Dynamics of PIWI-interacting RNAs in Disease and Medicine

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Abstract

Small RNA plays important role in regulation of gene expression and decides the cell fate. They are found to regulate mRNA transcript which in turn are responsible for translating into proteins. Recent discovery of PIWI-interacting RNAs revealed many mysterious dimensions of biology as they are known to regulate "the ancient relics" called as transposons or "jumping genes". Previously PIWI-piRNA pathway was known to be germline specific but recent studies shown their strong role in somatic cells and neurons in diverse organism. Hence, this review is the study of multifaceted role of piRNA in transposon regulation with the compilation of their recent discovery in different cell types in order to provide a strong understanding of their role in Disease diagnosis and medicinal therapeutics to research fraternity.

Keywords : *PIWI-interacting RNA Transposons, neurons, Disease Diagnosis, Medicinal Therapeutics*

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1.1 Background

P-Element induced wimpy testis (PIWI) proteins are known to interact and guide a class of noncoding small RNA called as PIWI-interacting RNAs (piRNAs). Recent discovery of millions of PIWI interacting RNAs exposed a mesmerizing and unexpected dimension of molecular biology. piRNAs are first discovered in *Drosophila Melanogaster* germ line cells and it was found that they play major role in transposon silencing, genome rearrangement, spermatogenesis and maintaining germ line integrity but many recent evidences proposed their presence in somatic cells, neurons and cancer cells which illustrated their multifaceted function like epigenetic programming, stem cell function and whole body regeneration [1-2]. The recent role of piRNA and its function is reported in neurons suggest their strong role in neurodegenerative disorders like *amyotrophic lateral sclerosis* (ALS), Alzheimer's disease (AD), Parkinson's disease (PD), Rett's Syndrome and aging [3]. Further studies on disease associated piRNA lead us to significant study of neurodegeneration and

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help in development of novel therapeutics to contribute improvement in medicine, early prognosis and diagnosis as well. Recently Philip D. Zamore evidenced common biogenesis of piRNA across evolutionary broad range of animals which shows that if transposons are known as ancient relics as piRNAs also must have been associated with them and evolved differently in broad range of animal and different cell types.

1.2 Transposons "The ancient relics"

Transposons so called molecular parasites make up large percentage of genome volume in mammals and other lower organisms. These are known to be the ancient remnants accruing by time within the genome and responsible for influencing the genome by their property of mobilization. They composed 45% of the humans and one third of flies' genome and are known to be triggered by piwi-interacting RNAs (piRNAs), a class of very less known small non-coding RNAs responsible for maintaining genomic integrity, heterochromatin formation and epigenetic regulation of germ line organs in gonads of flies and other mammals.

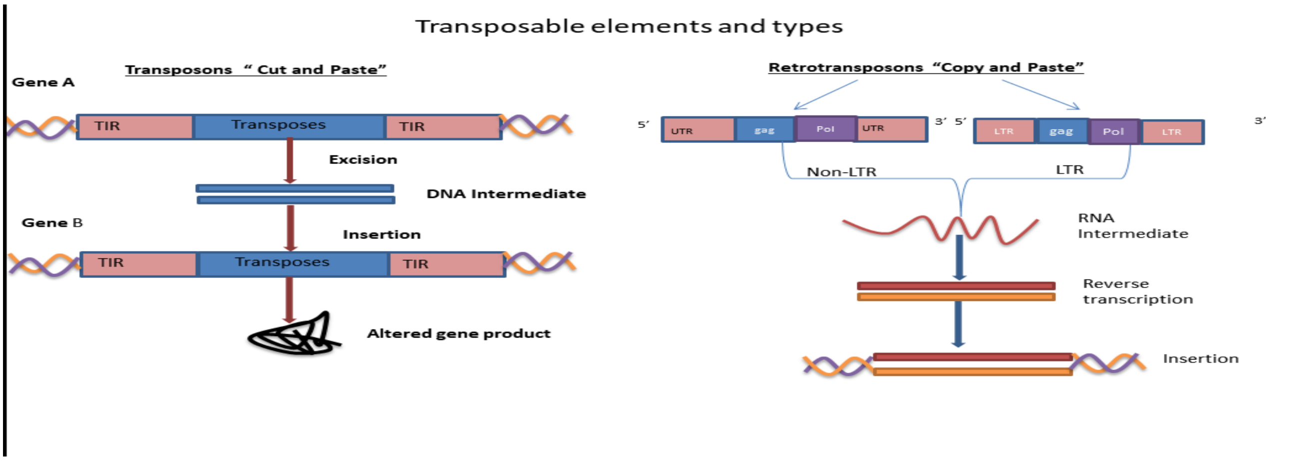


Figure 1.1: Transposable Elements and Types: A) showing Tranposons use "Cut and Paste" Mechanism B)

Retrotransposons "Copy and Paste" mechanism.

Transposons were first discovered in maize by Barbara Mcclintok in 1940 [4-5]. Transposable elements are genetic material also called as "jumping genes" which are able to multiply with in the genome by copying themselves to the other location and which leads to variation within the species as they are notorious enough to move from one genome location to another. Hence they are involved in genome rearrangements, genome size, and also lead to lethal or adaptive effects as well [6]. TE sequences are divided in to those with or without a RNA transposition intermediate, insertion mechanism, features of TE sequences i.e. internal coding and non-coding sequence, terminal repeats, autonomous and non-autonomous TEs i.e. TE relying on other elements to move due to lack of certain proteins which help them to move from one place to other. Biologically, TEs are classified into two main classes i.e. retrotransposons called as Class I TEs and DNA transposons i.e. Class II TEs. The former transpose from RNA intermediate by transcription and again by reverse transcription i.e. "copy and Paste Mechanism". It includes long terminal Repeat (LTR), Dictyostelium intermediate repeat sequence (DIRS – like elements), Penelope- like elements (PLEs), longinterspersed elements (LINEs) and Short Interspersed elements (SINEs). It ranges from 5-7 kb in drosophila which includes Copia, Gypsy, ZAM, Idefix and Tirant are infectious retrotransposon family found to infect other cells [7]. The latter can transpose via DNA intermediate. They are of four type i.e. Terminal inverted repeats (TIR), Crypton, Helitron and Maverick. These can increase DNA volume by getting replicated with the chromosome or by gap repair mechanism. Transposon have their own machinery to replicate and can behave autonomous and in non- autonomous way. It is depicted in previous studies that transposons maniac is handled by class of small RNA called as piwi-interacting RNA which are known to silence transposons in germline cells as they are responsible for incorporating genetic variations and genome defense mechanism recruits piRNA to handle the transposons expression and maintain the integrity of the genome. In female flies the piRNA are found to silence an endogenous retrotransposon called as *gypsy* in somatic follicle cells during oogenesis [8] and in males they are found to silence a gene called as stellate a gene involved in male sterility [16]. Later on *flamenco* a piRNA producing locus was identified which is responsible for controlling other TE families [17]. Hence piRNAs previously called as rasiRNA (repeat associated small interfering RNAs) controls the expression of TE in germline cells to maintain the integrity as well as variation in the generations.

1.3 PIWI-interacting RNA (piRNA)

piRNAs are a class of non coding single stranded RNA with 24-36 nucleotides long which guide a mammalian class of Argonaute proteins called as PIWI proteins. Initially piRNAs were found in *Drosophila* testis and are named as rasiRNA in 2001 later a distinct class of piRNA is recognized as they were found to be associated with PIWI unlike other small RNAs. piRNAs are found to be different according to their source of origin 1) piRNAs are known to silence transposons and are repeat specific piRNAs, they are formed from remenants of transposons and further silence active transposons. 2) piRNAs which are formed from 3'UTR regions and are mainly found in somatic cells like ovarian somatic cells of flies and spermatocytes of mice. 3) piRNAs derived from long noncoding RNA their function is still not much known [1].

1.4 Argonaute Protein (PIWI Protein)

Argonautes are known for small RNA guiding protein as they are known to guide them to target. PIWI is a subfamily of Argonaute also called as AUB and AGO3 in flies which is expressed in both germline cells and somatic cells of gonads. It has mainly PAZ and MID domain which makes PIWI protein to associate with piRNAs specifically. As, PAZ and MID domain of PIWI binds specifically with piRNA by recognising their 5'U end. Hence they play major role in piRNA specific transposon silencing.

1.5 Biogenesis: PIWI-interacting RNA and transposon silencing in germline

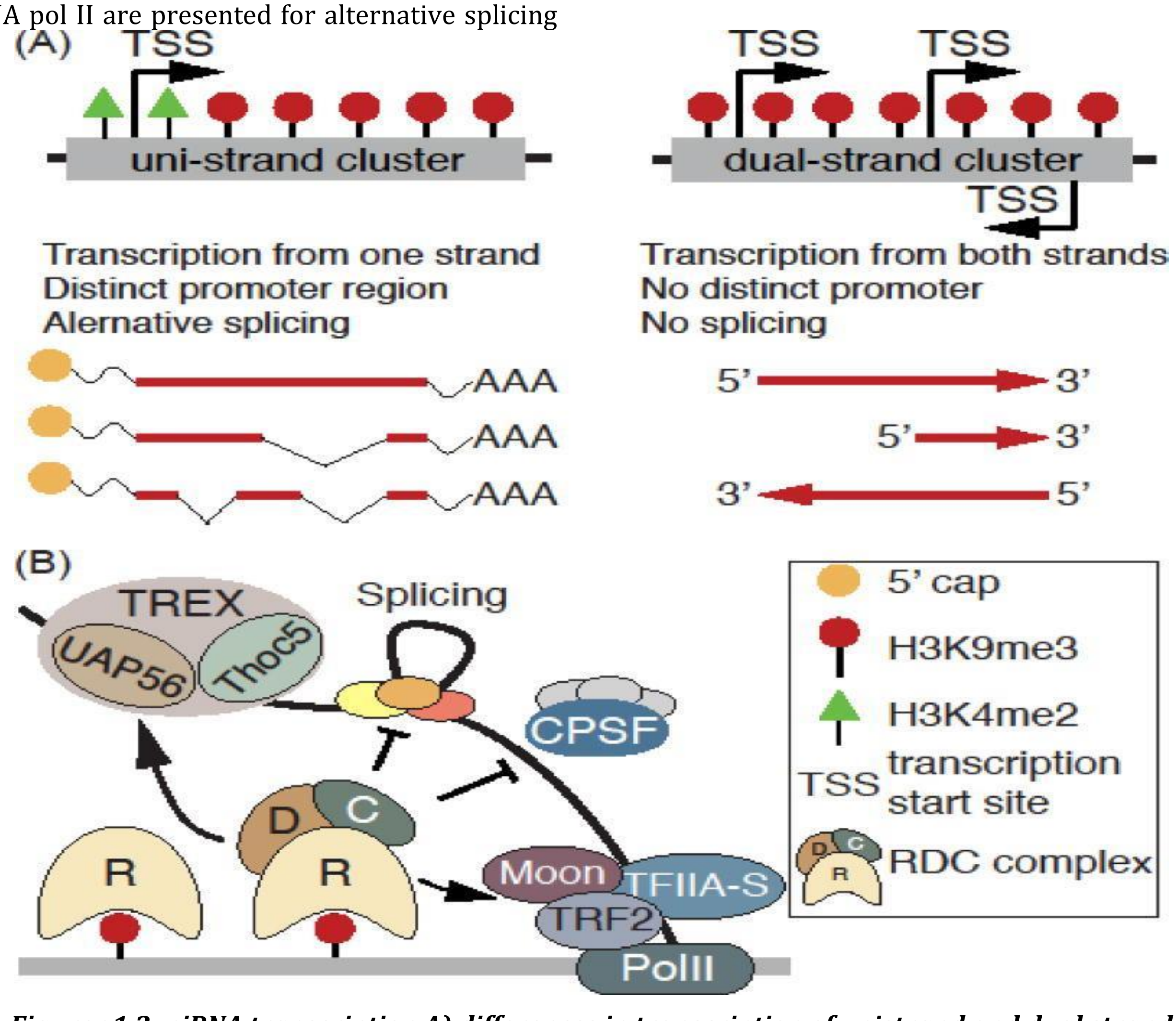
piRNA are class of small non-coding RNA ranging from 23-34nt in length present in all mammals including humans. These are found to bind with specific clade of Argonaut called as PIWI proteins. Many evidences suggest that piRNA biogenesis takes place in three phases in all organisms i.e. primary pathway, ping-pong mechanism and the phased primary pathway. The primary pathways produce pre-piRNA which is ready tobind with Piwi and Aub further ping-pong mechanism is initiated by primary pre-piRNA to produce secondary piRNAs and AGO3 slicer dependent trimming leads to production of secondary piRNA also termed as amplification of piRNA and increase the population of piwi bound piRNAs. This whole pathway is organized in a systematic manner to silence the transposon and require a range of proteins, transcription factor and determinants to procure piRNA biogenesis [18]. Phased primary pathway is also called as zucchini dependent pathway as, zuchhini is responsible for trimming and producing piRNA transcript of specific size. There are recent evidences which present the advances in piwi- piRNA biogenesis includes various studies like identification of *myb-A* transcriptions factor required for transcription of prepatchytene piRNA in mice from piRNA cluster rich regions

1.5.1 Foundations and accreditation of piRNA precursor existence

The piRNAs are characteristically distinguished from canonical RNAi pathways of miRNAs and siRNAs which include its dicer free processing and origin from single stranded precursors with the distinguished length of

23-34 nucleotides. These piRNAs are recognized by PIWI clad of ARGONAUTES containing structural features

with amino terminal domain, PAZ domain, MID domain and PIWI domain (animal specific). Other than this Argonauts are separated into three groups AGO, WAGO and PIWI clade which are associated with different small RNA classes. In flies PIWI is responsible for guiding piRNA to silence transposons. The existence and evolution of piRNA is a big mystery which needs a lot more studies. Various evidences revealed association of multiprotein complex is responsible for recognizing specific genomic landmark for transcribing piRNAs and it was found that piRNA precursors are transcribed from genomic clusters containing transposons fragments accumulated from years in the genome which in turn gives a clue of genetic memory of transposition invasion. This genetic memory lead us to a thought of adaptive immune response mechanism adapted by antisense primary piRNAs which are transcribed from clusters formed from the accumulation of transposons. The accumulation of transposon is found in various genomic locations which act as a trigger for piRNA silencing. It is predicted that moonshiner is recruited by piRNA cluster via heterochromatin protein-1 variant Rhino and moonshiner in turn recruits TBP related factor triggers piRNA transcription initiation [19]. Hence histone marks are responsible for triggering transcription in heterochromatin regions instead of any sequence motif. There are two types of piRNA cluster known till date known as unistrand and dual strand cluster according to their capacity of generating piRNAs. The main feature of unistrand cluster is formation of piRNA from only one genomic strand. *Flamenco* and 20A are two unistrand cluster known in D*rosophila* till date. 20A cluster is found in somatic follicle cells and germ cells but flamenco is limited to follicle cells repress gypsy-like transposons. The specific features of flamenco cluster give the idea of production and foundation of piRNA as these clusters are most known and prevalent. Flamenco is located in the boundary of euchromatin and heterochromatin region colonized with fragments of gypsy family TEs in minus strand. The promoter is marked with H3K4me2 and cluster body is surrounded by H3K9me2/3 marks and transcribed with RNA polymerase II [19]. The transcript formed by RNA pol II are presented for alternative splicing and transferred to Yb bodies via nuclear DotCOM (place for accumulation of *flam* transcript) [20].Dual strand cluster are predominantly found in germ cells of *Drosophila* produces piRNA clusters. They are majorly located in pericentromic and subtelomeric heterochromatin regions and boundaries of euchromatin regions. They are transcribed by RNA polymerase II. Recognizing transcription site on piRNA cluster and initializing transcription are hard-hitting process as it requires a multiprotein complex in germline cells.



Figurer 1.2: piRNA transcription A) differences in transcription of unistrand and dual strand cluster former use alternative splicing and latter uses no splicing. B) the complex required for dual strand transcription of piRNAs which majorly happens in germline cells.

(adopted from Huang X et al., 2017).

It was found that Rhi and deadlock and cuff (A RDC complex) interact with each other and act as anchor between the transcription site and other protein complex (TBP-TATA-box binding protein) which makes a platform for different proteins to get associated. Hence, Rhi-Del platform which binds with cuff (cap binding protein) and moonshiner (a link between RDC and TBP-TATA-box binding protein) guides other protein to loci of piRNA producing cluster as, H3k9me3 decoration is found everywhere in genome and this methylation is found in areas which are highly silent heterochromatin region but they only act as recognition site in case ofpiRNA transcription [21]. This complex not only initiates transcription of piRNAs but also suppress the termination and stabilization by deciding the timing of RNA polymerase II binding which finally makes antisense piRNA transcript for transposon silencing. Further it was also studied that TREX is also responsible for transcription of dual strand cluster as knockout experiments lowered the rate of piRNA transcripts and was found to be co-localized with RDC in nuclear foci [22]. Finally, the transcripts formed by dual stranded clusters do not go for capping and splicing as they are bounded by cuff (which prevents them for capping) and DEADBOX helicase U2AF65 associated protein which takes them to nuclear periphery where vasa escorts them to nauge for further processing.

1.5.2 Pre-piRNA processing in cytoplasm

In flies RNA piRNA transcript and requires rhino-Deadlock cutoff/moonshiner, U2AF65 associated protein (UAP56), a complex for transcription and stabilization of precursor piRNA 23-24] which are exported to cytoplasmic nauge for processing and transposon silencing in germline cells of drosophila. The various export factors are associated to export piRNA from nucleus to nauge followed by piRNA processing and recently it is found that MOV10L1 (mouse homologue) [25]/armi (in drosophila) is a factor which is associated with piRNA processing. MitoPLD/ zucchini is found to play a major role producing 5'end of precursor piRNA and produce specific 5'U bias for loading into PIWI protein. Zucchini a homolog of mice MitoPLD is found in flies which trims the piRNA which are generated from clusters rich in transposons [26-29]. Historically pre-piRNA formation is zucchini dependent also called as primary biogenesis of piRNA but it can be also be called as zuchhini dependent phased piRNAs as, it is also found that 3'end formation of piRNAs is also deployed by *zuc* and exhibit a signature of phased piRNAs. Hence primary piRNA biogenesis can also be called as zuchhini dependent phased piRNA. Further piRNA maturation take place with *nibbler* which participates in exonucleolytic maturation of piRNA and *papi* [23,30] has been implicated in formation of 3'end formation which is the last step of piRNA processing as it decides the size of piRNA and then mature PIWI associated piRNAs are methylated by HEN 1 to produce 2'o end of piRNA to make them single stranded stable structure and control transposons epigenetically [31-34].

The second phase which is also called as amplification of piRNA or ping-pong mechanism requires lot of determinants for its deployment. The ping pong signatures are also found in planarians [35], Koala genome[36], Hydra [37], Zebra fish and silk-worms [38]. The second phase of piRNA biogenesis is followed by transcription and stabilization of primary precursor in germline cells of *Drosophila Melanogaster* which require a protein complex called Rhino-Deadlock-Cutoff which stabilize and flag them for piRNA processing in Yb in case of follicle cells and nauge in case of germline cells bodies [39]. Both the phases are interconnected as the primary piRNA are exported from the processing site of cytoplasm to nucleus once again for ping-pong amplification. The primary piRNA are trimmed very specifically with zucchini which makes the way for phased piRNA as trimming independent [40]. Zucchini interplays during primary pathway of piRNA biogenesis and Tudor interplays in secondary pathway of piRNA biogenesis i.e. during ping-pongamplification Tudor protein plays a major role. Piwi-piRNA complex interact with TUDOR family for amplification. Several TDRDs such as Spn-E, Tudor, Krimper, and tejas are found to be important for PIWI functioning [41-42]. The mature piRNA-Piwi complexes have different chance as the mode of localization of Argonaute is different. Piwi localize into nucleus and Aub localize in perinuclear cytoplasm hence there can be two forms in which piRNA can carry out silencing i) Transcriptional gene silencing, ii) post transcriptional gene silencing which is also involved in ping-pong mechanism. Researchers identified 1U bias in piRNAs strands bound with Aub derived from antisense piRNA clusters and 10A bias in sense piRNA strand derived from transposons and are bond with Ago3 and whole process occurs in nauge a perinuclear germline structure reveals the un-spliced amplification of piRNA called as ping-pong mechanism which leads to silencing of transposons without any slicer enzyme. Ping-pong cycle leads to adaptive and sequence specific mechanism of PIWI-piRNA mediated silencing which do not require any slicer but needs TUDOR family to mediate its function and maintain its stability [43-44]. So, in germline cells piRNA biogenesis occurs in nauge and their licensing is different from somatic cells.

Ping-pong amplification occurs in germline cells of flies, the mechanism increases the piRNA population and responsible for dicer independent splicing. The production of phased

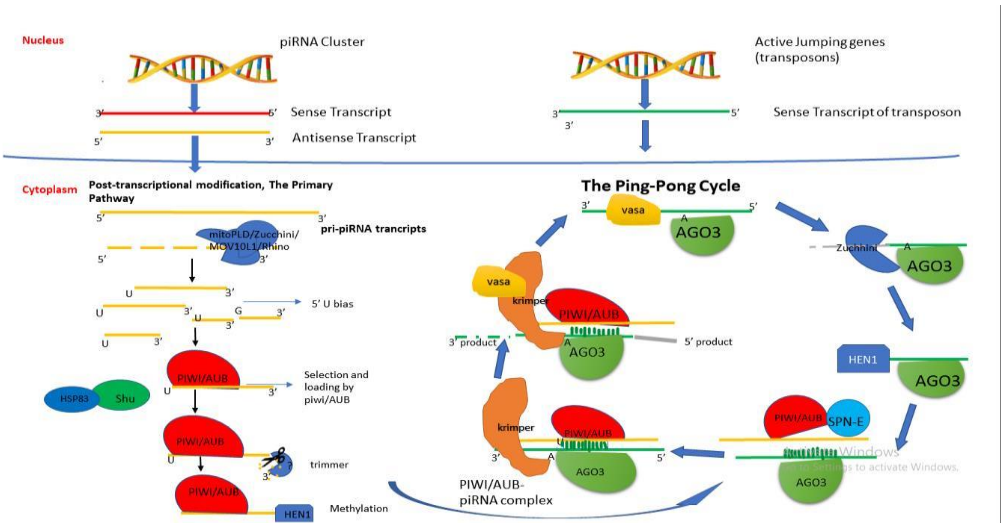
piRNA occurs by specific binding and overlaps of 1U bias of AUB associated piRNAs and 10A bias of Ago3 associated piRNAs. Many computational studies revealed the 10nt overlap between AUB-Ago3 associated sequences i.e. AUB is bound to 1U biased piRNAs and Ago3 is bound to 10A bias piRNA and their 10 nt. interaction is revealed by analyzing sequences computationally using various tools. This interaction of Aub- Ago3 bounded piRNA leads to formation of more piRNAs from the transposons transcripts. This amplification mediates from biogenesis of piRNA to its target silencing hence it plays very important role in transposon silencing via piRNAs. The primary piRNA produced from clusters are associated with AUB have the capacity to slice transposons transcript at the specific position with its slicer activity and the piRNAs which are produced have a 5' end and are in sense orientation of transposons and their loading and association with Ago3 is followed by maturation of piRNA includes trimming of their 3'end by unknown nuclease and forms a complex of Ago-piRNA which is again ready to detect and cleave more transcript of piRNA cluster to form antisense piRNA which are identical to target transcripts. In somatic cells maturation of Aub associated piRNAs also occurs via zucchini by trimming 3'end and production of phased piRNA occurs and these are loaded into PIWI to create adaptive silencing of transposons.

1.5.3 PIWI-piRNA biogenesis in soma

In somatic cells specifically in ovarian cells of drosophila melanogaster piRNA transcription takes place from the unistrand cluster and PIWI is expressed but AUB and AGO3 is not expressed. The piRNAs which are transcribed from unistrand clusters are same as that of formed in dual strand clusters of germline i.e. 23-34 nucleotide in size and 2'o methylated at 5' and 3' end. As structurally the piRNAs transcribed from unistrand clusters of somatic cells are same as that of germline dual strand cluster but, the process of transcription isentirely different. The well-known unistrand cluster is named as *flamenco* has its own promoter with H3K9me2 enables RNA polII to transcribe clusters and with that the cluster body is decorated with H3k9me3 which shows the heterochromatin region. The promoter lacks TATA box but contains an initiator (Inr) motif and downstream promoter (DPE). The promoter region also ports a binding site of transcription factors called as Cubitus interruptus (Ci) in the upstream region -515 to -365 of transcription start site (Yamanaka S et al.,

2014; Mohn F et al., 2014; Pane et al., 2011; Goriaux C et al., 2014). Co-immunoprecipitation experiments by

various authors discovered cytoplasmic localization of ARMI and YB which are mainly responsible for stabilizing piRNAs in somatic cells. Hence, the main protein involve in guiding the piRNA to sense transcript of transposons in somatic cells is PIWI protein and a putative helicase called as ARMI, a nuclease called as zucchini and a TUDOR protein called as YB is responsible for stabilization and biogenesis of piRNA. Unlike germline cell somatic cell piRNA do not undergo ping-pong mechanism for their amplification. Before piRNA assemble in Yb it was also found that the transcripts derived from different piRNA cluster loci are accumulated at one place on a single foci named as DOT Com. As the piRNA transcript derived from flamenco goes through alternative splicing and accumulated in a single nuclear structure of follicle cells called as Dot COM, the transcript are transferred from here to YB for further processing by different protein complexes. [48].



1.6 PIWI-piRNA in cancer

Cancer is most common and dreadful disease accounts for millions of deaths yearly, due to its late detection and reoccurrence [49] after treatment highlights the need for novel prognostic biomarker identification [50]. Recent discovery of piRNA in silencing transposons by interacting with various proteins and their domains in carrying out silencing shows its functions and mechanism in cancer. piRNA are responsible for silencing hence PIWI/piRNA complex interacts to form Transcriptional gene silencing and post transcriptional gene silencing and aberrant piRNA expression lead to many cancer types like lung cancer, breast cancer, colorectal cancer, kidney cancer, Hematological malignancy and Glioblastoma. At the time of transcription the complex interactwith many proteins and transcriptional factors so, the overexpression of piRNAs lead to inhibition of apoptosis or aberrant expression like over expression of piR-021285 inhibits BC (Breast cancer) cells apoptosis [52], similarly piRNA-823 expression increases DNA methylation and inhibits tumor suppressor P16INK4 expression [57]. Many piRNAs also interact with mRNAs, long non coding RNAs and pseudogenes to mediate post transcription gene silencing and leads to suppression of cancer like piR-55490 binds to mTOR causing suppression of lung cancer cells. Similarly piR- 651 upregulation leads to tumor proliferation and apoptosis inhibition. piR-3481 and piR-52200 upregulation promotes cell proliferation by perturbing AMPK phosphorylation of ATM-AMPK-p53 pathway in lung cancer [59]. In Gastric cancer piR-651 up-regulates cell proliferation and piR-823 down regulates proliferation of cancer cells and weakens methylation of tumor supporter genes [208, 54]. In colorectal cancer piR-1245, piR-54265, piR-025551, piR-823 promotes cell proliferation and growth by inhibiting apoptosis by regulating serine threonine phosphorylation and STAT3 phosphorylation. It is found that piR-32051, piR-39894 and piR-43607 are up-regulated and expressed during metastasis and high tumor state during kidney cancer [55-56]. piR-823 and piR-651 are also up-regulated in Hematological malignancy and promotes proliferation and cell cycle progression [57-59]. piR-52207 up- regulation is responsible for cell proliferation and tumor genesis by binding target mRNA (NUDT4,MTR) in ovarian cancer [60]. As, piRNA works in upstream of different regulatory networks shows their importance and for early cancer diagnosis and cancer detection. Aberrant PIWI expression is also seen in different cancer types like PIWIL1 up-regulated is responsible for DNA hypo methylation in lung cancer, regulate signaling pathway of gastric cancer, and used as a prognostic markers for predicting colorectal cancer [61-63], PIWIL2 is responsible in cervical cancer and renal cancer [64-65], PIWIL3 differential regulation leads to gastric cancer and multiple myeloma [66] and PIWIL4 are regulation expression leads to triple negative breast cancer [67]. Advanced detection of technologies can help to detect the piwi/piRNA expression in normal and cancer stages. PIWI/piRNA during biogenesis and function interact with different proteins but their overexpression or under expression may lead to many effects like phosphorylation in cellular signaling pathways, DNA methylation at a specific loci and specific interaction with mRNA leads to aberrant expression of genes.

1.7 PIWI-piRNA in neuron

Evidences revealed the function of piRNA in germline and their key role in maintaining fertility [68, 39]. Many strong evidences showed the presence of Piwi/Hiwi/Mili proteins and piRNAs in somatic cells like neurons in brain and kidney in mice [69]. Lee et al., reported distribution of piRNA in brain for example Miwi expression was identified in Hippoccampus region of mice by in Situ hybridization and confirmed by real time PCR. piRNA knockout studies by Lee et al. also shown reduced spine density in axons. Rajasethupathy et al. (2012) observed piRNA in neurons of Aplysia and interpreted their unique biogenesis pattern [70]. The amount of piRNA in brain is one tenth of the amount of piRNA in germline evidenced by [71-72, 59]. A neurotransmitter named as serotonin (5 Hydroxytryptamine) highly synthesized in neurons of Central Nervous System responsible for regulation of mood, apetite and sleep and its modulation alter the level of piwi/piRNA complex in Aplysia brain. CREB2 a cAMP responsive element binding protein 2 responsible for inhibitory constraint ofmemory also regulated by piwi/piRNA complex and hence responsible for memory loss and various memory disorders. As the complex interferes with the serotonin dependent methylation of conserved promoter sequence of CREB2 which is further responsible for the formation of synaptic plasticity and memory storage [73]. These evidences strongly depict the interference of Piwi/piRNA in neuronal development and memory storage on flies, mice and further in other higher organisms which in turn create a future enigma of involvement of piRNAs in neurodegenerative diseases like Alzheimer’s, Huntington’s, Parkinson’s and Brain tumor to many other neuronal disorders. It was studied that brain tumors also had a high number of transposons and which gives a clue of presence of piRNA in tumors and hypothesis was confirmed by doing knockout experiment of piwi/Aub in drosophila suppresses the tumor growth.

1.8 DNA methylation and piRNA in neurodegenerative Disorders

piRNA dependent DNA methylation and Demethylation takes place in mammalian and germ line cells. Many recent studies depicted role of piRNA in DNA methylation of retrotransposons [74-75, 16]. Itou et al., identified role of piRNA/ PIWI protein in DNA methylation by silencing EGFP transgenes through induction of artificial piRNA and validating the results by bisulphite sequencing. However it is elucidated that piwi specific piRNA are mainly evolve from embryonic piRNA clusters and intergenic piRNA clusters. The latter are present in somatic cells including neuronal cells too. Recent research challenges these beliefs by designing an experimental system containing antisense EGFP transgenes which are methylated by producing artificial piRNA. In the same line DNA methylation is also a major cause of many neurodegenerative disorders like Alzheimer’s, Dementia, Parkinson’s, Rett Syndrome. Recent studies prove epigenetics as a major player in mediating neurodegenerative disorders. Many genes involved in maintaining neuronal plasticity and differentiation are epigenetically deregulated which gives a glimpse of involvement of small non coding RNAs including piRNAs in neurodegenerative disorders. Latest studies performed DNA methylation analysis of Alzheimer's, Dementia with Lewy bodies, Parkinson’s and observed that neurodegenerative disorders shares same aberrant cpG methylation. The disruption of DNA methylation by piRNA (DQ599803) transcribed from piRNA cluster which is essential for neuronal plasticity and neurodegenerative disorders [73].

1.9 Conclusion

piRNAs are not only specific to germline but their presence in other somatic cells illustrates their involvement in many diseases not only limited to fertility or sterility but also to various memory related disorders and may be some neurodegenerative disorders. The androgens also play a strong role in regulating the proteins and determinants involved in biogenesis of piRNA like, Estrogen upregulates the transcription factor MYB which is involved in transcription of piRNA clusters and Testosterone upregulates various components of nauge/YB like TUDOR protein. Similarly piRNA are also depicted to be found in brain of mammalian tissue. Various researches show the role of piRNA in memory storage and also they take part in DNA methylation which is further responsible for causing neurodegenerative disorders. This debate demonstrated presence of piRNA in somatic cells other than germline cells. Although there is a remarkable progress in understanding theimpairment of piRNA biogenesis and piwi proteins, but still many questions arise and remain elusive inline. Is there any reproductive disease in which interference of these piRNAs occurs, as knockout effects of any determinant of piRNA machinery maximally leads to sterility? How and why other RNAs escape from these piRNA machinery importantly mRNA? There are certain homologs of PIWI named as piwi like protein in humans, do they also interfere in piRNA biogenesis if not than what is there function? As Androgens and myb family components also play a key role in regulating the genes and proteins of piRNA machinery and take part in cell cycle and cell proliferation so, there are some links which warrants the role of piRNAs in cancer, and it is of great interest to examine mystery of piRNA and piwi proteins in neuronal disease and cancer would inhibit tumorogenesis. As many deep sequencing studies revealed presence of piRNA expression signatures in different carcinoma tumors but brain tumor studies are still elusive and unexplored. This review is mainly focused on identifying role of piRNAs in brain development and memory storage.

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