

Forum

The potential role of the mobile and non-coding genomes in adaptive response

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The tightly regulated feedback loops linking small RNAs (sRNAs) and transposable elements (TEs) offer the opportunity for an adaptive response to changing environments at the molecular level. Environmentally induced changes in TE and sRNA profiles may affect expression of coding genes and trigger an organismic and trans-generational response. Understanding this link may provide a mechanistic explanation for how species can adapt to changing climates and may offer novel molecular targets for biomedical and agricultural applications.

Introduction

As our environment is changing, it is pivotal to improve our understanding of the organismal response at all levels. Environmental disturbances can trigger mobilisation of TEs in the genome where TE insertions introduce *de novo* mutations and possibly cause a change in sRNA expression [1]. Such a response has the potential to generate feedback loops (Figure 1) that could lead to phenotypic plasticity and rapid adaptation and potentially affect organismal fitness by altering gene expression [2]. Such a mechanism also offers potential targets for species management, conservation, and biomedical applications (Table 1).

PIWI interacting RNAs and TEs: germline guardians and disruptors

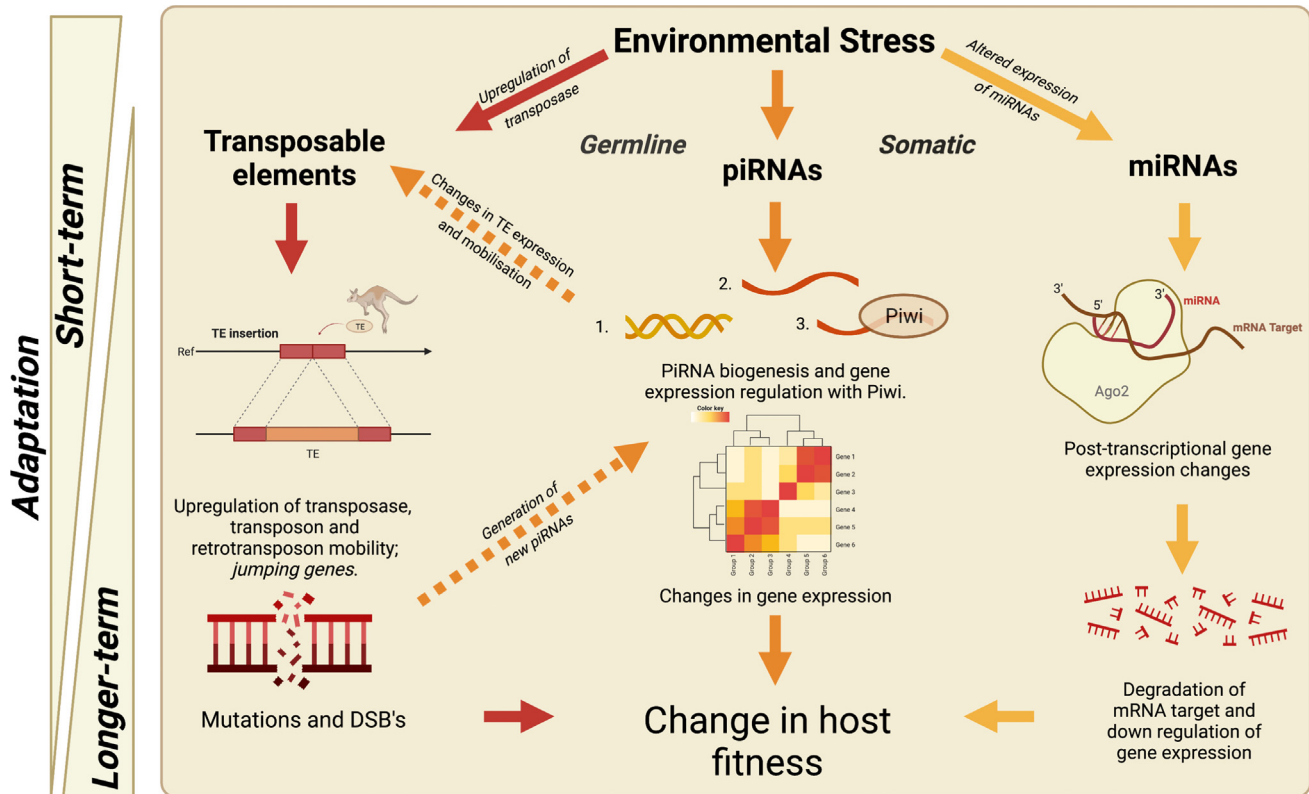
PIWI-interacting RNAs (piRNAs) are short, 24–30 nucleotide molecules, and their expression can be induced by TE insertions (Figure 1), which can affect gene expression and cause the possible disruption of the surrounding gene expression. Additionally, piRNAs can affect mRNA expression at the post-transcriptional level through partial complementarity. piRNAs have been found to play a pivotal role in the development of testes and ovaries and a deregulation of piRNA gene silencing mechanisms can lead to sterility and prevent successful reproduction. A knockout of the piRNA processing protein Ziwi (Zebrafish piwi homologue) for example results in the lack of germ cell differentiation and a silencing of the germ line [3]. Furthermore, destabilisation of TEs can affect trans-generational fitness through increasing genomic instability caused by varying levels of transposon transcripts [4].

piRNAs protect the animal germline through silencing of TEs and prevent integration of TEs into the germline genome through cooperation with small interfering RNAs (siRNA) [2]. Miniature Inverted Repeat Transposable Element (MITE) regions for example are often located near genic regions and can putatively affect gene expression through sRNA-mediated epigenetic mechanisms [5]. The composition of TEs in the genome varies widely across taxa and may range from approximately 10% in the *Arabidopsis thaliana* genome to over 80% in the maize cultivar *Zea mays* in plants. In animals, TEs comprise 37% of the genome in mouse *Mus musculus*, 46% in humans, and >50% in zebrafish *Danio rerio* [6]. The presence of TEs in a wide range of taxa highlights the potential for conserved mechanisms across kingdoms for TE regulation. TEs are incredibly diverse and can exert an effect on gene expression through mobilisation and insertion near to or into a gene, generating mutations that can be deleterious [1,7].

piRNAs can bind to TE-derived sRNAs and interfere at the transcriptional and post-transcriptional levels to silence TE activity [3,8,9]. Heat stress in *Caenorhabditis elegans* for example induces the mobilisation of Tc1/*mariner* TEs causing DNA double-strand breaks because of elevated Tc transposase expression and transposon excision [9]. These DNA breaks and mutations caused by TE invasion can change sRNA profiles by generation of new piRNA clusters and provide a potential adaptive stress–response feedback loop [2,6,9,10].

Heat stress is known to affect piRNA biogenesis and related signalling [11]. Genes that were upregulated in a heat treatment showed a significant overlap with gene expression in *prde-1* mutant *C. elegans* lacking most piRNAs grown at 20°C. The changes in gene expression caused by heat stress could therefore reflect reduction in piRNA biogenesis leading to reduced piRNA-mRNA mediated gene silencing and result in the upregulation of specific genes. The fitness of progeny exposed to higher temperatures was affected for up to three subsequent generations after last heat exposure [11]. *C. elegans* housed in a higher-temperature environment saw a partial rescue in motif-dependent piRNA activity when also infected with pathogenic bacteria. Both temperature and bacterial modifications led to persisting changes in gene expression even when the environmental stimulus was returned to the normal state. Studying the genes directly targeted by piRNAs in response to environmental change is a promising avenue to improve our understanding of the underlying mechanisms.

Another effect of heat stress induced *mariner* transposon activity in *C. elegans* is the induction of double-strand DNA breaks during the prophase of meiotic cell divisions, which are propagated by recombination repair mechanisms and result in decreased male fertility [9]. These genomic disruptions were caused by *Tc1/mariner*



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Figure 1. Overview of the interplay between transposable elements (TEs), PIWI interacting RNAs (piRNAs), and miRNAs in gene regulation and its potential role in adaptive responses to environmental stress. TE response can lead to mutations through double-strand breaks (DSBs) and can sometimes lead to generation of new piRNA clusters. piRNAs regulate TEs through silencing mechanisms. Both piRNAs and miRNAs can regulate gene expression through post-transcriptional silencing. Schematic created with BioRender.com.

TEs, which were more common in spermatocytes than ovaries, with 25-fold increase in double-strand breaks seen in heat-stressed males. The environmental triggers of TE excision and why spermatocytes are more vulnerable warrants further investigation.

Failure of silencing mechanisms to protect the genome leads to reactivation of TE neighbouring genes in the genome. This reactivation can be triggered by environmental changes including pollution, radiation, thermal, and endocrine disruption, and lead to higher mutation rates and wider genetic variation [12]. TE mutagenesis can lead to accelerated evolution through chromosomal rearrangement and epigenetic

changes. Heat-shock is known to activate retrotransposons in *Drosophila*, where mild heat stress causes increased activity of the transposase gene and transposon *mariner-Mos1*, a gene that contains heat shock elements, in somatic cells. Some piRNA pathways are known to be heat sensitive, particularly *mariner* TE pathways [9]. This is important as heat shock proteins have been shown to be activated following heat stress and trigger mobilisation of *mariner-mos1* transposons [1].

In response to environmental stress, many piRNA pathway factors are rapidly evolving. In *Drosophila* for example, the rapidly evolving protein *Cutoff (Cuff)* plays a putative role in modulating piRNA precursor

transcription and in turn activation of transposons precursors and trigger adaptive responses to long-term environmental stress [10]. C-terminal binding proteins (CtBPs) suppress canonical transcription of transposons and piRNAs. When *Cuff* forms stable complexes with CtBPs it inhibits the action of CtBPs and reduces noncanonical transcription and activates canonical transcription thus balancing CtBP activity. This balance could be affected by environmental stress induced TE mobilisation and supports the idea that the interaction and combined activation of sRNA- and TE-involving mechanisms lead to (potentially adaptive) responses to environmental changes (Figure 1).

Table 1. Overview of environmental impact on TE and sRNA expression across the animal and plant kingdom. The examples in this table highlight how piRNA, TE and miRNA regulation and genome disruption can have applications for species management and disease research, e.g. in agriculture (*Z. mays*), warming water bodies (*M. reevesii* and *D. rerio*), diet (*Drosophila melanogaster*), disease and mutagenesis potential (*Homo sapiens* and *C. elegans*) and conservation opportunities.

Species	Gene/sRNA/TE	Function/role	Potential applications	Refs
<i>D. rerio</i>	Zwi1	piRNA processing	Regulation of TEs	[3]
<i>D. melanogaster</i>	Mariner-Mos1	TE, transposase gene Activated by heat-shock	Induction of mutations	[1]
<i>C. elegans</i>	Tcf1/mariner	TE	Induction of DNA double-strand breaks	[9]
<i>Z. mays</i>	Helitron DNA TE	TE, gene capture	Gene duplication	[6]
<i>H. sapiens</i>	Alu SINE retrotransposon	TE, retrotransposon Causes haemophilia by disrupting exon 14 of human factor VIII gene	Germline mutations Exon interruption	
<i>H. sapiens</i>	L1 retrotransposon	TE, retrotransposon Causes colon cancer by disruption of APC gene	Somatic mutations Exon interruption	
<i>D. melanogaster</i>	miR-9a-5p	miRNA social environment and sex-bias ratio dependent expression	Species conservation, potential in sperm competition	[14]
	miR-9b-5p	miRNA, enriched in male		
	miR-278-3p	miRNA, enriched in male		
	miR-970-3p	miRNA, enriched in male		
<i>M. reevesii</i>	Sox9	Sex-related; testis	Species management and sex-determination	[15,16]
	DMRT1	Sex-related; testis. Target of miR-200a-3p		
	BMP7	Sex-related; testis		
	AMH	Sex-related; ovary		
	miR-200a-3p	Sex-related; downregulated in males	Species management, hormone regulation. and sex-determination	
	miR-138-5p	Sex-related. Targets AMH gene; steroid hormone antagonist		

miRNAs and germ cell development in changing environments

Another family of sRNAs are micro RNAs (miRNAs) – short 18–23 nucleotide molecules generated through cleavage of precursors. Mature miRNAs can complementarily bind to mRNA transcripts at the 3' UTR and regulate gene expression at the post-transcriptional stages [13]. miRNA expression has been shown to be affected by environmental stressors leading to alterations in gene expression profiles. In *Drosophila* for example, miRNA profiles in sperm collected from males from long-term selection lines exposed to varying sex ratios (equal sex ratio, male bias, or female bias for 38 generations) varied by 11 differentially expressed miRNA [13]. Notably, *miR-9b-5p* – an miRNA that is enriched in male primordial germ cells only – was reduced in the sperm from

males sampled from female-biased lines compared to males from male-biased lines [14]. Whether these differentially expressed miRNAs affect the following generations is currently unclear and warrants further study.

Another example of a changing environment affecting miRNA regulation is temperature-dependent sex-determination (TSD). TSD occurs during embryo development and environmental factors such as temperature can affect the ratio of male and female offspring. The specific regulatory mechanisms for TSD are unclear, but miRNAs are one putative mechanism involved through the regulation of hormone expression [15,16]. In Reeve's Pond turtles *Mauremys reevesii*, 60 miRNAs are differentially expressed in ovaries and testes during sexual maturation [16]. The genes targeted

by the differentially expressed miRNAs include *Sox9*, *DMRT1*, and *BMP7*, which are sex-related and expressed in testes. *DMRT1* is expressed in male testes and is a predicted target of *mre-miR-200a-3p*, which is downregulated in males. In females, *mre-miR-138-5p* is upregulated in ovarian tissues and is predicted to target the gene *AMH*, which in mammals is absent in male testes and present in ovaries. In contrast, Reeve's Pond turtles have high levels of *AMH* in testes, and low levels in ovaries. This is confirmed by the finding of at total 1594 differentially expressed genes between adult testes and ovaries with nine highly differentially expressed including *AMH* [15]. The gene expression data further suggests that hormone synthesis and gametogenesis in Reeve's Pond turtles are directly affected in TSD and that miRNAs are antagonistic regulators of

steroid hormones, keeping a balance between male and female sex determination pathways [15,16]. However, it is currently unclear what exactly leads to the differential expression of miRNAs in the first place. Given that the environment has putative effects on miRNA expression, miRNAs may be acting in feedback loops where environmental triggers regulate gene expression of key hormone and metabolic pathways to initiate shorter- and longer-term adaptive responses and alterations.

Concluding remarks

Climate change is strongly selecting for adaptive evolutionary responses in the genome. The non-coding regions of the genome may be playing a key role in such adaptive responses, providing adaptive *ad hoc* fitness advantages in some instances, but potential costs in others. The combined analysis of sRNA and TE profiles in environmentally stressed model organisms will provide key insights about their interactions and may uncover opportunities for biomedical and technological advances by revealing sRNA targets for sex-determination genes and regulatory mechanisms in the germline. This could

lead to improved tools for conservation and ecology but also agriculture and stock breeding.

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Declaration of interests

No conflicts of interest are declared.

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