

## Minireview

## PIWI Proteins and piRNAs in the Nervous System

Kyung Won Kim\*

Convergence Program of Material Science for Medicine and Pharmaceuticals, Department of Life Science, Multidisciplinary Genome Institute, Hallym University, Chuncheon 24252, Korea

\*Correspondence: kwkim@hallym.ac.kr

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**PIWI Argonaute proteins and Piwi-interacting RNAs (piRNAs) are expressed in all animal species and play a critical role in cellular defense by inhibiting the activation of transposable elements in the germline. Recently, new evidence suggests that PIWI proteins and piRNAs also play important roles in various somatic tissues, including neurons. This review summarizes the neuronal functions of the PIWI-piRNA pathway in multiple animal species, including their involvement in axon regeneration, behavior, memory formation, and transgenerational epigenetic inheritance of adaptive memory. This review also discusses the consequences of dysregulation of neuronal PIWI-piRNA pathways in certain neurological disorders, including neurodevelopmental and neurodegenerative diseases. A full understanding of neuronal PIWI-piRNA pathways will ultimately provide novel insights into small RNA biology and could potentially provide precise targets for therapeutic applications.**

**Keywords:** neurological disorders, non-coding RNA, posttranscriptional gene silencing, small RNA, transposable elements

## INTRODUCTION TO THE PIWI-piRNA PATHWAY

PIWI-interacting RNAs (piRNAs) are small non-coding regulatory RNAs and fall into the same biological category as microRNAs (miRNAs) and small interfering RNAs (siRNAs) (Aravin et al., 2006; Girard et al., 2006). piRNAs form a complex with the PIWI protein and can be found in both vertebrates

and invertebrates. piRNAs are distinct from miRNAs in several features, the first is size (piRNAs are generally 24 to 32 nucleotides in length rather than 21 nucleotides), second a lack of sequence conservation, and third independence from Dicer for biogenesis (Vagin et al., 2006; Weick and Miska, 2014). piRNAs often show a bias for a 5' uridine (Brennecke et al., 2007; Ruby et al., 2006; Stein et al., 2019) and have 2'-O-methylation at the 3'-end (Horwich et al., 2007; Kirino and Mourelatos, 2007; Ohara et al., 2007; Peng et al., 2018; Saito et al., 2007; Tang et al., 2016) (Fig. 1). piRNA is the most diverse class of regulatory RNAs in general. According to piRBase, the number of unique piRNA sequences in the mouse is over 68 million; in *Drosophila*, over 41 million; and in *Caenorhabditis elegans*, more than 28,000 (Wang et al., 2019). piRNAs are often found in clusters throughout the genome and have been seen to be enriched in the germlines (Brennecke et al., 2007; Parhad and Theurkauf, 2019; Ruby et al., 2006). They are derived from the RNA transcripts of transposons, protein-coding genes, and specific intergenic loci (Aravin et al., 2006; Girard et al., 2006).

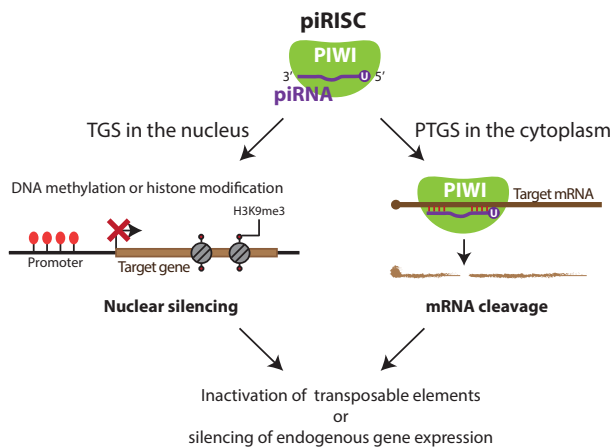
PIWI proteins belong to the Argonaute/Piwi family (Carmell et al., 2002). Like all Argonaute proteins, PIWI proteins contain two RNA-binding domains: the N-terminal PAZ domain and the C-terminal Piwi domain (Cerutti et al., 2000) (Fig. 2A). The Piwi domain has endonuclease activity that allows it to cleave RNA. The PIWI protein was initially described in *Drosophila*, and its name, P-element-induced wimpy testis (PIWI), was assigned as a result of the destructive effect on testis development observed in PIWI knockout (Lin and Spradling, 1997). PIWI proteins are highly conserved among

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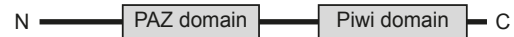


**Fig. 1. The molecular mechanisms of the PIWI-piRNA pathway.** The PIWI protein and piRNA form a complex, called piRNA-induced silencing complex (piRISC). piRISC regulates targeted RNAs, including transposable elements and endogenous target mRNAs, via transcriptional gene regulation (TGS) or post-transcriptional gene regulation (PTGS). TGS is often mediated through the recruitment of a chromatin methylation complex to the nucleus inducing a heterochromatin state, which serves as a repressive mark. TGS can also be mediated by recruiting DNA methyltransferases. PTGS is often mediated by the endonuclease activity of the PIWI protein resulting in cleavage of the target mRNA in the cytoplasm.

animals (Carmell et al., 2002). In *Drosophila*, there are three PIWI proteins, named PIWI, Aubergine (Aub) and Argonaute 3 (Ago3); and humans have four PIWI homologs, PIWIL1 (for PIWI-like RNA-mediated gene silencing1; also called HIWI1 for Human PIWI), PIWIL2 (also called HILI for HIWI-like), PIWIL3 (also called HIWI3), and PIWIL4 (also called HIWI2). There are three PIWI homologs in mice, PIWIL1 (also called MIWI for Mouse PIWI), PIWIL2 (also called MILI for MIWI-like), and PIWIL4 (also called MIWI2); and in *C. elegans*, there is only one functional PIWI, PRG-1 (for PIWI-related gene) (Carmell et al., 2002; Gunawardane et al., 2007) (Fig. 2B). Similar to the effects observed in *Drosophila*, *piwi* mutants result in germline defects, leading to sterility in *C. elegans* (Batista et al., 2008; Wang and Reinke, 2008) and mice (Carmell et al., 2007; Deng and Lin, 2002; Kuramochi-Miyagawa et al., 2004). Therefore, PIWI proteins play a crucial role in fertility and germline development across both vertebrate and invertebrate animals.

The PIWI-piRNA pathway relies on the specificity provided by the piRNA sequence which recognizes its RNA targets using base-pairing complementarity (Gou et al., 2014; Rouget et al., 2010; Saito et al., 2007; Shen et al., 2018; Zhang et al., 2018), while the PIWI protein functions as the effector (Tolia and Joshua-Tor, 2007). PIWI-piRNA complexes silence their target genes at both the transcriptional level and at the posttranscriptional level (Di Giacomo et al., 2013; Toth et al., 2016; Weick and Miska, 2014) (Fig. 1). Gene silencing at the transcriptional level often occurs through the recruitment of repressive chromatin modifications to genomic target loci

**A** PIWI protein



**B**

Human	Mouse	<i>Drosophila</i>	<i>C. elegans</i>
PIWIL1 (HIWI)	PIWIL1 (MIWI)	PIWI	PRG-1
PIWIL2 (HILI)	PIWIL2 (MILI)	Aubergine (Aub)	–
PIWIL3 (HIWI3)	–	–	–
PIWIL4 (HIWI2)	PIWIL4 (MIWI2)	Argonaute 3 (Ago3)	–

**Fig. 2. PIWI proteins.** (A) Domains of the PIWI protein. (B) PIWI homologs expressed in various model organisms.

(Ashe et al., 2012; Le Thomas et al., 2013; Shirayama et al., 2012; Sienski et al., 2012) and *de novo* DNA methylation (Aravin et al., 2008; Kuramochi-Miyagawa et al., 2008). Gene silencing at the posttranscriptional level often occurs through the cleavage of targeted mRNA transcripts by PIWI's endonuclease activity (De Fazio et al., 2011; Kim et al., 2018a; Kim et al., 2019; Reuter et al., 2011; Yu et al., 2019).

Given that one of the major roles of the piRNA pathway is the inactivation of transposable elements, impairment of this pathway can lead to overexpression of transposable elements, which may result in increased genome instability and thus germ cell defects and sterility. However, many animals produce piRNAs that do not target transposon sequences. For example, the vast majority of piRNAs produced in *C. elegans* do not match specific transposon sequences (Batista et al., 2008; Das et al., 2008; Wang and Reinke, 2008), and numerous piRNAs produced at the pachytene stage of murine spermatogenesis (i.e., piRNAs bound to PIWIL4) do not match transposon sequences (Li et al., 2013). The presence of these non-transposon-related piRNAs indicate the presence of additional targets and functions for the piRNA pathway that are independent of transposon silencing. In addition, several recent studies have demonstrated that the expression of PIWI and piRNAs is not limited to the germline, but instead occurs more broadly in other tissues (Rojas-Rios and Simonelig, 2018). Numerous recent studies have revealed that PIWI and piRNAs have non-gonadal somatic roles including embryonic patterning in fly (Rouget et al., 2010), metabolic homeostasis in the fly adult fat body (Jones et al., 2016) and stem cell self-renewal in the planarian neoblast (Kim et al., 2019; Reddien et al., 2005), as well as numerous roles in neuronal cells. This review focuses on the neuronal functions of the PIWI-piRNA pathway (Fig. 3) and the implication of its dysregulation in human neurological disorders. Readers are also recommended to consult several excellent recent reviews focusing on piRNA biology which include in depth descriptions of piRNA biogenesis and the mechanisms of piRNA-driven gene regulation (Czech et al., 2018; Ozata et al., 2019; Rojas-Rios and Simonelig, 2018; Weick and Miska, 2014).






Organism	Neuronal roles of the piRNA pathway	References
<i>C. elegans</i> 	Inhibition of axon regeneration following injury Promotion of foraging behavior (nictation) Regulation of transgenerational epigenetic inheritance	(Kim et al., 2018a) (Lee et al., 2017) (Moore et al., 2019; Posner et al., 2019)
<i>Drosophila</i> 	Regulation of genomic heterogeneity	(Perrat et al., 2013)
<i>Aplysia</i> 	Promotion of long-term facilitation	(Rajasehupathy et al., 2012)
Mouse 	Brain development Behavior and memory formation Schwann cell response following injury	(Lee et al., 2011a; Zhao et al., 2015) (Leighton et al., 2019; Nandi et al., 2016) (Sohn et al., 2019)
Rat 	Inhibition of axon regeneration following injury	(Phay et al., 2018)

Fig. 3. The roles of the piRNA pathway in neurons of various organisms.

## NEURONAL EXPRESSION OF PIWI AND piRNAs

PIWI proteins and piRNAs have been identified in various somatic tissues including neural cells, although it should be noted that their expression levels are significantly lower in these tissues than in the germline. A number of studies have detected the expression of piRNAs in the mammalian brain (Dharap et al., 2011; Ghosheh et al., 2016; Lee et al., 2011a; Nandi et al., 2016; Perera et al., 2019; Phay et al., 2018). In mouse brain tissues researchers have identified approximately 30,000 neuronal piRNAs, and interestingly, these neuronal piRNAs are most like PIWI2 (MIWI) associated piRNAs found in mouse testes (Ghosheh et al., 2016). These somatic piRNAs are shorter in length and tissue-specific, with increased occurrence of unique piRNAs in the hippocampus when compared to the germline (Perera et al., 2019). Hippocampal tissues showed the highest expression of piRNAs of any of the somatic tissues tested, followed by the brain cortex, kidney, and liver (Perera et al., 2019). These findings suggest that the role of neuronal piRNAs might be associated with neurogenesis and learning and memory.

## NEURONAL FUNCTIONS OF PIWI AND piRNAs

### Axon regeneration

Like other organisms, *C. elegans* piRNAs, PIWI/PRG-1, and other proteins involved in the piRNA pathway are abundant in the germline and their absence usually results in sterility (Batista et al., 2008; Wang and Reinke, 2008; Weick et al., 2014). My findings revealed a novel role for the neuronal piRNA pathway in axon regeneration, which acts in a cell-autonomous manner despite the extremely low expression of PIWI and other piRNA components (Kim et al., 2018a). A number of groups have done large-scale genetic screens to identify the regulators of axon regeneration in *C. elegans* (Chen et al., 2011; Hammarlund et al., 2009; Kim et al., 2018b; Nix et al., 2014) and it was one of these screens that

revealed the inhibitory role of the piRNA pathway in peripheral axon regeneration (Kim et al., 2018a). Loss-of-function mutants in a subset of the components from the piRNA pathway result in enhanced axon regrowth in adult animals. Notably, components of the piRNA transcription and maturation step, but not the nuclear transcriptional silencing step, are required for the inhibition of axon regeneration (Kim et al., 2018a). In addition, the endonuclease activity of PIWI was found to be critical in its inhibitory role in axon regeneration. Together, these results suggest that the neuronal piRNA pathway can inhibit axon regeneration via the elimination of target mRNAs using PIWI's endonucleolytic cleavage ('slicing') in the cytoplasm rather than nuclear silencing.

Importantly, this inhibitory role in axon regeneration seems to be evolutionarily conserved as a reduction in a PIWI-like protein in cultured adult rat sensory neurons increases axonal regrowth after injury (Phay et al., 2018). In this study, PIWI1 (MIWI) was detected in rat nervous tissue lysates, and its knockdown enhanced axon regrowth in cultured peripheral neurons after axonal injury. In addition, the authors also detected several neuronal piRNAs in rat sciatic nerve axoplasm and found that many of them are differentially expressed after nerve injury (e.g., piR-1199) (Phay et al., 2018). Another independent study in adult rat brains showed that numerous piRNAs are differentially expressed following transient focal ischemia in the rat cerebral cortex (Dharap et al., 2011).

Furthermore, PIWI1 (MIWI) is expressed in the mouse Schwann cell and the expression was dramatically reduced following sciatic nerve injury (Sohn et al., 2019). Additionally, thousands of piRNAs were differentially expressed following nerve injury in this model, and one piRNA (piR 009614) even enhanced the migration of Schwann cells.

Together, these nematode and rodent studies suggest that the piRNA pathway may contribute to neuron or Schwann cell responses during peripheral nerve injury. Further studies should be done to determine the exact mechanisms behind PIWI and piRNA mediated axon regeneration and regen-

eration as well as to elucidate the specific piRNAs and their targets which regulate these neuronal responses after nerve injury.

### Foraging behavior

Another likely role for PIWI and piRNAs in the neuron has been described in *C. elegans*. Under unfavorable environmental conditions, nematodes can undergo an alternative developmental stage called the 'dauer' or 'diapause' stage (Cassada and Russell, 1975; Hu, 2007). Dauers are developmentally arrested and non-feeding, but they are motile and explore the environment in search of food (Cassada and Russell, 1975). Sometimes the dauer stage nematodes exhibit a unique behavior called nictation in which a worm stands on its tail with its head swaying in the air. This behavior seems to enable dauers to interact with motile animals and allow it to be carried to new environments. Nictation is regulated by a specific set of cholinergic neurons in *C. elegans* (Lee et al., 2011b). Junho Lee's group at Seoul National University undertook a quantitative trait locus study and identified a specific locus containing a piRNA cluster that is responsible for the nictation behavior (Lee et al., 2017). In addition to the presence of this piRNA cluster, they also found that PIWI/PRG-1 is required for the regulation of nictation (Lee et al., 2017).

### Learning and memory

In *Aplysia*, the neuronal piRNA pathway regulates memory-related synaptic plasticity. Eric R. Kandel's group at Columbia University generated small RNA libraries from *Aplysia* central nervous system neurons and found unexpectedly abundant expression of piRNAs (Rajasekharan et al., 2012). Knockdown of PIWI or a certain piRNA (i.e., piR-F) was sufficient to impair long-term facilitation, which is a form of neural plasticity and memory. The authors showed that PIWI/piRNA complexes facilitate serotonin-dependent methylation in the CREB2 promoter region (CREB-2 is an inhibitory gene in *Aplysia* memory formation; Bartsch et al., 1995), leading to enhanced long-term synaptic facilitation (Rajasekharan et al., 2012).

Another recent study selectively disrupted PIWI proteins by simultaneous knockdown of two *piwi* genes PIWIL1 (MIWI) and PIWIL2 (MILI) in mice hippocampal tissues and found enhanced contextual fear memory in these mice (Leighton et al., 2019). In addition, selective hippocampal knockdown of PIWIL2 (MILI) in mice resulted in hyperactivity, while there was no observable phenotype for PIWIL1 (MIWI) disruption (Leighton et al., 2019). Consistent with these findings, Nandi et al. (2016) showed that *Piwil2* knockout in mice resulted in hyperactivity. These results suggest that the hippocampus of adult mice is sufficient to regulate hyperactivity and that this regulation is mediated by the piRNA pathway.

### Transgenerational epigenetic inheritance

Transgenerational epigenetic inheritance (TEI) refers to the phenomenon whereby epigenetic information is preserved in multiple generations even when the initiating environment or genetic event is no longer present (Boskovic and Rando, 2018). Environmental challenges, including high temperature

(Casier et al., 2019b), starvation (Rechavi et al., 2014), and osmotic stress (Burton et al., 2017), can produce responses that are maintained across generations. In addition, researchers have shown that acquired memory events like traumatic stress (Gapp et al., 2014) and conditioned fear responses (Dias and Ressler, 2014) can also be inherited for up to two generations in mice.

Although the molecular and cellular mechanisms underlying TEI are largely unknown, several mechanisms have been identified as mediators of epigenetic inheritance, including DNA methylation, histone modification, and small RNAs (i.e., siRNAs and piRNAs) (Boskovic and Rando, 2018; Casier et al., 2019a). piRNAs have been shown to affect the epigenetic inheritance via the induction of paramutations in the heritable RNA interference (RNAi) pathway (Ashe et al., 2012; de Vanssay et al., 2012; Hourri-Ze'evi et al., 2016; Sapetschnig et al., 2015; Shirayama et al., 2012). In the germline of *C. elegans*, piRNAs have been shown to initiate stable, heritable epigenetic silencing (Ashe et al., 2012; Shirayama et al., 2012). However, in the inheritance of somatic phenotypes, including acquired behavior, the heritable information needs to be passed from somatic cells to germ cells in order to be transmitted to the next generation. Here, this review will summarize two recently published *C. elegans* studies that describe transgenerational inheritance of behaviors and address how neuronal small RNAs control behavioral changes in response to environmental cues (Moore et al., 2019; Posner et al., 2019).

*C. elegans* is initially attracted to pathogenic *Pseudomonas aeruginosa*, but, within hours of exposure, the nematode learns to avoid it (Moore et al., 2019). Moore et al. (2019) found that worms not only learn to avoid this pathogen but they can pass on this avoidance behavior to their offspring without their prior contact with the bacterium. Interestingly, they found that these worms passed this behavior down to their progeny for up to four generations, which confers an obvious survival advantage. In order to sense and avoid this pathogenic bacterial species, active engagement from the nervous system is required (i.e., induction of *daf-7/TGF- $\beta$*  ligand in ASI sensory neurons) (Moore et al., 2019).

Strikingly, *C. elegans* PIWI/PRG-1 is specifically required for the inheritance of this learned pathogenic avoidance behavior. Mutants of *piwi* show normal pathogenic learning after *Pseudomonas* training, but the progeny of trained *piwi* mothers were defective in their avoidance of *Pseudomonas*. In *piwi* mutants, the activity of the responsible neuron was also abolished (i.e., no induction of *daf-7/TGF- $\beta$*  ligand expression in ASI neurons). In addition, exposure to *Pseudomonas* induced changes in the expression levels of a large group of piRNAs and several miRNAs. Thus, *C. elegans* PIWI is likely required to mediate the altered gene expression profiles in the offspring's neurons needed to mediate the transgenerational inheritance of this adaptive behavior. Additionally, the endogenous siRNAs pathway downstream of PIWI/PRG-1 mirrored these results. RNA dependent RNA polymerase (RdRP), which synthesizes secondary siRNAs, and nuclear RNAi, which mediates chromatin modification, were also required for the transgenerational inheritance of pathogenic learning (Moore et al., 2019).

Another groundbreaking discovery by [Posner et al. \(2019\)](#) provided insights into the small RNA-based mechanism of how the nervous system communicates with the germline to affect animal behavior transgenerationally. The authors first identified neuronal small RNAs and focused their research on the endo-siRNAs produced in an RDE-4 (RNAi deficient-4) dependent manner (RDE-4 is a double-stranded RNA-binding protein required for the biogenesis of certain small RNAs including piRNAs ([Tabara et al., 2002](#)). Neuronal RDE-4 expression induces neuron-specific RDE-4-dependent small RNA expression, and leads to changes in germline amplified small RNAs and gene expression for multiple generations. They tested one such germline gene, *saeg-2*, and showed that neuron-specific expression of RDE-4 resulted in the downregulation of *saeg-2* in the germline, and that this change was also maintained in a number of progeny generations. Moreover, the chemotaxis defect of *rde-4* mutants can be partially rescued by supplementation with neuronal RDE-4 for at least three generations, likely by controlling the activity of germline small RNAs and their regulation of germline genes ([Posner et al., 2019](#)). Indeed, silencing *saeg-2* leads to partial rescues of the chemotaxis defect of *rde-4* mutants. Together, [Posner et al. \(2019\)](#) demonstrated that small RNAs in the nervous system regulate germline genes allowing modification of specific behaviors in multiple generations. These findings suggest that small RNAs, particularly piRNAs, play a critical role in the TEI of learned behavior, which appears to strengthen the animal's survival. One of the proposed mechanisms for the transmission of learned behaviors in progeny animals is the physical transport of small RNAs from neurons to germ cells resulting in heritable genetic changes. However, this still needs to be tested and verified.

## piRNA DYSREGULATION AND NEUROLOGICAL DISEASES

### Neurodevelopmental disorders

Transposable elements can replicate and insert themselves into new genomic locations. This feature contributes significantly to the evolution of genomes but can also result in DNA breaks and illegitimate recombination, and therefore poses a significant threat to genomic integrity ([Belancio et al., 2008](#)). Excessive damage to the germline genome results in sterility. Thus, the piRNA pathway operates in the germline to control the activity of transposable elements.

The PIWI-piRNA pathway plays a role in brain development, and its regulation has emerged as a key factor in the development of various neurological disorders. In rodents, PIWIL1 (MIWI) is expressed in multiple brain tissues, including the hippocampus and cortex, and controls dendritic spine development and morphogenesis ([Lee et al., 2011a](#); [Zhao et al., 2015](#)). In addition, PIWIL1 (MIWI) also functions in promoting neuronal polarization and radial migration during neurogenesis ([Zhao et al., 2015](#)). In humans, active transposition occurs during neurogenesis, which provides genomic diversity between neurons ([Bodea et al., 2018](#); [Erwin et al., 2014](#)). Transposition-driven genomic heterogeneity is also observed in the brains of *Drosophila* ([Perrat et al., 2013](#)). Fly transposition predominantly occurs in memory-relevant

neurons within the mushroom body, which is a critical region for learning and memory, similar to the mammalian hippocampus. Intriguingly, a lack of PIWI proteins, Aubergine and Argonaute 3, correlates with elevated transposon expression in these neurons ([Perrat et al., 2013](#)). This suggests that PIWI/piRNA-mediated suppression of transposons may be an evolutionarily conserved mechanism that contributes to differential transposition rates in brain regions involved in learning and memory. Permitting transpositions may contribute to behavioral variability between individuals in the population, but accumulation of disruptive transpositions throughout life may also contribute to neural decline and the pathogenesis of various neurological diseases ([Erwin et al., 2014](#)).

In fact, *de novo* mutations of PIWIL2 (MILI) and PIWIL4 (MIWI2) significantly correlate in children with autism spectrum disorders from simplex families ([Iossifov et al., 2014](#)). piRNA dysregulation has also been proposed as the underlying mechanism in a severe neurodevelopmental disorder, Rett Syndrome (RTT). Mutations in or altered expression of the methyl-CpG binding protein 2 (MeCP2) is a well-known cause of Rett Syndrome ([Amir and Zoghbi, 2000](#); [Amir et al., 1999](#)). [Saxena et al. \(2012\)](#) found that global piRNA levels are higher in MeCP2 knockout mouse cerebellum tissues. It is worth noting that the neuronal activity of transposable element LINE-1 is increased with decreasing MeCP2 activity ([Muotri et al., 2010](#)). Therefore, Rett patients carrying MeCP2 mutations have increased susceptibility to transposition, which may plausibly be the result of piRNA dysregulation.

### Neurodegenerative disorders

Growing evidence suggests that the dysregulation of the piRNA pathways results in genomic instability of neurons leading to the development of various neurodegenerative disorders, including amyotrophic lateral sclerosis (ALS) and tauopathies. Abnormal expression of PIWI has been linked to the pathogenesis of ALS which primarily affects motor neurons. Mutations in RNA-binding proteins, including FUS and TDP-43, have been identified as causative events in ALS ([Lagier-Tourenne and Cleveland, 2009](#); [Zhao et al., 2018](#)). In a *Drosophila* ALS model (i.e., FUS knockdown), pre-piRNAs are atypically induced and translocated, eventually accumulating in the cytoplasm of the affected neurons. This aberrant expression appears to be mediated by fly PIWI homolog, Aubergine ([Wakisaka et al., 2019](#)).

Tauopathies refer to neurodegenerative diseases linked with the pathological aggregation of the Tau protein in neurofibrillary tangles in the brain. One of the potential pathological mechanisms of tauopathy is related to PIWI/piRNA regulation ([Sun et al., 2018](#)). It has been demonstrated that pathogenic Tau aggregation-induced decondensation of heterochromatin and reduction in PIWI and piRNAs activity results in transposable element dysregulation, causing neuronal cell death in these neurodegenerative tauopathies ([Frost et al., 2014](#)). In addition, numerous piRNAs were found to be up-regulated in human brain samples from Alzheimer's disease patients and many of the predicted mRNA targets of such piRNAs were significantly downregulated ([Qiu et al., 2017](#); [Roy et al., 2017](#)). This makes piRNA regulation a potentially interesting area for the development of novel therapeutic

strategies for the treatment of neurodegenerative diseases.

## CONCLUSIONS AND FUTURE PERSPECTIVES

Mounting evidence challenges the notion that PIWI proteins and piRNAs function solely to regulate the activity of transposable elements in the germline. This pathway has now been implicated in the regulation of the expression of endogenous genes in various somatic cells, including neurons. The PIWI-piRNA pathway has been shown to be involved in various neuronal events, including brain development, genomic heterogeneity, neuronal response following injury, behavior, memory formation, and TEI of acquired traits. Dysregulation of the PIWI-piRNA pathway has been implicated in the pathology of various neurological disorders, including neurodevelopmental and neurodegenerative diseases. In many cases, the comprehensive molecular mechanisms underlying activity and regulation of the neuronal piRNA pathway remain largely unknown. Future research will need to evaluate regulatory targets of the piRNA pathway in order to understand its contribution to neuronal gene regulation. Although manipulation or analysis of numerous piRNA sequences remains a technological challenge, rapidly growing genome editing and genome-wide technologies are likely to contribute to our future understanding of the neuronal piRNA pathway. We are just beginning to untangle the influence of this regulatory pathway in the neurons. Understanding the precise mechanisms employed by this pathway should help develop specific piRNAs based therapeutic strategies that could be used in the treatment or diagnosis of various neurological disorders.

### Disclosure

The author has no potential conflicts of interest to disclose.

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### ORCID

Kyung Won Kim <https://orcid.org/0000-0002-8252-6203>

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