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

Alice M. Godden1,* and Simone Immler1,*

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 Zwi (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 a piRNA 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...
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-involved mechanisms lead to (potentially adaptive) responses to environmental changes (Figure 1).

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.
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.

Acknowledgments
This manuscript was supported by funding from the Natural Environment Research Council (NE/S011188/1) and the European Research Council (SELECTHAPLOID - 101001341) to SI.

Declaration of interests
No conflicts of interest are declared.

References
1. Jardim, S.S. et al. (2015) Effects of heat and UV radiation on the mobilization of transposon mariner-Mos1. Cell Stress Chaperones 20, 843–851
2. Shpir, S. et al. (2014) Euchromatic transposon insertions trigger production of novel pi- andendo-siRNAs at the target sites in the Drosophila germline. PLoS Genet. 10, e1004358
3. Houwing, S. et al. (2007) A role for Pwi and piRNAs in germ cell maintenance and transposon silencing in Zebrafish. Cell 129, 69–82
4. Spichal, M. et al. (2021) Germ granule dysfunction is a hallmark and mirror of Pwi mutant sterility. Nat. Commun. 12, 1420
5. Wei, L. et al. (2014) Dicer-like 3 produces transposable element-associated 24-nt sRNAs that control agricultural traits in rice. Proc. Natl. Acad. Sci. U. S. A. 111, 9877–9882
6. Huang, C.R. et al. (2012) Active transposition in genomes. Annu. Rev. Genet. 46, 651–675
7. Reich, G.E. et al. (2019) Stress response, behavior, and development are shaped by transposable element-induced mutations in Drosophila. PLoS Genet. 15, e1007900
8. Brennecke, J. et al. (2007) Discrete small RNA-generating loci as master regulators of transposon activity in Drosophila. Cell 128, 1099–1103
9. Kuharewicz, N.A. et al. (2020) Elevated temperatures cause transposon-associated DNA damage in C. elegans spermatocytes. Curr. Biol. 30, 5007–5017 e4
10. Patward, S.S. et al. (2020) Adaptive evolution targets a piRNA precursor transcription network. Cell Rep. 30, 2672–2685 e6
11. Bélanger, T. et al. (2018) The piRNA pathway responds to environmental signals to establish intergenerational adaptation to stress. BMC Biol. 16, 103
12. Wells, J.N. and Feschiotte, C. (2020) A field guide to eukaryotic transposable elements. Annu. Rev. Genet. 54, 539–551
13. Hayashi, K. et al. (2008) MicroRNA biogenesis is required for mouse primordial germ cell development and spermatogenesis. PLoS One 3, e1738
14. Hotzy, C. et al. (2021) Evolutionary history of sexual selection affects microRNA profiles in Drosophila sperm. Evolution 75, 310–319
15. Xiong, L. et al. (2019) Transcriptome sequencing and comparative analysis of adult ovary and testis identify potential gonadal maintenance-related genes in Mauremys reevesii with temperature-dependent sex determination. PeerJ 7, e6557
16. Xiong, L. et al. (2020) Comparison of adult tests and ovary microRNA expression profiles in Reeves’ Pond turtles (Mauremys reevesii) with temperature-dependent sex determination. Front. Genet. 11, 133