toaurignacian, although at sites including Le Piage and Isturitz in France, the Protoaurignacian underlies the Aurignacian (*11*, *12*). The best record of early symbolic artifacts in Europe comes from the Swabian Aurignacian and from the Aurignacian of southwestern France and several other regions, and not from the Protoaurignacian.

It is not clear, however, whether the term Protoaurignacian has much meaning. For example, the purported Protoaurignacian assemblage of Krems-Hundssteig has been shown to be a mixed assemblage containing a strong component of artifacts from the Gravettian, an Upper Paleolithic cultural group that postdates the Protoaurignacian by ~10,000 years (*13*). Also, artifacts such as split-based bone points and carinated scapers, often viewed as hallmarks of the Aurignacian, have been found at sites attributed to the Protoaurignacian (see the figure). This is the case with the split-based bone points at Trou de la Mère Clochette, Arbreda, and Fumane (*14*). Similarly, lithic assemblages with mixed signatures have been recovered from Crimea, Romania, and the Basque Country (*15*). The concept of the Protoaurignacian is thus not as robust as the article by Benazzi *et al*. implies.

While the research reported by Benazzi *et al*. is a welcome step forward in establishing the narrative of colonization of Europe by modern humans, the archaeological and human fossil records will almost certainly prove to be more complex and fascinating than our current models suggest. It is only through carefully excavating sites and establishing high-resolution regional signatures of the events and processes of the last glacial cycle that a reliable picture of the spread of modern humans and the extinction of Neandertals will come into focus. ■

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RNA

Phased piRNAs tackle transposons

Tertiary piRNAs help silence dangerous DNA elements

By **Haruhiko Siomi**[1] *and* **Mikiko C. Siomi**[2]

Transposable elements are dominant inhabitants of the eukaryotic genome. Their ability to mobilize and insert anywhere in the genome can cause mutations that are generally detrimental to the host. This makes them key targets for silencing. Small RNAs called PIWI-interacting RNAs (piRNAs) are one means to attack transposons. They constitute the largest class of small RNAs, but the mechanisms by which they are generated are not completely clear. On pages 812 and 817 of this issue, Mohn *et al*. (*1*) and Han *et al*. (*2*), respectively, propose a revised model for piRNA biogenesis that increases the diversity of piRNA sequences and thereby expands the range of targets they can silence.

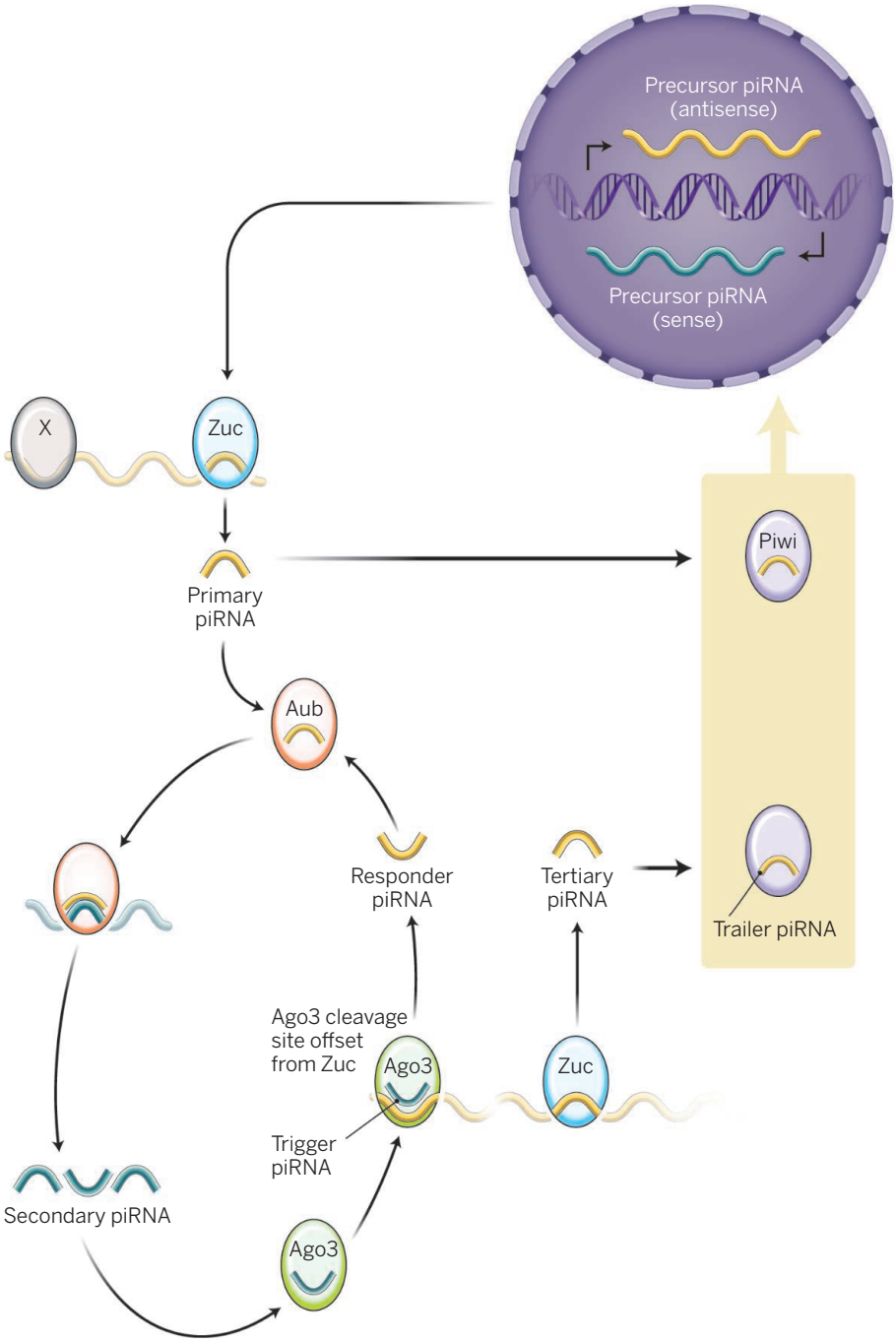
In animals, piRNAs are generated in germline cells, which means their effects can be transmitted to offspring. Most piRNAs are derived from genomic regions called piRNA clusters, which also harbor a large number of truncated transposable elements (*3*). In the germ cells of *Drosophila melanogaster*, single-stranded precursor transcripts (antisense) are produced from these clusters (see the figure). This precursor piRNA is then cleaved by an endonuclease Zucchini (Zuc), to generate short primary piRNAs (26 to 31 nucleotides long). The processing of cluster transcripts occurs in the cytoplasm, and mature piRNAs are loaded onto the PIWI proteins Ago3, Aub, and Piwi (*4*). Primary piRNAs have a bias toward having uridine at their 5′ ends (1U bias), are largely antisense with regard to corresponding transposable element sequences, and are loaded onto Aub and Piwi. Aub-bound primary piRNAs, together with Ago3, then initiate a "ping-pong cycle," which generates secondary piRNAs by cleaving complementary targets (both sense and antisense single-stranded RNA). Cleavage not only determines the 5′ end of secondary piRNAs but also consumes transposable element transcripts, thereby silencing transposons (*3*, *5*). Secondary piRNAs loaded onto Ago3 show 10-nucleotide complementarity at their 5′ ends with Aub-bound primary piRNAs and possess a sense bias with adenosine at the 10th nucleotide (10A bias). Piwi does not participate in the ping-pong cycle because once loaded with piRNAs, it is imported into the nucleus to repress transposable elements by modifying chromatin (*6*). This cycling model amplifies only ping-pong pairs of piRNAs and is therefore insufficient to explain the extraordinary sequence diversity of piRNAs.

Using state-of-the art bioinformatics, Mohn *et al*. and Han *et al*. independently found that Piwi- and Aub-, but not Ago3-bound piRNAs, displayed notable sequence "phasing." By analyzing small RNA populations from *Drosophila* germ cells (from ovaries) that are depleted of Rhino, a factor required for dual strand piRNA cluster transcription (*7*–*9*), Mohn *et al*. noticed a distinct nucleotide start site in piRNA profiles. These sites may correspond to piRNA biogenesis initiation sites, and the piRNAs that contain them are called "responder piRNAs." Most responder piRNAs occupy Aub and are complementary to Ago3-bound piRNAs, with a 10-nucleotide overlap at their 5′ ends. These Ago3-bound secondary piRNAs may trigger production of responder piRNAs. Surprisingly, piRNAs originating downstream of the responder piRNAs, called "trailer piRNAs," mostly associate with Piwi.

> ***"...PIWI-interacting RNAs (piRNAs) are one means to attack transposons."***

Mohn *et al*. observed that the 3′ ends of responder piRNA are immediately followed by 5′ ends of Piwi-bound trailer piRNAs. Remarkably, trailer piRNAs show phasing with a ~27-nucleotide interval, though both the amounts of trailer piRNA and phasing accuracy decrease as the distance from the trigger site increases. Phased trailer piRNAs also show a striking 1U bias. These findings support an intriguing model in which piRNA 3′ ends are defined by a cleavage event that preferentially occurs immediately upstream of a U residue, which corresponds to the 5′ end of the next piRNA.

[1]*Department of Molecular Biology, Keio University School of Medicine, Tokyo 160-8582, Japan.* [2]*Graduate School of Science, The University of Tokyo, Tokyo 113-0032, Japan. E-mail: awa403@keio.jp, siomim@bs.s.u-tokyo.ac.jp*



Expanding the piRNA arsenal. In *Drosophila* germ cells, Ago3-bound secondary piRNAs act as triggers to produce responder piRNAs that bind to Aub. This initiates production of trailer piRNAs (through Zuc-dependent phased tertiary RNA production) that mostly bind to Piwi. Protein X may determine piRNA cluster transcripts among cellular RNAs and initiate phased piRNA production by cleaving the cluster transcripts. A similar ping-pong–independent mechanism may also exist in ovarian somatic cells.

ILLUSTRATION: C. SMITH AND V. ALTOUNIAN/*SCIENCE*

Thus, 5′- and 3′-end formation of piRNAs is mechanistically coupled, precluding a 3′-trimming event to form mature piRNAs. Trailer piRNAs are selectively lost in ovaries lacking Zuc, suggesting that Zuc determines both the 3′ and 5′ ends of most germline Piwi-bound piRNAs. By sequencing analysis of cleaved transcripts, Han *et al.* also concluded that production of Piwi- and Aub-bound phased piRNAs depends on Zuc.

The findings of Mohn *et al.* and Han *et al.* also suggest that cleavage in the ping-pong cycle by trigger piRNA (bound to Ago3) produces not only a corresponding ping-pong partner piRNA but also piRNAs from the remaining 3´ portion of the cleaved transcripts: Once a cleaved 3′ transcript with 1U is loaded onto Piwi, it follows repeated rounds of cleavage with a ~27-nucleotide interval. The interval may be determined by a combination of Piwi's footprint, Zuc cleavage of Piwi-bound trail sequences, and the preference of Piwi to bind 1U. Although Zuc-dependent piRNAs are viewed as primary piRNAs, trailer piRNAs, which occupy the vast majority of Piwi, may reasonably be called "tertiary" piRNAs because their production depends on secondary piRNAs. Therefore, primary piRNAs make secondary piRNAs, which make phased "tertiary piRNAs." What initiates this sequential pathway is Aub-bound antisense primary piRNAs, which produce Ago3-bound sense "trigger piRNAs" in the ping-pong cycle. This raises the question of how such antisense primary piRNAs are selectively produced.

Both Mohn *et al.* and Han *et al.* also observed phased piRNAs in ovarian somatic cells where only the primary piRNA production pathway operates, indicating that Zuc-dependent phasing can be initiated without the ping-pong cycle. Because purified Zuc exhibits no nucleotide preference for its cleavage site (*10*, *11*), ovarian somatic cells may rely on factors replacing the function of Ago3-trigger piRNA complexes to determine piRNA biogenesis initiation sites on particular transcripts. This ping-pong–independent mechanism should also be available in germline cells to supply Aub-bound antisense primary piRNAs that produce Ago3-bound trigger piRNAs.

Mohn *et al.* and Han *et al.* demonstrate that Zuc-mediated 3′ end formation of piRNAs is also evident in mice, suggesting that primary piRNA phasing is mechanistically conserved in mammals. Thus, important future challenges are to identify factors that endow specificity to Zuc both in germline and ovarian somatic cells to trigger 3′-directed and phased piRNA production in the vast array of the transcriptome, and to elucidate the mechanisms that funnel most Zuc-cleaved transcripts onto Piwi. ■

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Haruhiko Siomi and Mikiko C. Siomi

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