Molecular biology

A tail of RNA interference

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It emerges that strings of nucleotides are added to messenger RNAs that are undergoing silencing in nematode worms. The composition of these nucleotide tails promotes the formation of small RNAs that drive heritable gene regulation. **See p.283**

Discovered in the minuscule nematode worm *Caenorhabditis elegans* nearly 30 years ago, small RNAs have been implicated in a surprising range of biological processes, from antiviral defence in plants to cancer in humans[1]. In *C. elegans*, these RNAs can be transmitted from one generation to the next, providing a non-DNA-based mechanism for heritable gene silencing, in which messenger RNAs are inhibited or degraded[2]. But the molecular details underlying the phenomenon have remained elusive. On page 283, Shukla *et al.*[3] describe an enzyme that converts even seemingly innocuous mRNAs into templates for the formation of small RNAs and mediators of transgenerational gene silencing.

In 1987, a genetic mutation was identified in *C. elegans* that activates transposons[4] — abundant but normally inactive genes that can replicate and reinsert themselves at new locations in the genome, causing mutations. Twelve years later, a mutation was found[5] that deactivates a gene-silencing phenomenon called RNA interference (RNAi). The two mutations had the same physical effects on the worm, revealing a crucial role for RNAi in transposon silencing.

The mutations were later mapped[6] to a single gene, *RNAi-defective-3* (*rde-3*). The protein encoded by this gene, RDE-3, belongs to a family of enzymes that extend the ends of DNA and RNA by adding strings of untemplated nucleotides (that is, those not copied from existing DNA or RNA). But the specific role of RDE-3 remained a mystery. More recently, RDE-3 was shown to add strings of alternating uridine (U) and guanosine (G) nucleotides to RNA ends, forming poly(UG) tails[7]. Could this RNA-tailing activity underpin the molecular mechanisms of transposon silencing and RNAi?

Typically, RNAi is initiated by double-stranded RNA. When introduced into a cell, either experimentally or naturally, double-stranded RNA is chopped up by enzymes into small interfering RNAs (siRNAs). These, like other classes of small RNA, associate with Argonaute proteins, either to guide sequence-specific degradation of matching mRNAs or to repress their translation into protein. This effectively silences the genes that encode those mRNAs. Shukla and colleagues found that, when they injected stranded RNA into the *C. elegans* germ line (the tissue that produces reproductive cells), poly(UG) tails were appended to the matching cellular mRNA. Importantly, the addition of poly(UG) tails — a process aptly named depended on *rde-3*.

But are poly(UG) tails simply markers of RNA degradation, or do they have a direct role in RNAi? In a key experiment, the authors attached RNA tails of various nucleotide compositions to single-stranded mRNA fragments produced *in vitro*, and then introduced them into *C. elegans*. The RNA fragments appended with poly(UG) tails, but not other compositions, were potent triggers for gene silencing.

In *C. elegans*, primary siRNAs produced during the initial stage of RNAi trigger a second phase, in which secondary small RNAs called 22G-RNAs are synthesized from the target mRNA by enzymes called RNA-dependent RNA polymerases[2]. The 22G-RNAs probably act in a feedback loop to maintain small-RNA production and mRNA silencing (Fig. 1). Earlier work showed that *rde-3* is required for the formation of 22G-RNAs, but its specific role was unclear[6]. How an RNA is transformed into a substrate for 22G-RNA synthesis was also not understood. Could poly(UG) tails serve this function? Perhaps: Shukla *et al.* report that poly(UG)-tailed RNAs synthesized *in vitro* are bound by RNA-dependent RNA polymerases and function as templates for 22G-RNA production *in vivo*. So, poly(UG) tails might act as landing pads for RNA-dependent RNA polymerases.

Shukla *et al.* found evidence that mRNA was cleaved or trimmed before poly(UG) tails were added (Fig. 1). Cleavage might therefore prime an mRNA for pUGylation, possibly because the process of cleavage would remove another tail consisting of adenosine (A) molecules, which is added to most mRNAs to promote their stability and translation.

Taken together, the authors' work suggests a model in which mRNAs are bound by siRNAs and associated proteins, leading to their cleavage. RDE-3 adds poly(UG) tails to the cleaved

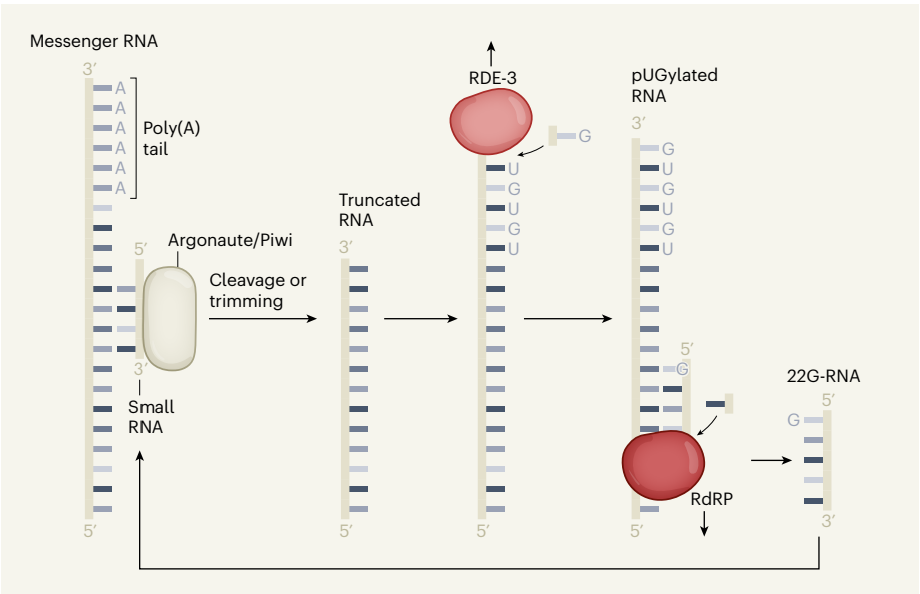

Figure 1 | RNA interference in nematode worms. Messenger RNAs can be silenced (degraded or their translation inhibited) by small RNA molecules. The small RNA that initiates mRNA silencing is anchored to an Argonaute/Piwi protein, and acts as a sequence-specific guide to direct mRNA cleavage or trimming, perhaps removing the mRNA's poly(A) tail. Shukla *et al.*[3] report that, in nematodes, the truncated RNA produced is bound by an RDE-3 enzyme, which adds a tail that consists of alternating uridine (U) and guanosine (G) nucleotides in a process called pUGylation. This pUGylated RNA acts as a 'template' for an RNA-dependent RNA polymerase (RdRP) enzyme. RdRP synthesizes secondary small RNAs, which are 22 nucleotides long and begin with a guanosine (22G-RNAs). The 22G-RNAs probably act to maintain mRNA silencing by having the same role as the initiating small RNA. Cycles of mRNA truncation, pUGylation and 22G-RNA synthesis drive transgenerational gene silencing.

mRNA end, enabling RNA-dependent RNA s to bind and synthesize 22G-RNAs templated from the mRNA. These 22G-RNAs act in the same way as the initial siRNA molecules, gene silencing.

Earlier work[8] implicated RDE-3 in the addition of poly(U) tails, rather than poly(UG) tails. The reason for the discrepancy between this work and the new findings is unclear, but it might reflect tissue-specific effects, such as those of germline compared with non-germline tissue. If so, it would suggest that RDE-3 can switch tailing modes, from poly(UG) to poly(U), depending on the cell type. A shortcoming of the new study is that the authors did not test whether poly(U) tails can also trigger RNAi, although they did show that other tail varieties cannot do so.

In *C. elegans*, RNAi underlies transgenerational epigenetic inheritance – a phenomenon in which changes in gene expression can be transmitted across three or more generations without changes in DNA sequence[2]. Strikingly, Shukla *et al.* show that a single dose of poly(UG)-tailed RNA injected into the worm germ line can trigger silencing of a matching gene for several generations. Through a series of simple genetic experiments, the authors found that cycles of pUGylation and 22G-RNA synthesis drive gene silencing from one generation to the next. This is a key breakthrough in our understanding of experimentally induced RNAi. But does pUGylation occur naturally?

Hundreds of *C. elegans* genes, including transposons, are naturally regulated by an RNAi pathway involving RDE-3 – hence the original link between RNAi and transposon silencing. Naturally occurring RNAi commonly involves a distinct class of small RNA, called Piwi-interacting RNAs (piRNAs). These piRNAs, like siRNAs, trigger the production of

"This is a key breakthrough in our understanding of experimentally induced RNA interference."

22G-RNAs and heritable gene silencing. Shukla *et al.* identified poly(UG) tails on several natural RNAi targets, including transposons. This is enticing evidence that pUGylation is not restricted to experimental RNAi, and might have a broad role in regulating gene expression. But the phenomenon will have to be explored on a wider scale to uncover how central it is to the various pathways involving small RNAs.

Is pUGylation unique to nematodes? In ciliates – a group of unicellular, nucleus-bearing organisms – poly(U) tails promote RNA-dependent RNA polymerase activity during RNAi[9]. But whether pUGylation occurs in ciliates, and what function poly(UG) tails might serve in organisms that lack RNA-dependent RNA polymerases (such as mammals), is unclear. RDE-3 has potential counterparts in species ranging from yeast to humans[6], and artificial forms of it can add poly(UG) tails to RNAs, even in distantly related organisms, such as yeast and frogs[7]. Shukla and colleagues' study paves the way for the identification of poly(UG)-tailed RNAs in other species, and the exploration of their roles in the production of small RNAs and other biological processes.

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