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Genome Defense Against Transposable Elements and the Origins of Regulatory RNA

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Abstract Under selective pressure to contain the harmful effects of transposition, genomes have evolved multiple RNA-based mechanisms for regulating transposable elements (TEs). In this chapter, we describe a number of examples of RNA-based TE defense mechanisms. Once established, these RNA-mediated TE silencing mechanisms, such as RNA interference by miRNAs, may come to be used to regulate host genes. It is becoming possible to reconstruct evolutionary transitions demonstrating how specific TE defense mechanisms were co-opted to provide additional regulatory complexity for host genes. For instance, we have recently shown how miRNAs may have evolved from siRNA encoding TEs. Here we propose another specific model, the transcript infection model, whereby TE insertion dynamics can couple RNA-mediated repression mechanisms to the regulation of host genes.

Abbreviations

dsRNA	Double-stranded RNA
miRNA	MicroRNA
MITE	Miniature inverted repeat transposable element
piRNA	Piwi-interfering RNA
PTGS	Post-transcriptional gene silencing
rasiRNA	Repeat-associated small interfering RNA
RISC	RNA-induced silencing complex
RNAi	RNA interference
siRNA	Small interfering RNA
TAS	Telomeric associated sequence
TE	Transposable element
TIR	Terminal inverted repeat
UTR	Untranslated leader region

1 The Ascent of Regulatory RNA

Two competing epistemologies aim to explain the origins of fundamental changes in scientific thought. Karl Popper was a champion of the principle of

falsification, whereby any observation inconsistent with a theory necessitates immediate rejection of that theory (Popper 1959). For Popper, falsifiability distinguished science from non-science. Thomas Kuhn, on the other hand, held that established theories only give way under the weight of an accumulation of multiple anomalies that cannot be explained through the accepted world-view (Kuhn 1962). When this occurs, a paradigm shift ensues resulting in both a new theory and a radically altered world-view. The realization that RNA molecules play a fundamental role in regulating eukaryotic gene expression represents such a paradigm shift in biology (Britten and Davidson 1969). In fact, it took no fewer than three independent discoveries, each in a different phylum, before the broader implications of RNA-mediated gene silencing were fully appreciated and biologists were finally able to accommodate RNA as a regulatory agent alongside protein factors of the classic cis-trans gene regulation model.

RNA-mediated gene silencing was first discovered when botanists working with transgenic plants began to notice a number of confusing gene silencing phenomena (reviewed in Matzke and Matzke 2004). What was then called "cosuppression" was found to occur when plant transgenes involved in pigment synthesis were over-expressed, resulting in silencing of both the transgenes and the homologous plant genes (Napoli, Lemieux and Jorgensen 1990; van der Krol, Mur, Beld et al. 1990). Cosuppression was later determined to occur post-transcriptionally, and while the mechanism underlying these gene silencing events remained unclear, it seemed to be related to sequence interactions that occurred between transgenes with similar (or identical) sequences and/or between transgenes and related plant genes (de Carvalho, Gheysen, Kushnir et al. 1992; van Blokland, van der Geest, Mol et al. 1994). Models of what became known as post-transcriptional gene silencing (PTGS) in plants first articulated the connection between gene silencing and 1-RNA-dependent RNA polymerase, 2-small RNA species and 3-double-stranded RNA (dsRNA) (Lindbo, Silva-Rosales, Proebsting et al. 1993). A few years later, the connection between plant PTGS and sequence-specific RNA-mediated endonucleolytic degradation of mRNA was more definitively established (Metzlaff, O'Dell, Cluster et al. 1997).

In the mean time, a similar transgene induced gene silencing phenomenon, dubbed "quelling", was observed for the fungus *Neurospora crassa* (Romano and Macino 1992). As was seen for plants, exogenous *Neurospora* pigment genes were found to be silenced by homologous transgenes, and the effect appeared to be copy-number dependent. Ultimately, a quelling-defective mutant phenotype (Cogoni and Macino 1997) was shown to be linked to an RNA-dependent RNA polymerase gene (Cogoni and Macino 1999), confirming more speculative models as to the mechanism of RNA-mediated gene silencing.

Finally, observations of complementary antisense RNA sequences pointed to a possible regulatory role for RNA in animals (Lankenau, Corces and

Lankenau 1994), and later RNA-mediated gene silencing was definitively proven in the flatworm *Caenorhabditis elegans*, where it was called RNA interference (RNAi) (Fire, Xu, Montgomery et al. 1998). A major contribution of this work was the demonstration of the role of dsRNA in sequence-specific gene silencing. Fire et al. also showed that a tiny amount of dsRNA was sufficient to cause RNAi, pointing to the likely involvement of amplification in the process. So while the plant and fungal efforts came first, the subsequent *C. elegans* work both unified the understanding of PTGS, quelling and RNAi, and much like Kuhn may have predicted, served as the tipping point after which the fundamental understanding of how eukaryotic genes are regulated was forever changed.

2
RNAi and Genome Defense

Early work on PTGS in plants was also critical in establishing the connection between RNA-mediated gene silencing and genome defense against invading genetic elements. In fact, not long after PTGS was discovered, it became clear that viral sequences could both initiate, and be subject to, PTGS. This was revealed when tobacco plants expressing a viral coat protein encoding transgene recovered from infection with the virus and then became resistant to subsequent viral infection (Lindbo, Silva-Rosales, Proebsting et al. 1993). In addition to virus transgene stimulation of PTGS, plants were also shown to use PTGS as a defense against naturally occurring viral infections (Covey, Al-Kaff, Lángara et al. 1997; Ratcliff, Harrison and Baulcombe 1997). Together, these data led to the notion that a number of gene silencing mechanisms may have originally evolved as genome defense mechanisms that could guard against the harmful effects of viral infection and/or transposition (Matzke, Mette and Matzke 2000). Once these global regulatory mechanisms were established, they could have been co-opted by the host to add an additional layer of regulatory complexity for its own genes. For instance, if an invading genetic element integrated in the flanking region of a host gene, it could change the expression of the adjacent gene as the element sequence was acted on by various host repression mechanisms (Matzke, Mette and Matzke 2000). In addition, the machinery that evolved to defend against invading genetic elements could just as easily be used to regulate host genes, as we believe to be the case for microRNAs (Piriyapongsa, Marino-Ramirez and Jordan 2007). We elaborate on these scenarios and make specific predictions on the insertion patterns and regulatory effects of mobile genetic elements in the section outlining our "transcript infection" model.

Shortly after the role of dsRNA in RNAi was uncovered in *C. elegans*, a connection was made between RNA-mediated gene silencing and trans-

posable elements (TEs). In *C. elegans*, RNAi was initially related to repression of TEs when RNAi deficient mutants were shown to lose the ability to repress Tc1 elements in the germline (Ketting, Haverkamp, van Luenen et al. 1999; Tabara, Sarkissian, Kelly et al. 1999). The specific mechanism underlying Tc1 silencing by RNAi was related to the presence of dsRNAs that are formed when terminal inverted repeat (TIR) sequences at the ends of the elements base pair with each other (Sijen and Plasterk 2003). This base pairing occurs when full-length Tc1 elements are expressed as RNA; since the TIR sequences at the ends of the element are complementary to each other in the single stranded RNA molecule, they can fold into "snap-back" structures forming dsRNA (Fig. 1). The bound TIR dsRNA sequences are cleaved by the RNAi endonucleases to yield short interfering RNAs (siRNAs) that silence element expression via sequence-specific degradation of complementary Tc1 mRNAs, to which they bind. siRNAs encoded by the *MuDR* family of maize elements were also shown to repress TE activity of the same family of elements via specific targeted mRNA degradation (Slotkin, Freeling and Lisch 2005). Thus, TE sequences are both the initiators and the targets of RNAi by siRNAs. Considering these TE-siRNA data, together with the plant findings on PTGS and viral resistance, RNAi was designated as "the genome's immune system" (Plasterk 2002).

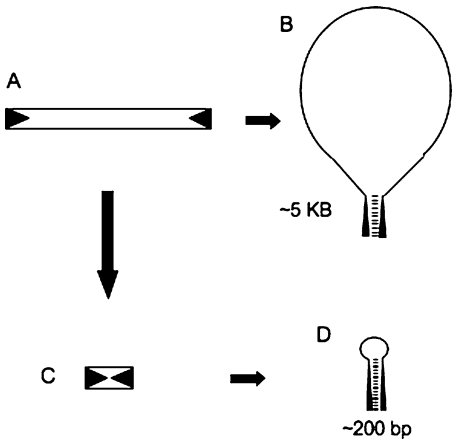

Fig. 1 Model for the siRNA-to-miRNA evolutionary transition. **A** Full-length (autonomous) DNA-type TEs encode siRNAs from snap-back dsRNA regions. (**B**) Formed by the bound terminal inverted repeats (TIRs). **C** Miniature inverted-repeat transposable elements (MITEs) are non-autonomous deletion derivatives of full-length DNA-type TEs, which contain TIRs and short internal regions. **D** MITEs expressed as RNA fold to form hairpin structures that resemble pre-miRNAs. A number of human and plant miRNA genes a derived from MITEs

3
TEs and microRNAs

While the relationship between TEs and RNA-mediated silencing via siRNAs has been appreciated since RNAi was first studied, the TE origins of a related class of regulatory RNAs, microRNAs (miRNAs), were only recently uncovered. miRNAs are also short ($\sim$ 22–25 bp) RNA molecules with a functional role in RNAi analogous to that of siRNAs (Ambros 2004; Bartel 2004). Single stranded mature miRNA sequences with regulatory activity are processed from longer transcripts by two rounds of RNA cleavage. In animals, a relatively long pri-mRNA is cleaved near the base of a hairpin (stem-loop) structure by the enzyme Drosha to yield a $\sim$ 70–90 bp pre-miRNA hairpin. The pre-miRNA hairpin is exported from the nucleus to the cytoplasm and a mature miRNA sequence is then cleaved from the dsRNA stem region by the endonuclease Dicer. Dicer is the same endonuclease that processes siRNAs from longer dsRNA sequences. In plants, both of the mature miRNA biogenesis steps are catalyzed by the related endonuclease Dicer-like1 in the nucleus. Mature miRNAs associate with the RNA-induced silencing complex (RISC), and together the miRNA–RISC targets mRNAs for regulation. miRNA target specificity is determined by partial complementarity with the 3′-untranslated region (UTR) sequence of the mRNA, and regulation is achieved by translational repression and/or mRNA degradation. miRNAs have been implicated in a variety of functions, including developmental timing (Lee, Feinbaum and Ambros 1993; Reinhart, Slack, Basson et al. 2000), apoptosis (Brennecke, Hipfner, Stark et al. 2003), and hematopoetic differentiation (Chen, Li, Lodish et al. 2004).

The relationship between TEs and miRNAs was discovered when a number of miRNA genes were shown to be derived from TE sequences (Mette, van der Winden, Matzke et al. 2002; Smalheiser and Torvik 2005; Borchert, Lanier and Davidson 2006; Piriyapongsa and Jordan 2007; Piriyapongsa, Marino-Ramirez and Jordan 2007). In the human genome, a group of related miRNA genes was found to be derived from the Made1 family of TEs (Piriyapongsa and Jordan 2007). Made1 elements (Morgan 1995; Oosumi, Belknap and Garlick 1995; Smit and Riggs 1996) are members of a specific class on DNA-type TEs known as miniature inverted-repeat transposable elements (MITEs) (Bureau and Wessler 1992; Bureau and Wessler 1994). MITEs are short non-autonomous derivatives of full-length DNA-type elements (Feschotte and Mouches 2000; Feschotte, Zhang and Wessler 2002). Full-length DNA-type elements are typically several kb in length and contain a single open reading frame, which encodes the transposase enzyme that catalyzes transposition, flanked by two TIR sequences on either end of the elements (Fig. 1A). As is the case with the Tc1 elements of *C. elegans* (Sijen and Plasterk 2003), full-length transcripts of DNA-type elements can fold into "snap-back" structures with the two TIRs forming a dsRNA region (Fig. 1B). This dsRNA region can

be processed to yield siRNAs that silence expression of the elements. MITEs are shorter sequences of ~ 80–500 bp, which lack the internal ORF of full-length elements but retain the TIRs (Fig. 1C). In other words, MITEs are closer to being palindromes, and read through transcription of MITEs will lead to RNA sequences that can fold into hairpin structures reminiscent of the pre-miRNA the sequences processed by Dicer to yield mature miRNAs (Fig. 1D).

The difference in structures that produce siRNAs versus miRNAs may be related to the different lengths of the transcripts that are processed including both the bound dsRNA regions and the intervening regions. For instance, longer dsRNA regions are most likely to yield multiple siRNA sequences, while shorter dsRNA hairpins, with small loops, will yield a single miRNA sequence. This may have to do with steric hindrance, i.e., physical-spatial constraints, placed on the dsRNA endonucleolytic machinery that cleaves small hairpins. In other words, RNA endonucleases can scan along longer dsRNA structures to yield multiple siRNAs, but they may only be able to cleave a single miRNA from a short hairpin sequence. It is also worth noting that TE-related dsRNA could be derived from sense and antisense transcripts generated from convergent transcription of the same element and/or read-through transcription of dispersed elements from both directions. Such dsRNA sequences would be expected to yield siRNAs.

The relationship between full-length DNA-type elements and siRNAs on the one hand, and MITEs and miRNAs on the other, led to the articulation of a specific model for how miRNAs could have evolved from siRNA encoding TEs in a stepwise manner (Piriyapongsa and Jordan 2007). As illustrated in Fig. 1, this model posits that siRNAs were first processed from the two TIRs of full-length elements bound as dsRNA. Later, as derivative MITEs evolved from full-length elements and proliferated in the genome, the same RNA endonucleolytic processing machinery cleaved the dsRNA from the hairpin stem regions yielding mature miRNA sequences. A corollary prediction of the TE-miRNA origins model holds that evolutionary intermediates may exist as TE sequences that encode both siRNAs and miRNAs. This prediction was tested and confirmed by an analysis of *Arabidopsis thaliana* and *Oryza sativa* (rice) genomic sequence and expression data (Piriyapongsa and Jordan 2008).

In Arabidopsis and rice, there are in fact a number of examples of individual TE insertions that encode both siRNAs and miRNAs. Dual siRNA-miRNA encoding TEs can be expressed as read-through transcripts from the intronic regions of spliced RNA messages. These TE-transcripts can fold to form the hairpin (stem-loop) structures characteristic of miRNA genes along with longer dsRNA regions that are typically processed as siRNAs. Taken together with a recent study showing Drosha independent processing of miRNAs from Drosophila introns (Ruby, Jan and Bartel 2007), and phylogenetic analysis indicating that Dicer is more ancient than Drosha (Cerutti and Casas-Mollano 2006), these results indicate that ancestral miRNAs could

have evolved from TEs prior to the full elaboration of the miRNA biogenesis pathway. Later, as the specific miRNA biogenesis pathway evolved, and numerous other expressed inverted repeat regions came to be recognized by the miRNA processing endonucleases, the host gene related regulatory functions of miRNAs emerged. In this way, host genomes were afforded an additional level of regulatory complexity as a by-product of TE defense mechanisms. The siRNA-to-miRNA evolutionary transition is representative of a number of other RNA-based regulatory mechanisms that initially evolved to silence TEs and were later co-opted to serve as regulators of host gene expression (Girard and Hannon 2008).

4
Repeat-Associated Sequences and piRNAs

In the past few years alone, fundamental TE-research from a number of different labs has significantly expanded our understanding of transposon silencing mechanisms and host genome evolution. Thanks to the completion of the *Drosophila melanogaster* heterochromatin sequencing project, which provides deep insights into the structure and organization of heterochromatin (Smith, Shu, Mungall et al. 2007), the availability of inexpensive mass sequencing technologies such as 454 pyrosequencing, and novel bioinformatics tools, the TE community has revolutionized their concepts on TE biology (recently reviewed in: Aravin, Hannon and Brennecke 2007; Hartig, Tomari and Forstemann 2007; O'Donnell and Boeke 2007).

Whereas in earlier models the actual molecular key-players for TE-silencing were enigmatic, the current TE-silencing model is based on members of Argonaute proteins that play a pivotal role in TE silencing (Aravin, Hannon and Brennecke 2007). These proteins belong to the so-called Argonaute superfamily, bind distinct classes of small RNAs and form the core of the RNA-induced silencing complex (RISC), which is the RNA-interference effector complex (Tolia and Joshua-Tor 2007). Argonaute proteins segregate into two functionally and evolutionarily distinct clades, the Ago clade and the Piwi clade. Whereas in fission yeast and plants only Ago clade proteins are found, ciliates and slime molds encode exclusively Piwi clade proteins. Animal genomes typically contain both clades, and hence, with the further functional dissection of Argonaute proteins, it is becoming clear that the phylogenetic division of Argonautes reflects their underlying biology.

The Ago clade proteins complex with siRNAs and miRNAs, which both derive from dsRNA precursors (Tolia and Joshua-Tor 2007). Whereas miRNA-Ago complexes interfere with the translation and stability of protein-coding mRNAs, by which fine-tuning of gene expression is accomplished, siRNA-Ago complexes are targeted against exogenous viral parasites in animal systems (Wang, Aliyari, Li et al. 2006). In *C. elegans* however, some endogenous

siRNAs were found that are likely to participate in host gene regulation as well (Ruby, Jan, Player et al. 2006).

The Piwi clade proteins are found in all animals examined so far but their expression is tightly restricted to the germline. Whereas the three Piwi proteins Aubergine, Piwi, and AGO3 are all expressed in both Drosophila male and female germline (Cox, Chao, Baker et al. 1998; Saito, Nishida, Mori et al. 2006; Brennecke, Aravin, Stark et al. 2007; Gunawardane, Saito, Nishida et al. 2007), expression of the mammalian Piwi homologues MIWI (PIWIL1), MILI (PIWIL2), and MIWI2 (PIWIL4) is restricted to the mouse testes (Kuramochi-Miyagawa, Kimura, Yomogida et al. 2001; Deng and Lin 2002; Kuramochi-Miyagawa, Kimura, Ijiri et al. 2004; Carmell, Girard, van de Kant et al. 2007). Consequently, Piwi loss-of-function mutants generally exhibit massive defects in germ cell development of male and female Drosophila (Cox, Chao, Baker et al. 1998) and during mouse spermatogenesis (Deng and Lin 2002; Kuramochi-Miyagawa, Kimura, Ijiri et al. 2004; Carmell, Girard, van de Kant et al. 2007).

In addition to their fundamental functions in germline development in animals, Piwi proteins play a pivotal role in post-transcriptional silencing of genomic parasites such as TEs (Aravin, Naumova, Tulin et al. 2001; Savitsky, Kwon, Georgiev et al. 2006; Vagin, Sigova, Li et al. 2006). In Piwi loss-of-function mutants of Drosophila, massive bursts of TE transcripts were observed (Aravin, Naumova, Tulin et al. 2001). Later, a novel class of small 25- to 27-nucleotide RNAs with homology to repetitive elements, called repeat-associated small interfering RNAs (rasiRNAs) was discovered (Aravin, Lagos-Quintana, Yalcin et al. 2003). Direct experimental evidence for the conserved functional interaction between Piwi proteins and rasiRNA came from immunoprecipitations of Piwi complexes in Drosophila (Saito, Nishida, Mori et al. 2006; Vagin, Sigova, Li et al. 2006; Brennecke, Aravin, Stark et al. 2007; Gunawardane, Saito, Nishida et al. 2007), Zebrafish (Houwing, Kamminga, Berezikov et al. 2007) and mice (Kim 2006). Due to their tight association with Piwi proteins, and their evolutionary conservation throughout the animal kingdom, rasiRNAs were renamed to Piwi-interfering RNAs (piRNAs).

The Piwi piRNA system forms a separate post-transcriptional regulatory network distinct from the canonical RNAi and miRNA pathways, since Piwis have never been observed to complex with miRNAs in any organism (Aravin, Gaidatzis, Pfeffer et al. 2006; Girard, Sachidanandam, Hannon et al. 2006; Saito, Nishida, Mori et al. 2006; Brennecke, Aravin, Stark et al. 2007). Similar to miRNAs, the piRNAs carry a 5′-monophosphate group and exhibit a preference for a 5′-uridine residue (Aravin, Gaidatzis, Pfeffer et al. 2006; Girard, Sachidanandam, Hannon et al. 2006; Lau, Seto, Kim et al. 2006). Unlike animal miRNAs, but similar to plant miRNAs, piRNAs carry a 2′-*O*-methyl modification at their 3′ ends added by a Hen-1 family RNA methyltransferase (Hartig, Tomari and Forstemann 2007). The Piwi-piRNA pathway is largely independent from the function of Dicer that is the key-player of miRNA and

siRNA pathways and is clearly distinctive in terms of evolutionary age and mode of selection.

Whereas some miRNAs are conserved over millions of years by coevolving in concert with their host-derived target genes under purifying selection, piRNAs evolve rapidly and even closely related species harbor different repertoires of piRNAs cocktails (Aravin, Gaidatzis, Pfeffer et al. 2006; Girard, Sachidanandam, Hannon et al. 2006; Lau, Seto, Kim et al. 2006). These data suggest that piRNAs are under rapid adaptive evolution in concert with horizontally invading and vertically expanding genomic parasites (Aravin, Hannon and Brennecke 2007). Consequently, Piwi proteins that are tightly linked to dynamic piRNAs should also evolve rapidly in an adaptive manner similar to the situation found in centromere-specific histone variants and their hyperdynamic satellite DNA targets (Henikoff, Ahmad and Malik 2001; Malik, Vermaak and Henikoff 2002) or the antiviral DNA-editing enzyme APOBEC3G and their viral targets (Sawyer, Emerman and Malik 2004).

With the isolation and characterization of Piwi-associated piRNAs by immunoprecipitation and large scale sequencing, their genomic mapping analysis revealed a limited set of discrete loci that could give rise to most piRNAs (Brennecke, Aravin, Stark et al. 2007; Gunawardane, Saito, Nishida et al. 2007). These so-called piRNA clusters map to the pericentromeric or telomeric heterochromatin, range from several to hundreds of kilobases, are devoid of protein-coding genes, and are densely occupied by eroded remnants of formerly active TEs and other repeats. Similar piRNA clusters were found in mammals (Aravin, Gaidatzis, Pfeffer et al. 2006; Girard, Sachidanandam, Hannon et al. 2006; Lau, Seto, Kim et al. 2006) and zebrafish (Houwing, Kamminga, Berezikov et al. 2007).

As one of the most extensively studied piRNA clusters, the flamenco locus, serves as a model system for expanding our understanding on transposon silencing. Thanks to elegant genetic studies, this locus has been mapped to the X-chromosomal pericentromeric heterochromatin of Drosophila as a key-regulator for controlling transposon activity (Pelisson, Song, Prud'homme et al. 1994; Prud'homme, Gans, Masson et al. 1995). Mutations in this master locus give rise to transcriptional derepression and transpositional bursts of formerly silenced retrotransposons, such as gypsy, Idefix and ZAM (Pelisson, Song, Prud'homme et al. 1994; Bucheton 1995; Prud'homme, Gans, Masson et al. 1995; Desset, Meignin, Dastugue et al. 2003; Meignin, Dastugue and Vaury 2004). Subsequently, Pelisson and colleagues demonstrated that flamenco-mediated silencing of gypsy is dependent on the presence of the Piwi protein (Sarot, Payen-Groschene, Bucheton et al. 2004). As deduced from recent immunoprecipitations and large-scale sequence analyses (Brennecke, Aravin, Stark et al. 2007), the main fraction of Drosophila piRNAs were bound by and isolated from the Piwi protein complex in the germline maps almost exclusively to the minus strand of the flamenco locus (Brennecke, Aravin, Stark et al. 2007; Gunawardane, Saito, Nishida et al. 2007). This piRNA

master locus spans a region of 179-kb and consists of eighty-seven percent of nested mobile DNAs of many TE families. Importantly the three retrotransposons ZAM, Idefix and gypsy were all found in the minus strand orientation within the flamenco locus (Brennecke, Aravin, Stark et al. 2007). This remarkable strand asymmetry suggests that the heterochromatic flamenco locus is expressed via follicle cells in the germline as a long primary transcript composed of numerous TE-remnants, all in antisense orientation.

In the course of two independent piRNA studies, the authors detected a remarkable strand bias of piRNAs derived from each of the three Piwi complexes (Brennecke, Aravin, Stark et al. 2007; Gunawardane, Saito, Nishida et al. 2007). Whereas Piwi and Aubergine proteins preferentially bind piRNAs corresponding to the antisense strand of transposons, Ago3 complexes almost exclusively with piRNAs of TEs in sense orientation. Surprisingly, a unique complementary relationship between sense and antisense piRNAs was observed over 10 nucleotides in the 5′ ends between Aubergine- or Piwi- and Ago3-associated piRNAs. Based on these observations, both research groups concluded that Piwi-mediated cleavage events generate new piRNAs in a self-reinforcing amplification cycle for piRNA generation named the "Ping-Pong" model (Fig. 2) (Brennecke, Aravin, Stark et al. 2007; Gunawardane, Saito, Nishida et al. 2007). According to this model, initiation of the cycle begins with processing of primary piRNAs, transcribed by defective TE-remnants in the heterochromatin. These early piRNAs are antisense to active, euchromatic TE copies and bind either Piwi or Aubergine. Triggered by partial complementarity in their 5′ ends, the Aubergine/Piwi-piRNA complexes target and cleave their active euchromatic transposon-counterparts and generate new sense piRNAs that bind Ago3. Next, the sense piRNA-Ago3 complex directs another cleavage event of a heterochromatic piRNA cluster transcript creating a new antisense piRNA capable of binding to Piwi and Aubergine. This model allows the efficient amplification of weak piRNA signals in the absence of a RNA-dependent RNA polymerase, which is an essential enzyme for RNAi signal-reinforcement in fission yeast, plants and nematodes (Cogoni and Macino 1999; Dalmay, Hamilton, Rudd et al. 2000; Sijen and Kooter 2000), but is absent in Drosophila and vertebrates (Schwarz, Hutvagner, Haley et al. 2002). Clearly, piRNAs provide an excitingly dynamic system for acquiring adaptive immunity against invading genomic parasites.

A second Drosophila piRNA cluster that has been genetically linked to TE control corresponds to the telomeric associated sequence (TAS) repeat on the X-chromosome (X-TAS). Insertions of one or two P elements into X-TAS are sufficient for silencing in trans P element mobility in the P-M hybrid dysgenesis system (Ronsseray, Lehmann and Anxolabehere 1991; Marin, Lehmann, Nouaud et al. 2000; Stuart, Haley, Swedzinski et al. 2002) and to reactivate P-transposition in mutants of the Piwi family (Reiss, Josse, Anxolabehere et al. 2004; Josse, Teysset, Todeschini et al. 2007). The exact insertion positions were mapped and sequenced within X-TAS repeats (Karpen

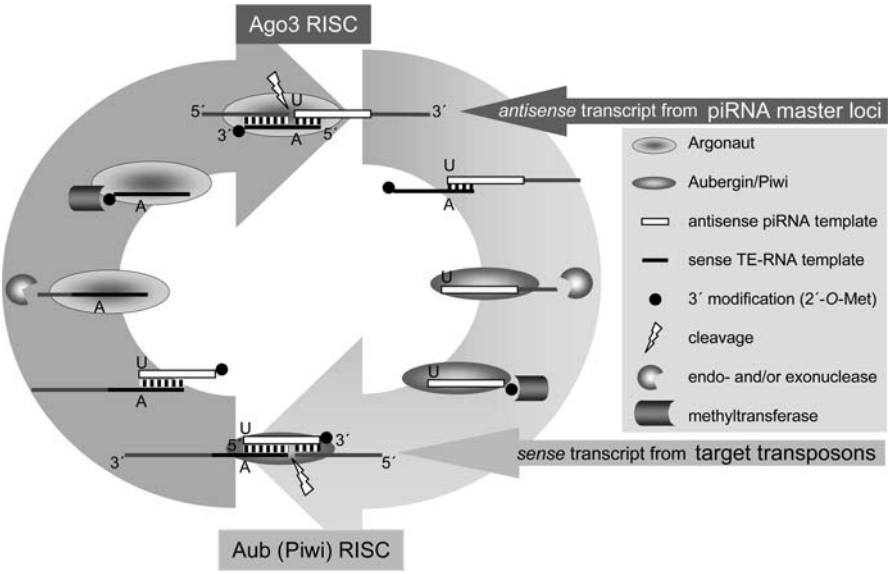

Fig. 2 The "Ping-Pong" model for piRNA biogenesis. The model was first proposed by Brennecke et al. 2007 and Gunawardane et al. 2007 and the figure is modified after Hartig, Tomari and Förstemann 2007. *Bottom*: Transposon-derived sense transcripts expressed from multiple genomic sites are targeted and cleaved by Piwi or Aub RISC loaded with a piRNA guide. The cleaved transcript is only partly degraded and serves as a specified "hunting-bait" for Ago3 RISC. *Top*: This complex in turn cleaves the antisense transcripts that originate from the master control loci. Again, the cleaved RNA serves as a bait for Piwi or Aub RISC. Thus, sense and antisense transcripts fuel an amplification cycle in which the 5′ ends of piRNAs are defined by RISC cleavage. In this model, the 3′ends are shortened by an endonuclease and/or exonuclease, subsequently 2′-*O*-Me-modified by a methyltransferase

and Spradling 1992; Ronsseray, Lehmann, Nouaud et al. 1997; Boivin, Gally, Netter et al. 2003) and multiple small RNAs homologous to X-TAS were isolated by (Brennecke, Aravin, Stark et al. 2007). Unfortunately, in the cases of both Drosophila strains, the strain that was analyzed for piRNA expression (Brennecke, Aravin, Stark et al. 2007; Gunawardane, Saito, Nishida et al. 2007) and the strain sequenced for the Drosophila heterochromatin sequencing project (Hoskins, Carlson, Kennedy et al. 2007; Smith, Shu, Mungall et al. 2007) are devoid of P elements, since in both cases P element-free lab strains (M strains) were used in the analyses.

Despite our current lack of sequence information on P element-derived piRNA in the germline of Drosophila, it seems very likely that in more recent *D. melanogaster* strains harboring P elements in the subtelomeric cluster the X-TAS locus is actually driving the expression of such integrated P elements in an antisense orientation. Similar to the Ping-Pong model (Brennecke, Aravin, Stark et al. 2007; Gunawardane, Saito, Nishida et al. 2007) de-

termined from the pericentromeric flamenco piRNA cluster, the heterochromatic X-TAS antisense-P-transcripts could be targeted by the Piwi protein complex, which binds euchromatic sense-P-transcripts in a sequence specific manner by complementarity and chops them into short piRNAs that further trigger the sequence-specific degradation of novel P-transcripts.

Since parasitic P elements have invaded natural *D. melanogaster* populations within the last 50–100 years by horizontal transfer from neotropical Drosophila host species (Kidwell 1983), the piRNA mediated resistance locus was born immediately when P elements inserted stochastically into X-TAS of the subtelomere and were trapped. Through this random insertion event, a new TE-specific immune locus was generated, which is now expressing RNA-based "antibodies" against recently invading and selfishly expanding euchromatic P elements (O'Donnell and Boeke 2007), providing an adaptive, vertically transmitted immunity against P-mobility to the following generations. Hence, the classic P element system presents a perfect model system for studying the exceptional dynamics of the interplay between genome parasites and hosts even over a relatively short time scale.

5
Transcript Infection Model

The data described above, emerging from experimental analyses of a number of different RNA-based regulatory systems, all point to TEs as being initiators of, and targeted by, RNA-mediated antisense gene regulation. While the impetus for the evolutionary origins of these distinct regulatory systems was the need to repress TEs, in many cases host genomes have co-opted the regulatory complexity afforded by RNA-based systems to control expression of their own genes. Here, we propose a specific model that links the RNA-mediated repression of TE sequences to the regulation of host genes via "transcript infection."

Transcript infection occurs when a TE inserts inside of, or adjacent to, a host gene (Fig. 3). The abundance of TEs, coupled with their transpositional activity, ensures that many gene-associated insertions of this kind will occur. Such insertion events may allow for the production of chimeric transcripts that include both host gene and TE sequences. Most studies to date have emphasized the functional consequences of TE insertions inside genes or in upstream proximal promoter regions. Our transcript infection model rests on TE insertions that occur downstream (3′) of host genes. These downstream insertions may lead to substantial regulatory effects by inducing antisense regulation of nearby host genes.

In Fig. 3, we illustrate a number of specific scenarios by which transcript infection via 3′ TE insertions could lead to the repression of host genes. For instance, a 3′ TE insertion oriented antisense to the host gene could give

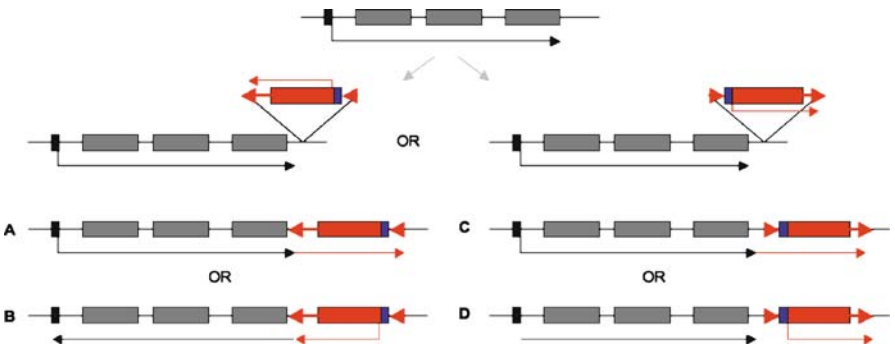

Fig. 3 Transcript infection model. TEs insert downstream of host genes in the antisense or sense orientation. Antisense oriented downstream TEs can result in host gene sense-TE antisense chimeric transcripts promoted by the host gene (**A**) or TE sense-host gene antisense chimeric transcripts promoted by the TE (**B**). Sense oriented downstream TEs can result in host gene sense-TE sense chimeric transcripts promoted by the host gene (**C**) or independent transcripts promoted by the host gene and the TE (**D**). The implications of these distinct scenarios for RNA-mediated host gene regulation are detailed in the text

rise to a chimeric transcript, promoted by the host gene, consisting of sense host gene transcript fused to an antisense TE transcript (Fig. 3A). In this case, Piwi and Aubergine could complex with the antisense TE sequence and titrate off the host transcript, leading to down regulation or loss of function. This would be analogous to a TE-induced auto-immune disease. On the other hand, if transcription is promoted by the antisense oriented 3′ TE insertion, a read-through transcript consisting of both sense TE and antisense host gene sequence could be produced (Fig. 3B). This chimeric transcript could be targeted for miRNA interference mediated by Argonaute and Dicer proteins or silencing via the piRNA pathway. In either case, there would be a reduced level of host transcript dependent upon the transcriptional activity of the TE promoter.

Sense oriented 3′ TE insertions would lead to different predictions regarding RNA-mediated regulation of host genes. For instance, the host gene promoter could drive transcription of chimeric mRNAs that include both sense host gene and sense TE sequences (Fig. 3C). The sense TE sequences could be targeted by the piRNA pathway via Ago3. This would cause depletion of the fused host gene transcript similar to what was proposed for the antisense oriented 3′ insertions. However, if there is independent transcription of the host gene and the 3′ sense TE insertion, then one would neither expect any transcriptional interference, nor, consequently, an effect on host gene expression (Fig. 3D).

There are a few caveats with respect to the details of the transcription infection model that we would like to describe. For instance, in order for TE transcription infection events to exert regulatory effects, antisense TE transcripts incorporated into a sense primary gene transcript would need to be

complexed by Piwi and Aubergine in the piRNA pathway. It is possible that only some of the antisense TE transcripts produced in this will feed into the piRNA pathway. There may be some determining factor that influences such an outcome such as the stage of chimeric expression and/or the temporal and spatial availability of triggering cellular factors. A deeper understanding of such factors will have to await further experimental proof. In addition, sense TE transcripts fused with primary gene transcripts will only be incorporated into the piRNA or miRNA regulatory pathways if piRNAs or miRNAs against that particular element already exist. Finally, there are substantial differences in the number (density) of TE sequences in different species and these differences may well relate to the probability of the transcription infection model to exert substantial regulatory effects.

Consistent with the transcript infection model, we recently showed that human TEs at the 3′ ends of genes are prone to produce antisense transcription and that these TE antisense promoters are evolutionarily conserved, suggesting some regulatory function (Conley, Miller and Jordan 2008). Given the availability of complete genome sequences, along with TE annotations and high-throughput transcript mapping, it should be possible to more directly test the predictions of the transcript infection model.

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