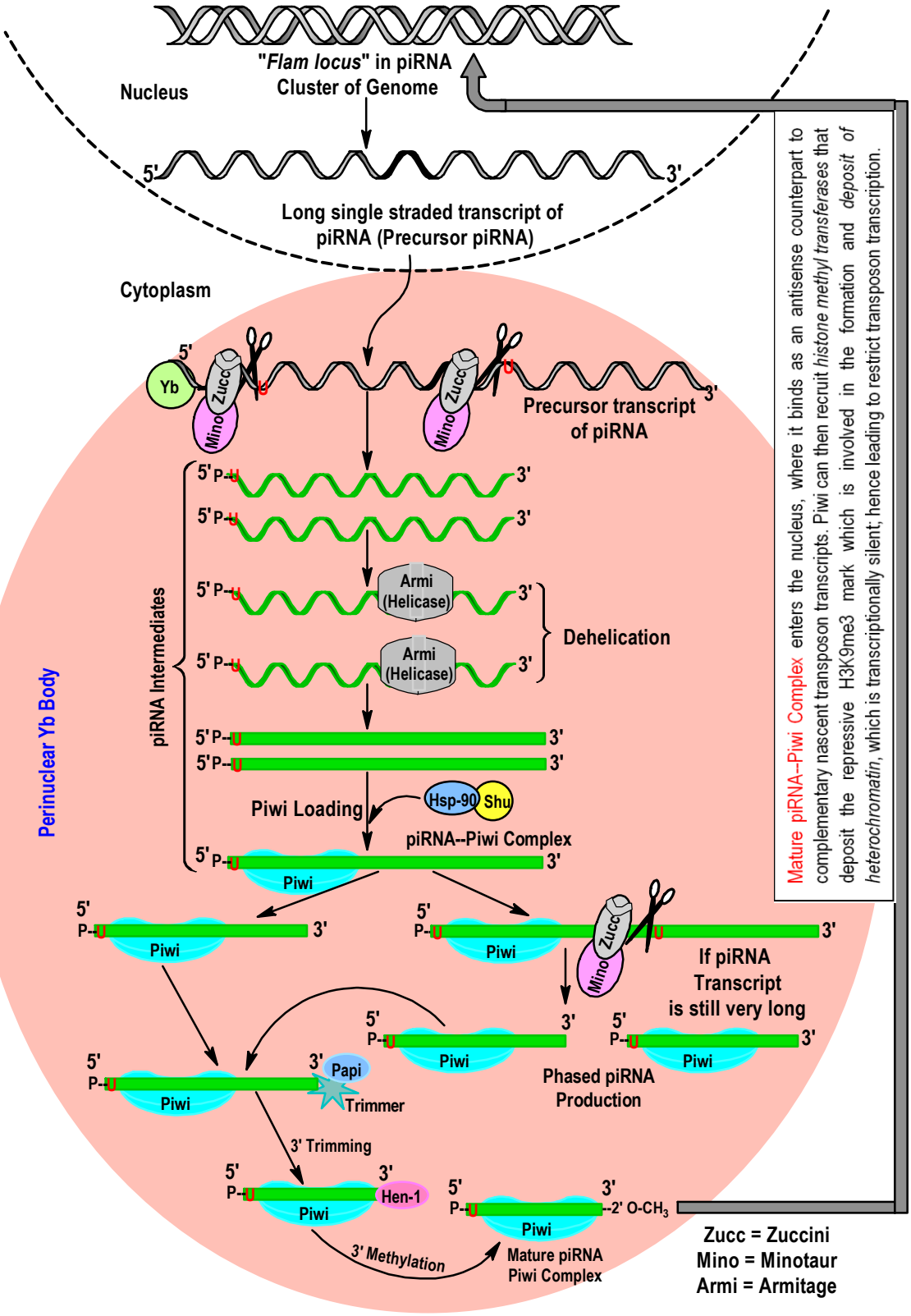

Fig. 5: Primary pathway of biogenesis and maturation of piRNA in follicle (somatic) cells of *Drosophila melanogaster*.

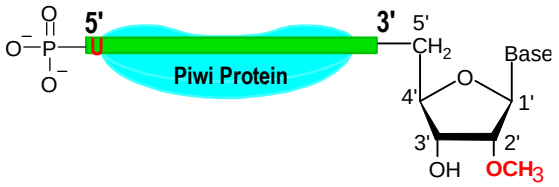

Fig. 6: Structure of mature piRNA-Piwi complex.

make mature piRNA-Piwi complexes (Fig. 6). Mature piRNA-Piwi complexes then enter inside the nucleus to exert silencing. Most factors required for *de novo* piRNA biogenesis are also found attached with the outer mitochondrial membrane, providing a clue for the central role played by mitochondria in the process of piRNA biogenesis.

Once piRNA-Piwi complex enters the nucleus, it binds to complementary nascent transposon transcripts and causes silencing. At the transcriptional level, piRNA-Piwi complex directly modifies chromatin structure and histone proteins in the nucleus *via* recruiting the histone methyl transferases that accumulates the repressive H3K9me3 (modified histone-3 protein) which restrict transposon transcription by depositing the heterochromatin to the cluster loci.

(ii) *Secondary Processing Pathway* (*Ping-Pong Cycle*) *of piRNAs in Germ Cells*:

In germ cells of *Drosophila*, maturation of piRNAs is carried out in nuage (instead of Yb body) i.e., another perinuclear, non-membranous structure through both primary and secondary pathways. While 'uni-strand' cluster of *flamenco* in follicle cells generate single stranded piRNAs which are predominately antisense to transposon's sense RNA; most of the germline piRNAs are produced from both strands ('dual-strand' clusters) yielding transcripts from both sense (*cis*) and antisense (*trans*) strands. After being transcribed, they are exported to Nuage for further processing and maturation.

While primary piRNAs have a bias toward uridine (U) at their 5' end (1U bias); secondary piRNAs show 10-nucleotide complementarity with primary piRNAs at their 5' ends and possess a sense bias with adenosine (A) at the tenth nucleotide (10A bias) (Fig. 7). *Drosophila* contains three distinct PIWI genes (i.e., *ago3*, *aubergine* (aub), and *piwi*). From among them, *piwi* and *aub* are required for both male and female fertility; whereas, *ago3* is essential for female fertility only. *aub* and *ago3* cleave their target transposon transcripts in the cytoplasm; whereas, *piwi* regulates its target transposons at the transcriptional level in the nucleus itself.

Latest studies revealed that *Aub* and *Ago3* are the main proteins involved in the processing and activation of piRNA through primary and secondary (ping-pong cycle) both the pathway in germ line cells of *Drosophila melanogaster* (Fig. 7). *Aub* protein specifically attaches to antisense-strand piRNAs followed by its binding to sense piRNA precursors exhibiting complementarity of its 5' U to the 10 nt 3' Adenine (10nt A); and finally cleaves that. This cleavage generates the 5′ end of a new form of sense piRNAs that associate with *Ago3* to get activated; following which it shows an affinity for its complementary anti-sense piRNA precursors and carries out its cleavage. This cleavage events lead to the creation of antisense piRNAs, which then again loaded onto *Aub* protein. In brief, *Aub* binds to antisense-strand piRNAs and cleaves sense piRNA precursors; giving rise to sense piRNAs bound by *Ago3*. In contrast, *Ago3* binds to sense-strand piRNAs and cleaves antisense piRNA precursors, producing antisense piRNAs that again load onto *Aub*.

During this process of cleavage of sense and anti-sense piRNA precursors, translation of the sense RNA transcripts is shattered. Further, during this entire process, accuracy is maintained and the generation of a new piRNA possessing the exact same sequence (as was in initial piRNA that triggered the cycle) is produced. Since this is a

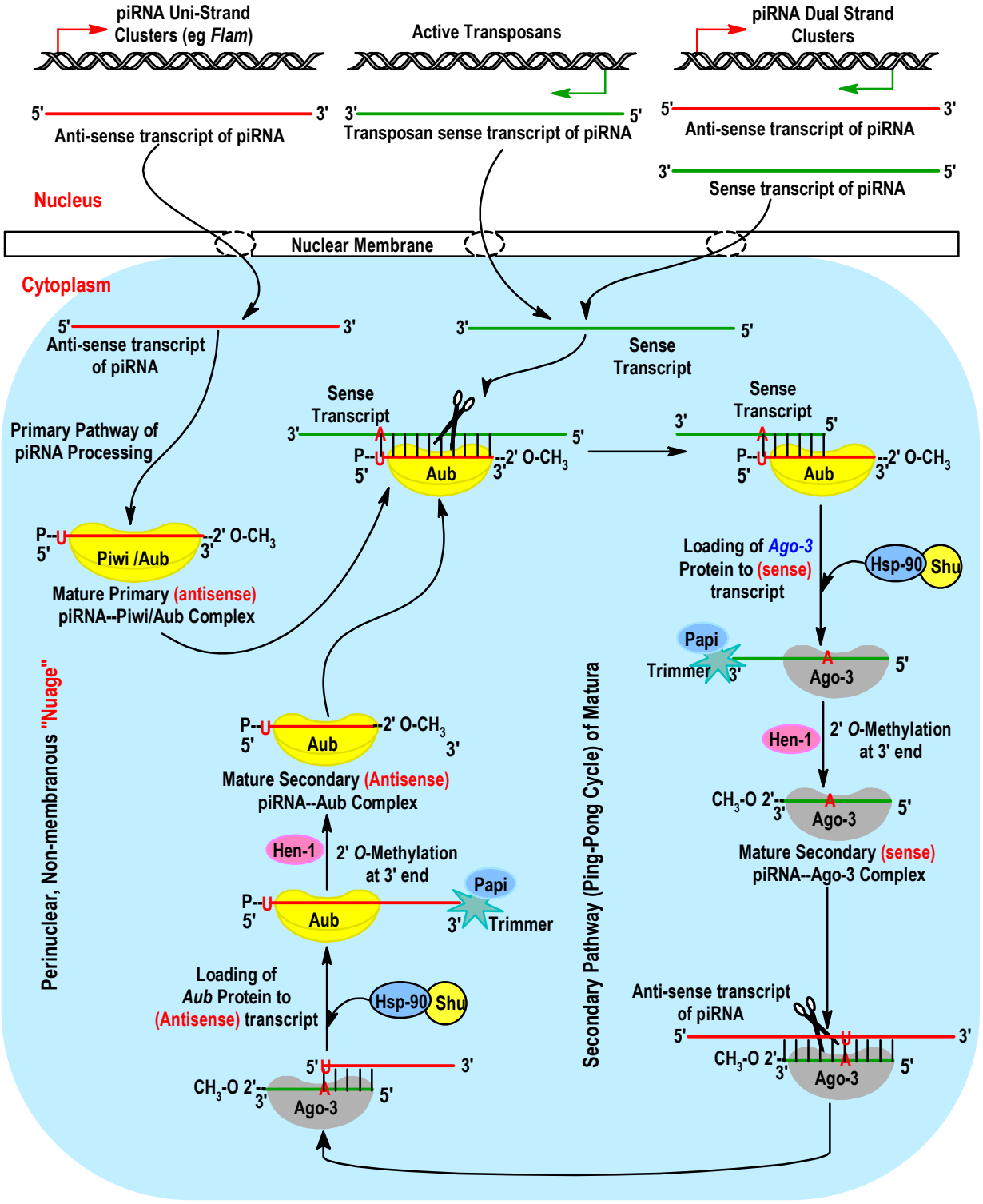

Fig. 7: Primary and secondary (Ping-Pong) pathways of maturation of piRNAa (and post-transcriptional gene regulation) in germ line cells of *Drosophila melanogaster*.

cyclic process (ping-pong cycle), very large number of piRNAs are produced in a very short time span.

Most of the information on piRNAs is obtained from model organisms such as *Drosophila*; however, continual progress has been made to

identify the piRNAs in other organisms of non-chordates and chordates including mammal with the help of small RNA sequencing (sRNA-seq) methods and available databases.

Many animals produce piRNAs that guide PIWI proteins to silence transposons and regulate gene expression (Czech and Hannon, 2016). piRNAs complementarity with transposons ensure genomic stability in the germline cells of animals (from smaller to higher animals including mouse and humans). piRNAs also protect somatic tissues from transposons and viruses (Gainetdinov *et al.*, 2018).

In mouse, MitoPLD (a protein belonging to Mitochondrial PhosphoLipase-Dsuper family) performs the function as homologue of Zuccini (of *Drosophila*) and act as the endonuclease during the piRNA biogenesis (Watanabe *et al.*, 2011). Similarly, the function of Armitage (of *Drosophila*) is carried out by MOV10L1 (Mov10-like RNA helicase-1) which belong to the RNA helicase family and unwind the piRNA transcript. Further, GPAT2 (Glycerol-3-Phosphate AcylTransferase 2) functions as Minotaur.

In germline cells of mammals (specially in testes), conspicuous classes of piRNAs perform separate and specific functions. Primary piRNAs are generated from long, single stranded RNAs transcribed from genomic loci called piRNA clusters. These long precursor transcripts are fragmented by endonucleolytic cleavage catalysed by the mitochondrial protein PLD6 producing tail-to-head, phased precursor piRNAs (pre-piRNAs). Each pre-piRNA begins with a 5' monophosphate, a pre-requisite for loading nearly all Argonaute proteins. Once bound to a PIWI protein, the 3' ends of pre-piRNAs are trimmed by the single stranded RNA exonuclease, Trimmer/PNLDC1. Finally, HENMT1 2'-O methylates the 3' ends and provide mature piRNAs. In the secondary pathway (i.e. ''ping-pong'' cycle) PIWI-catalyzed slicing (directed by piRNA) of a target transcript produces an RNA fragment bearing a 5' monophosphate which acts as a pre-piRNA precursor, binds to a PIWI protein, and ultimately generates a new secondary piRNA with 10-nt complementarity to the piRNA that produced it.

According to the current model, conspicuously separate mechanisms produce piRNAs in different type of cells and tissues and too in different animals. For example, fly oocytes and mouse embryonic male germ cells produce both primary and secondary piRNAs; and the secondary pathway unexpectedly initiates production of primary piRNAs. This means that slicing of a long precursor transcript catalysed by piRNA-directed PIWI protein generates secondary piRNA. This step is followed by generation of a series of phased pre-piRNAs, which then mature into primary piRNAs (Han *et al.*, 2015; Homolka *et al.*, 2015; Yang *et al.*, 2016).

However, unlike embryonic germ cells, pachytene piRNA biogenesis in post-natal male germ cells in mouse differs strikingly and proposes the current model because the majority of piRNAs are produced only by primary biogenesis after birth'' (Nishimura *et al.*, 2018).

Recently, Gainetdinov *et al.* (2018) have proposed a unified model for biogenesis of piRNAs in germ cells of mammals, insects, and likely most other animal (Fig. 8).

Uses of piRNAs:

(1) *Transposon Silencing*:

piRNAs were first reported for being as the defending tool against transposon mobilization in fly germline cells; which was later observed in the range from lower organisms to humans. The negative effects of transposons are as follows:

(i) Transposons, also called jumping genes, threaten the stability of other genes as they can "copy and paste" their own DNA in the host genome causing a series of deleterious consequences. For example, insertion of exon and also the introns may lead to alter the coding sequences and splicing patterns respectively; resulting in the production of deleterious proteins.

(ii) The insertions into 5' UTRs or 3' UTRs can affect post-transcriptional gene regulation.

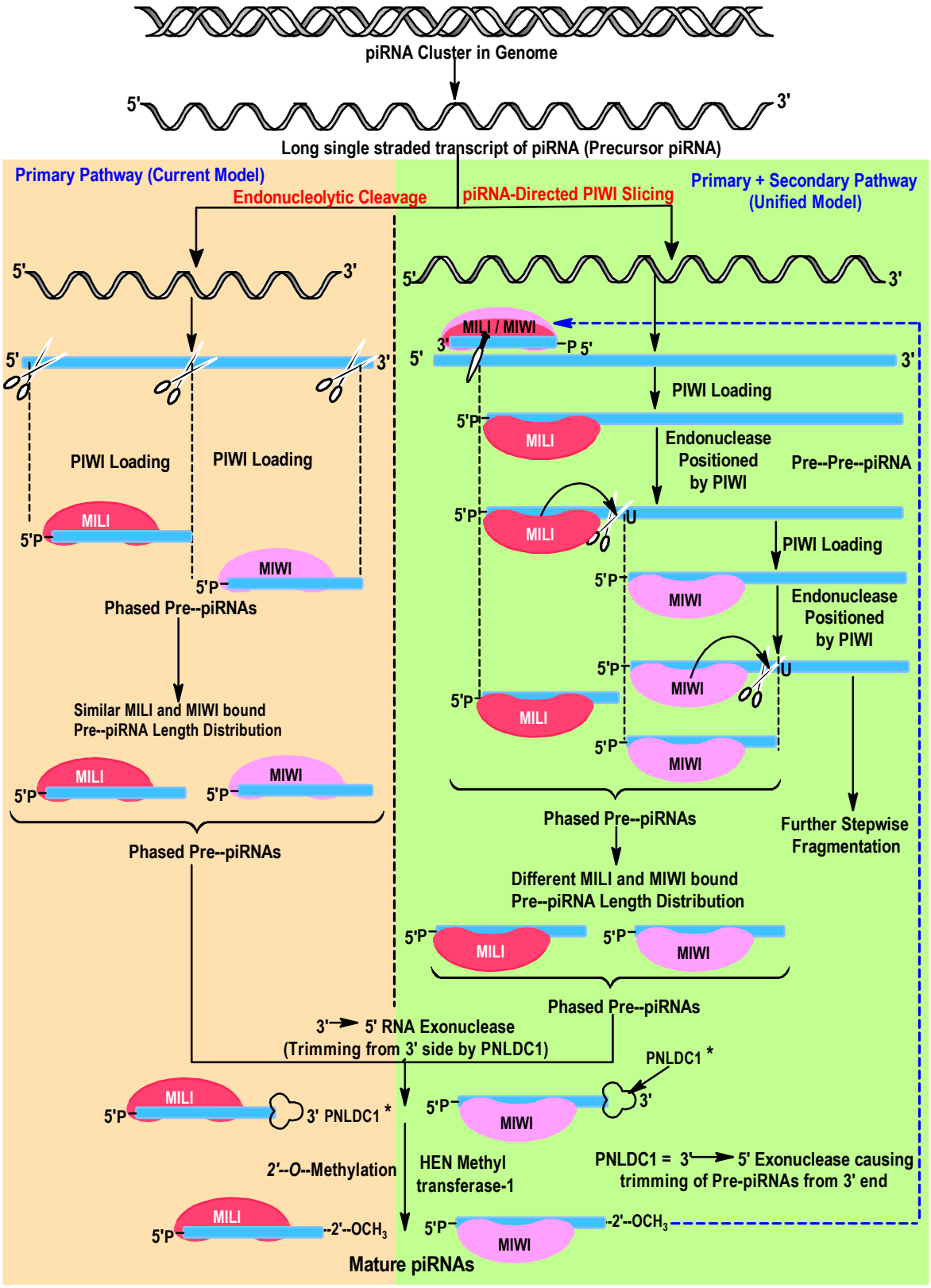

Fig. 8: current model for primary biogenesis in post-natal mouse testis and the unified model for piRNA biogenesis in animals.

BOX 4

The CpG sites or CG sites are the portions of DNA where a cytosine (C) nucleotide is followed by a guanine (G) nucleotide along its 5' → 3' direction. The genomic regions to which CpG sites occur with high frequency in called CpG islands. Cytosines in CpG island can be methylated to form 5-methylcytosines by enzyme DNA methyltransferases that add a methyl group. In humans, about 70% of promoters located near the transcription start site of a gene (proximal promoters) contain a CpG island.

(iii) Transposon insertions also lead to DNA nicks and double-strand breaks; moreover, errors in the repair of these lesions can lead to recombination between transposon repeats, triggering chromosomal duplication, deletion, translocation, and inversion.

The basic function of piRNAs was initially reported to silence the transposons and protect the germline genome from transposon attacks (Lewis *et al.*, 2018). The PIWI-piRNA complex accomplishes this at both transcriptional and post-transcriptional levels, depending on the specific type of PIWI proteins involved (Wang *et al.*, 2023).

At the transcriptional level, piRNA-Piwi protein complex modifies chromatin structure and histone proteins in the nucleus *via* the regulation of enzyme DNA methyltransferase (DNMT). In chromatin, DNMT methylates CpG islands in promoter regions of DNA, suppressing the transcription. Piwi proteins guide DNMT to bind with transposable elements or target genes (BOX 4). As a consequence, transposable elements or target genes are silenced. piRNAs and Piwi proteins also interact with histone methylation machinery and regulate the methylation of lysine residues of histone which causes to repress transcription. Further, during transcriptional gene silencing (TGS), piRNAs are transported to the nucleus where they bind to nascent transposon transcripts *via* base-pairing. PIWI proteins then recruit cofactors to inhibit transcription and cause the silencing of transposons effectively.

Degradation and post-transcriptional gene silencing (PTGS) by piRNAs are carried out through ping-pong cycle which is vital for transposon silencing through *Aub*-bound and *Ago3*-bound piRNAs (Czech *et al.*, 2018). Since the ping-pong cycle (Figs. 7, 8) generates secondary piRNAs, it causes degradation of transposon mRNAs leading to its silencing (Czech and Hannon, 2016). Many phenotypic abnormality and diseases are caused due to alterations in genome by transposons, which can then be taken care of by silencing them using piRNAs.

(2) *Therapeutic Uses of piRNAs*:

Since piRNAs are related with gene regulation, there has been growing interest in studying their roles in diagnosis and treatment of human diseases. A number of studies have revealed that any defect in biogenesis of piRNAs can do both, promote or suppress the occurrence and development of various diseases; especially cancers. Magnitude of expression and appearance of specific piRNA is used as biomarker for certain diseases of humans (Wu *et al.*, 2020).

(i) *For Targeting mRNA*:

piRNAs can target protein-coding sequences of mRNAs regulating the gene expression (Zhang *et al.*, 2015). This mechanism is sequence-driven, but not entirely sequence-specific and the targeting vary in different species; however, random mismatches within the piRNA sequence do not significantly affect its targeting efficiency (Vourekas *et al.*, 2016).

(ii) *piRNAs in Autoimmune Diseases*:

Autoimmune diseases are immune disorders in which body's immune system gets overactivated and recognises its own tissues as non-self (or antigen) and attack on them, leading to continuous damage to organs and tissues.

Table 2: Different Auto-Immune Diseases and Expression of piRNAs as Biomarker

Auto-immune Diseases	Symptoms	Expression of piRNAs as Biomarker
Rheumatoid arthritis (RA)	a chronic autoimmune disease that affects joints, causing pain, swelling, and stiffness.	piR-16,735, piR-4153 and piR-823. (Pleštilová *et al*., 2016)
Systemic lupus erythematosus (SLE)	Systemic lupus erythematosus (SLE) is a multisystem autoimmune disease that can severely affect women aged 10–50 years (Kaul *et al*. 2016) SLE can damage tissues and organs in the body, leading to various complications such as lupus nephritis (LN). Xiao *et al.* (2023).	piR-020244, piR-013323, piR-009000, piR-020364 and piR-001184, piR-019949 and piR-018624 (Flores-Chova *et al*., 2023)
Multiple sclerosis (MS)	Multiple sclerosis (MS) is a chronic neuroinflammatory disease. Normally, environmental and genetic risk factors are responsible for the MS; also, the adaptive immune system, particularly T cells and B cells, playing a crucial role (Thompson *et al*., 2018).	Kamenova *et al*. (2022) studied that endogenous miRNAs and piRNAs regulate MS genes and identified 50 piRNAs as potent regulators of 21 MS genes. Many of these genes are targeted by piRNAs. For example, MYC gene could be targeted by piR-16,771, piR- 17,232 and piR-1177.

The major risk factor for autoimmune diseases has been observed to be the locus for the major histocompatibility complex (MHC) (Jiang *et al.*, 2024). The regulation and balance of CD4+ T cells [specially T helper 1 (Th1) and T helper 2 (Th2) sub-sets] significantly affects the autoimmune inflammation *via* different cytokines (Charlton and Lafferty, 1995; Pisetsky, 2023). Some common examples of autoimmune diseases include rheumatoid arthritis, systemic lupus erythema-tosus, multiple sclerosis. For most of the autoimmune diseases, piRNAs get either up-regulated or down-regulated and act as the biomarkers. Table 2 shows the expression of various piRNAs as a biomarker during some major autoimmune diseases.

(iii) *piRNA and Other Diseases*:

piRNAs are also used as the biomarkers for the various other diseases to diagnose them. Type, timings and magnitude of piRNA expression helps to diagnose the various diseases.

(a) *piRNA in Viral Infections*:

Cells after infection, defend against pathogens through intrinsic immunity called cell-autonomous immunity (Majdoul and Compton, 2022). During invasion, pattern recognition receptors (PRRs) bind and identifies the pathogen specific molecular patterns and activate antiviral interferon and pro-inflammatory responses (Jiang *et al.*, 2024). Interferon induces the expression of interferon-stimulated genes (ISGs) through the JAK-STAT (Janus kinase-signal transducer and activator of transcription) signalling pathway. Most of the ISGs exhibit antiviral response, either by degrading viral nucleic acids or by inhibiting expression of viral gene.

In eukaryotes also genomes carry sequences originating from viruses, known as endogenous viral elements or EVEs (Ter Horst, 2019; Ogawa and Honda, 2022); which are abundantly available in piRNA clusters from across arthropods to many mammalian species (including humans). These EVEs transcribes and results in biogenesis of piRNA which can be antisense or complementary to ancient (infecting) viral sequences. In the event of re-infection, these EVE-originated piRNA can

Table 3: Expression of Different Types of piRNAs during Different Conditions of Tuberculosis (Zhang *et al.*, 2019)

Different stages of Tuberculosis	Number of piRNA Expressed	Types of piRNAs
Patient with active TB infections	Eleven piRNAs were DE between TB and the other three groups,	piR-001421, piR-018570 and piR-020582 were globally associated with TB.
Latent TB Infections (LTBI)	Seven (7) types piRNAs were differentially expressed (DE) between LTBIs and treated LTBIs groups.	These are--piR-017936, piR-019675, piR-019912, piR-020548, piR-020381, piR-020490 and piR-009059
Treated LTBI Patients		
Controlled (Healthy) Patients	Three types of piRNA were DE between LTBI and Controlled groups	piR-020381, piR-020490 and piR-009059

direct PIWI proteins to target (infecting) viral RNAs leading to effective silencing of them.

In humans, expression of specific piRNAs during AIDS helps to diagnose the disease; and it can be used as a diagnostic kit. In AIDS, after infection with the Human Immunodeficiency Virus (HIV), patient suffers a widespread loss of CD4+T cell. Doke *et al*. (2022) reported the conspicuous changes in magnitude of expression of piRNAs and PIWI proteins in human after primary exposure to HIV-1 Tat protein and cocaine. Martínez-González *et al.* (2020) have reported expression of 39 piRNAs from plasma extracellular vesicles in HIV/HCV co-infected patients.

Herpes simplex virus (HSV) causes herpes which is characterized by painful blisters or ulcers. It is a common infection with two main types: oral herpes (usually HSV-1, causing cold sores) and genital herpes (usually HSV-2, causing genital sores). Herpes is treatable, but not curable. Studies on the piRNA in HSV-1 infected human fibroblasts have revealed the 69 differentially expressed piRNAs (52 upregulated and 17 downregulated) involved in antiviral immunity functions (Wang *et al.*, 2023). piRNA transfection assays in HSV-1 infected fibroblasts revealed that piR-36 and piR-233 inhibited HSV-1 replication; while piR-36 and piR-041 promoted HSV-1 replication, suggesting piRNA involvement in the viral replication in host cells (Jiang *et al.*, 2024).

Li *et al.* (2021) studied the expression profiles of piRNAs in human rhinovirus (HRV)-infected H1-HeLa cells (HeLa cells are used by scientists to develop a cancer research method that tests whether a cell line is cancerous or not) and revealed that some downregulated piRNAs were positively associated with LINE-1 transcription of retro-transposons, implying a potential mechanism of piRNA in rhinovirus infection. Long Interspersed Nuclear Element-1 (LINE-1 or L1) is a retro-transposon group that constitutes 17% of the human genome and shows variable expression across various cell types.

Similarly, studies on possible role of piRNAs in the infection of human papilloma virus (HPV) and respiratory syncytial virus (RSV) have been made by Huang *et al*. (2021) and Corsello *et al.* (2022), respectively.

(b)*piRNA in Bacterial Infections*:

In case of bacterial infection also, differential expression of various types of piRNAs becomes the biomarker for the early, quick and accurate diagnosis. Some common bacterial infections are:

Tuberculosis (TB), which is an infectious disease caused by bacteria *Mycobacterium*

Table 4: Expression of different types of piRNAs in different types of cancers (Wu *et al.*, 2020)

Types of Cancer	Type of piRNAs	Expression Mode	Functional Role
Lung Cancer	piR-L-163	Downregulated	Inhibits cell migration and invasion
	piR-55490	Downregulated	Suppresses lung cancer cell proliferation
	piR-651	Upregulated	Promotes cell proliferation, migration, and invasion,
	piR-57125	Downregulated	Increases metastasis
	piR-34871, piR-35127, piR-46545, piR- 52200, and piR-57125,	Downregulated	Causes proliferation of cancer cells
Gastric Cancer	piR-823	Downregulated in Gastric cancer tissues	Inhibited cell proliferations, suppresses tumour growth,
	piR-32105, piR-58099, and piR-59056	Upregulated in Gastric cancer tissues	Increased proliferation of cells.
Liver Cancer	piR-LLi-24894, piR-LLi-30552, hsa-piR-020498, hsa-piR-013306	Expressed during different stages of Liver Cancer	Used as biomarker for different stages of Liver cancer
Colo-rectal Cancer	piR-1245	Consistent downregulation	Induces apoptosis
		Overexpression	Promoted Cell Proliferation
	piR-5937, piR-001311, piR-004153, piR-017723, piR-017724, piR-020365, piR-28876, piR-32105, piR-58099, and piR-59056	Downregulated	Used as Biomarker

tuberculii. De Araujo *et al*. (2019) identified 35 piRNAs from patients with active TB; latent TB infection (LTBI); treated patients of LTBI and healthy controls. The result clearly concluded the differential expression of different piRNAs; which can be used as an efficient biomarker for TB infection.

Zhang *et al.* (2019) studied the expression of piRNA in 20 patients of pulmonary tuberculosis and 20 healthy controls, through peripheral blood samples and concluded the presence of 777 differentially expressed piRNAs, with 192 exclusively expressed in TB patients and 142 exclusively expressed in healthy controls. Table 3 shows the expression of various piRNAs under the different conditions/stages of TB.

Another bacterial disease leprosy is caused by the bacteria *Mycobacterium leprae*, leading to chronic infection in the skin and peripheral nerve. There are 14 differentially expressed piRNAs between leprosy patients and healthy controls identified, with 5 piRNAs (piR-hsa-12,454, piR-hsa-1580, piR-hsa-21,131, piR-hsa-27,007 and piR-hsa-28,634) exhibited excellent predictive ability for leprosy (Pinto *et al.*, 2020).

Brucellosis is another bacterial infection caused by the *Brucella* species. It is a zoonotic disease (i.e. spreads from animals to humans). There are 7 upregulated piRNAs observed in brucellosis patients. Three piRNAs, *viz.* piR-000753, piR-001312 and piR-016742, could be the potential biomarkers for brucellosis (Wang *et al.*, 2022).

(c) *piRNA in Cancers*:

Incidence of cancer ranks the highest causing mortality among all in both the sexes globally. Though miRNAs have been largely studied in cancer pathogenesis, there is limited studies regarding the relationship between piRNAs and cancer therapeutics. Table 4 exhibits the role of various piRNAs in pathogenesis and biomarker efficiency for the cancer of different organs.

Ethical Statement

This is a review article hence no Ethical Approval needed.

Author Contributions

Author has read and agreed to the published version of the manuscript.

Conflict of Interest

The author declares no conflicts of interest.

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