SMALL RNAS

Transmitting silence through generations

The Miska and Mello laboratories shed light on the silencing mechanisms of Piwi-interacting RNAs (piRNAs) in *Caenorhabditis elegans* and on how silencing triggered by both piRNAs and environmental RNA interference (RNAi) is transmitted through generations.

In *C. elegans*, the effectors of RNAi are small interfering RNAs (siRNAs) that bind Argonaute (AGO) proteins to direct them to their targets. Primary siRNAs result from the processing of food-derived or injected double-stranded RNAs and activate RNA-dependent RNA polymerase to generate secondary siRNAs. Secondary siRNAs are usually 22 nucleotides long (22G-RNAs) and interact with worm-specific AGO proteins (WAGOs) to silence their targets. Endogenous WAGO–22G-RNA pathways silence many loci, yet how these pathways are triggered and how transgenerational inheritance is maintained remains controversial.

piRNAs function together with Piwi proteins of the AGO superfamily to silence transposons in the germ line. PRG-1 belongs to the Piwi family and is expressed exclusively in male and female germline cells along with endogenous piRNAs (which are 21 nucleotides long (21U-RNAs)).

To identify piRNA targets and the mechanisms by which piRNAs act on their targets in *C.elegans*, both groups used strains that carried a piRNA sensor — a GFP reporter gene that contained a short sequence complementary to an endogenous piRNA. Their studies indicated that silencing requires PRG-1 but also involves the generation of 22G-RNAs. Interestingly, these secondary siRNAs act on imperfectly complementary sites proximal to the piRNA target site and function to silence transcripts both at co-transcriptional and post-transcriptional levels. Thus, piRNAs can initiate silencing through imperfect base-pair interactions, which increases the potential number of targets for any given piRNA.

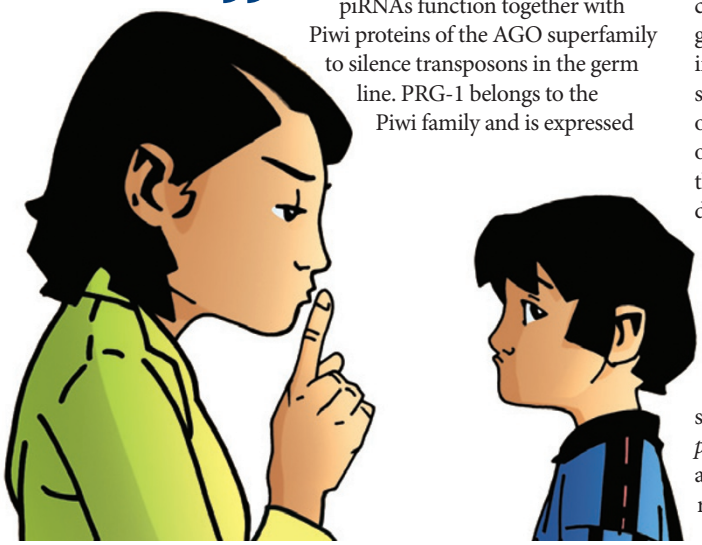
Mello and colleagues also observed that when single-copy transgenes consisting of an endogenous germline gene fused to a foreign sequence were inserted at a given chromosomal site, they could be either expressed or silenced depending on the length of the foreign sequence. They found that silencing was permanent and dependent on targeting of the foreign sequence by WAGO–22G-RNAs. This suggests that PRG-1 scans for foreign RNA sequences and initiates WAGO-dependent silencing in the germ line. In addition, single-copy transgenes were never silenced when inserted directly into a *prg-1* mutant, indicating that PRG-1 and piRNAs are essential for the initial recognition of the foreign sequence. Furthermore, they provided evidence for a self-recognition mechanism that protects endogenous sequences from being silenced by piRNAs. This mechanism is not known yet, but the authors suggest that it might involve a different type of AGO proteins known as CSR-1.

Importantly, both groups found that although PRG-1 initiates silencing, it is not necessary to maintain it through generations; maintenance depends on chromatin factors and the WAGO–22G-RNA pathway. Interestingly, when comparing the mechanisms underlying piRNA-mediated silencing and environmental RNAi, which are both transmitted through generations, Miska and colleagues found that they converge on a common nuclear RNAi and chromatin pathway. Indeed, they identify histone H3 Lys9 methylation, heterochromatin factors and nuclear RNAi defective 2 (NRDE-2) as factors required for transgenerational inheritance of both silencing triggers.

These findings add to the understanding of the intricate mechanisms of gene silencing and also provide an example of how acquired traits can be transmitted through generations. Importantly, they show that once a sequence is identified as foreign and silenced, an epigenetic memory is created that silences the foreign gene from one generation to the next. This mode of permanent silencing, referred to as RNA-induced epigenetic silencing (RNAe), could have profound implications for biological adaptation and evolution, as well as for health.

Kim Baumann

> “acquired traits can be transmitted through generations”



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ORIGINAL RESEARCH PAPERS Bagijn, M. P. *et al.* Function, targets, and evolution of *Caenorhabditis elegans* piRNAs. *Science* 14 Jun 2012 (doi:10.1126/science.1220952) | Shirayama, M. *et al.* piRNAs initiate an epigenetic memory of nonself RNA in the *C. elegans* germline. *Cell* **150**, 65–77 (2012) | Lee, H.-C. *et al. C. elegans* piRNAs mediate the genome-wide surveillance of germline transcripts. *Cell* **150**, 78–87 (2012) | Ashe, A. *et al.* piRNAs can trigger a multigenerational epigenetic memory in the germline of *C. elegans*. *Cell* **150**, 88–99 (2012)