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Argonaute proteins confer immunity in all domains of life

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Both eukaryotes and prokaryotes (archaea and bacteria) encode an arsenal of immune systems that protect the host against mobile genetic elements (MGEs) including viruses, plasmids, and transposons. Whereas Argonaute proteins (Agos) are best known for post-transcriptional gene silencing in eukaryotes, in all domains of life, members from the highly diverse Argonaute protein family act as programmable immune systems. To this end, Agos are programmed with small single-stranded RNA or DNA guides to detect and silence complementary MGEs. Across and within the different domains of life, Agos function in distinct pathways and MGE detection can trigger various mechanisms that provide immunity. In this review, we delineate the diverse immune pathways and underlying mechanisms for both eukaryotic Argonautes (eAgos) and prokaryotic Argonautes (pAgos).

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Introduction

Members of the Argonaute protein family are encoded in eukaryotes (eAgos) and prokaryotes (bacteria and archaea, pAgos) [1]. eAgos form two main phylogenetic clades that are broadly distributed in various eukaryotic lineages: the AGO (eAGO) clade and the PIWI (ePIWI) clade (Figure 1a). Compared with eAgos, pAgos show lower sequence and domain conservation [1,2]. Consequentially, pAgos are separated into three heavily branched main clades (long-A, long-B, and short pAgos, Figure 1a) [2,3]. Agos from all clades rely on a conserved general mechanism of action: they use small (14-35-nucleotide-long) single-stranded (ss) nucleic acid guides to bind complementary nucleic acid targets (Figure 1b) [4–13]. Yet, Agos from different clades rely on distinct types of guides (ssDNA or ssRNA) and guide biogenesis pathways. Furthermore, they bind different types of targets, and the outcome of guide-mediated target binding varies: depending on the specific Ago involved and/or the complementarity between the guide and target, the target is cleaved, or auxiliary proteins are recruited and/or activated [4,6,7,14–19].

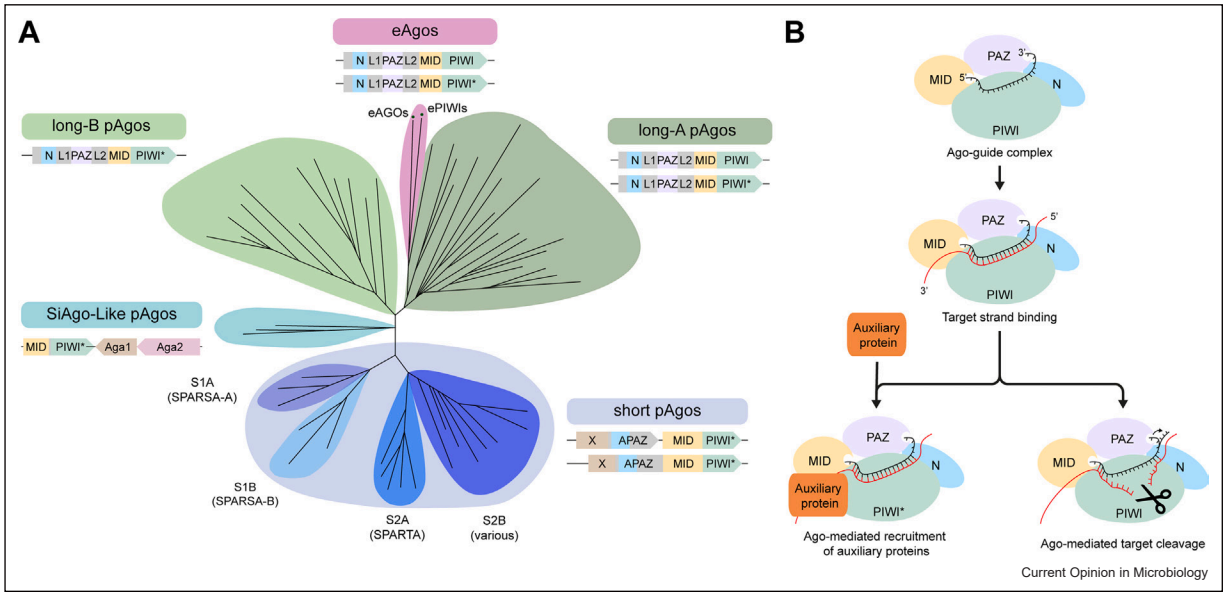
eAgos and long pAgos share an architecture with four domains and two ordered linker (L) segments: N–L1–PAZ–L2–MID–PIWI (Figure 1) [1,9,12,13,20]. The middle (MID) and PIWI–Argonaute–Zwille (PAZ) domains form binding pockets for the 5′- and 3′-ends of the guide, respectively. The P element-induced wimpy testis (PIWI) domain coordinates most contacts with the guide and target strands. In certain Agos, the PIWI domain contains a DEDX catalytic tetrad (where X can be H, D, or K) [12,19]. These Agos are referred to as 'catalytically active' or 'slicing' Agos, as they are capable of cleaving targets that are complementary to the guide. In general, eAgos that lack the DEDX tetrad rely on the recruitment of auxiliary proteins instead [20,21]. The function of the N-terminal (N) domain is less well understood and varies in different Agos. In certain eAgos, the N domain unwinds RNA duplexes during guide loading [22], while in certain pAgos, it contributes to guide/target duplex stability [23]. In contrast to eAgos and long pAgos, short pAgos are comprised of the MID and PIWI domains only, lack a DEDX tetrad, and generally form complexes with auxiliary proteins (Figure 1) [2,20].

While Agos are best known for their role in post-transcriptional gene silencing (RNA interference), this process has thus far only been observed in eukaryotes. In contrast, both eAgos and pAgos mediate programmable immunity against invading mobile genetic elements (MGEs), including viruses, plasmids, and transposons [7,16,17,24–28]. In this review, we delineate how Agos from distinct phylogenetic clades confer immunity and discuss differences and similarities in the underlying mechanisms.

Eukaryotic AGO proteins silence viruses

eAGO-clade eAgos (eAGOs) facilitate post-transcriptional gene silencing of endogenous mRNAs using microRNA (miRNA) or small interfering RNA (siRNA) guides (Figure 2) [18,29]. miRNAs are generally

Figure 1



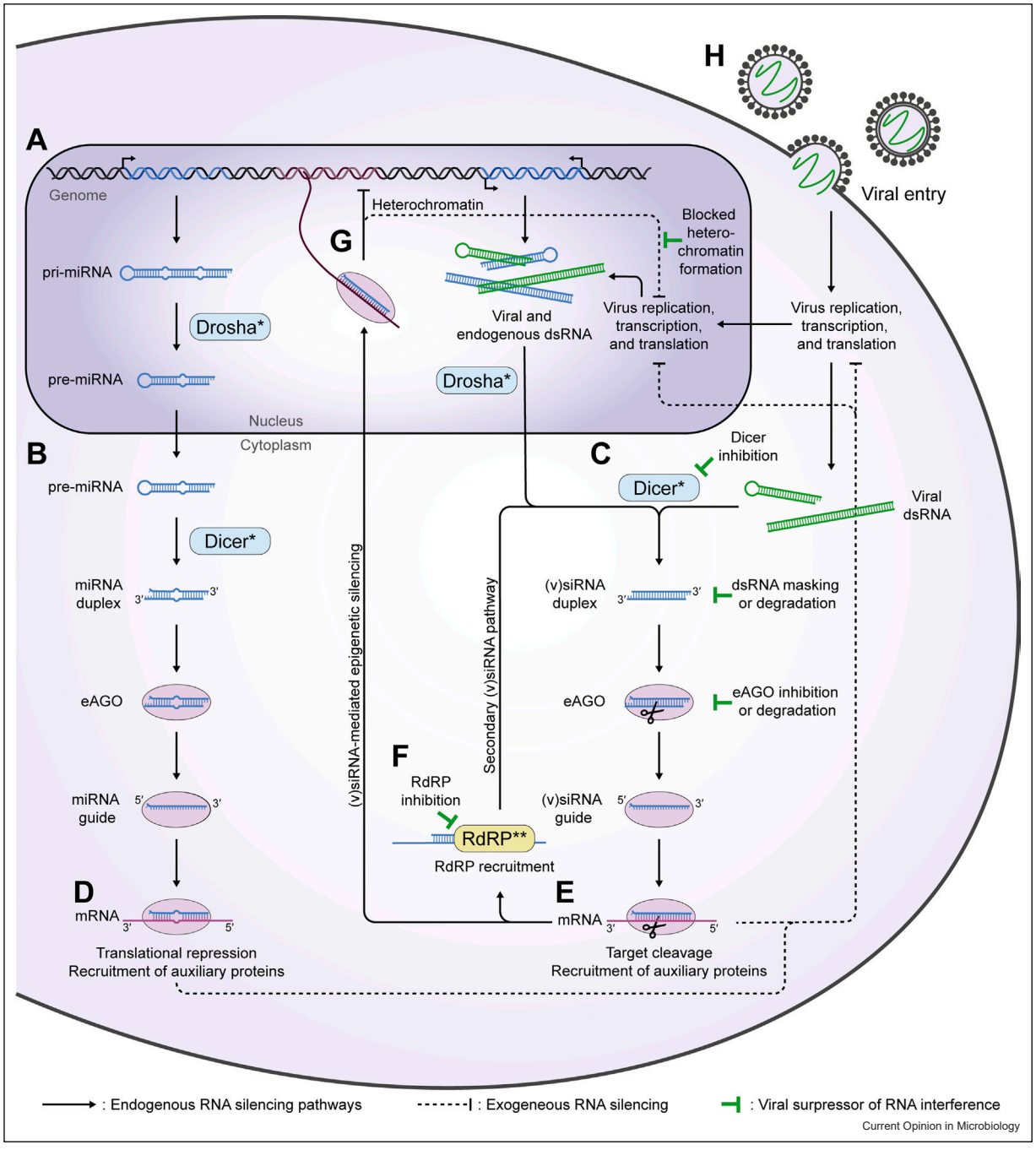
Phylogeny and general mechanisms of Ago proteins. **(A)** Schematic representation of the phylogenetic tree and domain composition corresponding to the main clades of eAgos and pAgos. N: N-terminal domain; L1 and L2: Linker segments 1 and 2; PAZ: PIWI-Argonaute-Zwille domain; MID: Middle domain; PIWI: P element-induced wimpy testis domain containing the catalytic DEDX tetrad (where X can be H, D, or K) required for target cleavage. PIWI*: PIWI domain with an incomplete DEDX tetrad. **(B)** General mechanism of Agos. Agos use a small nucleic acid guide to bind a complementary nucleic acid target. Depending on the specific Ago and complementarity between the guide and target, guide-mediated target binding results in recruitment and/or activation of auxiliary proteins (left) or in target cleavage (right). In certain Agos, the 3′-end of the guide is released from the PAZ domain during cleavage.
Schematic phylogenetic tree adapted from Ref. [20].

transcribed from the genome as long pri-miRNA double-stranded (ds)RNA hairpins with partial complementarity [30]. In metazoans (animals), the nuclear RNase III family enzyme Drosha processes pri-miRNAs to shorter pre-miRNAs, which are further processed by the cytosolic RNase III family enzyme Dicer. In plants, a single nuclear Dicer homolog processes miRNAs [31]. Dicer generates ∼22-bp miRNA:miRNA* duplexes with 2-nt 3′-overhangs, of which the miRNA guide strand is loaded onto eAGO, while the miRNA* passenger strand is released. eAGO/miRNA-mediated silencing of mRNA requires only limited guide/target complementarity. This allows a single miRNA to silence a wide range of mRNAs but prevents eAGO-mediated target cleavage [30].

siRNA biogenesis also relies on Dicer-mediated dsRNA processing. However, siRNA precursors are usually (almost) fully complementary dsRNAs, and siRNAs are generally fully complementary to their targets [32]. Consequentially, siRNA-guided mRNA binding facilitates target cleavage by slicing eAGOs. Nematodes, yeast, insects, plants, and mammals have endogenous siRNA pathways [33–36]. Besides silencing mRNA, siRNA-guided eAGOs can silence gene expression in the nucleus by recruiting proteins that promote heterochromatin formation [37,38]. In nematodes, yeast, and plants, mRNA targeting by primary eAGO–siRNA complexes can trigger generation of secondary siRNAs by the recruitment of an RNA-dependent RNA polymerase (RdRP) [39,40]. RdRP will directly generate siRNAs with a 5′-triphosphate group (in nematodes) or convert eAGO-targeted mRNA to a dsRNA product that functions as a substrate for Dicer to generate secondary siRNAs [29].

Beyond (post-translational) regulation of gene expression, eAGOs interfere with MGEs, including viruses and transposable elements in plants [25], nematodes [28], insects [26], and mammals [27]. eAGO-mediated MGE silencing primarily relies on the siRNA pathway. Transcription and replication of MGEs can give rise to different types of dsRNAs, including replication intermediates of RNA viruses, bidirectional transcripts, intramolecular paired viral RNA, structured viral RNAs, and viral dsRNA molecules synthesized by endogenous or virus-encoded RdRP. These viral dsRNAs are degraded by Dicer [41] or Drosha [42] as a first line of defense, but this also generates virus-derived siRNAs (vsiRNAs) that guide eAGOs to repress or degrade viral mRNA transcripts or RNA genomes [25–27]. Endogenous miRNAs can also be involved in MGE silencing: certain miRNAs regulate

Figure 2



General pathways mediated by eAGO-clade eAgos. **(A)** Endogenous miRNA and siRNA precursors are transcribed from the genome as dsRNA hairpins or long dsRNAs that are processed by nuclear Drosha*. **(B and C)** In the cytoplasm, Dicer* generates ~22-bp miRNA **(B)** or siRNA **(C)** duplexes with two-nucleotide 3′-overhangs. These duplexes are loaded onto eAGO and the passenger strand is released. **(D)** eAGO–miRNA complexes generally bind mRNA sequences with low complementarity, repress translation, and recruit auxiliary proteins that destabilize the target mRNA. **(E)** eAGO–siRNA complexes bind mRNA sequences with high complementary and cleave the target mRNA. **(F)** RdRP can be recruited to convert the target mRNA to a dsRNA duplex that can be processed by Dicer, resulting in generation of secondary siRNAs. **(G)** eAGO–siRNA complexes can recruit auxiliary proteins to promote heterochromatin formation in the nucleus. **(H)** After viral entry, virus-derived dsRNA molecules feed into the siRNA pathway resulting in the generation of vsiRNAs, which facilitate silencing of viral RNAs. Viruses encode VSRs that inhibit distinct steps of the siRNA pathway. *: In plants, miRNA processing is carried out by a single nuclear Dicer. **: RdRP-mediated generation of secondary siRNAs has been reported in nematodes, yeast, and plants. Please note that also RNA viruses encode RdRP for replication, which can be a source of dsRNA substrates for Dicer.

mRNA levels to promote antiviral responses, while other miRNAs directly target viral RNAs [27].

Eukaryotic PIWI proteins silence transposons

ePIWI-clade eAgos (ePIWIs) are best known for transposon silencing in animal germline cells (Figure 3) [43]. ePIWIs are guided by PIWI-interacting RNAs (piRNAs), which are generated from long ssRNA precursors in the cytoplasm in a Dicer-independent process. piRNA precursors include piRNA cluster transcripts [44,45], mRNA 3′-untranslated regions (UTRs), and noncanonical transcripts from heterochromatin-silenced transposons [46]. Initiation of piRNA maturation primarily relies on maternally inherited piRNAs. New piRNAs are generated through a self-amplifying process termed ping-pong [45]: initiator piRNAs guide ePIWI to cleave complementary precursor piRNAs to generate pre–pre-piRNAs. The 5′-end of these pre–pre-piRNAs are loaded into new ePIWIs while the 3′-end is cleaved by auxiliary nucleases (e.g. Zucchini in flies). The resulting responder pre-piRNAs are trimmed and 2′-O-methylated to form mature responder piRNAs. In turn, responder piRNAs guide ePIWIs to create new initiator piRNAs, comwait it pleting the ping-pong pathway [46]. In parallel, phased piRNA biogenesis takes place: the product of the cleaved pre–pre-piRNA can be loaded into ePIWIs and cleaved sequentially, resulting in the generation of trailing piRNAs from a single piRNA precursor [47].

ePIWIs silence transposons by transcript cleavage [48] or through the recruitment of auxiliary proteins that promote heterochromatin formation in the nucleus [48–50]. While the formed heterochromatin blocks canonical transposon transcription, it induces noncanonical transcription that results in the generation of additional piRNA precursors. In specific mosquitoes and Lepidoptera species, also virus-derived piRNAs (vpiRNAs) are generated and antiviral activity of ePIWI proteins is observed [51,52]. Yet, the mechanisms underlying antiviral ePIWI activity remain to be determined. Beyond silencing transposons and viruses, ePIWIs can also silence gene expression through mRNA cleavage [53] or recruitment of factors for deadenylation [54], and can increase translation by stimulating mRNA polyadenylation or translation activation [55,56].

Long prokaryotic Argonautes interfere with plasmid and bacteriophage DNA

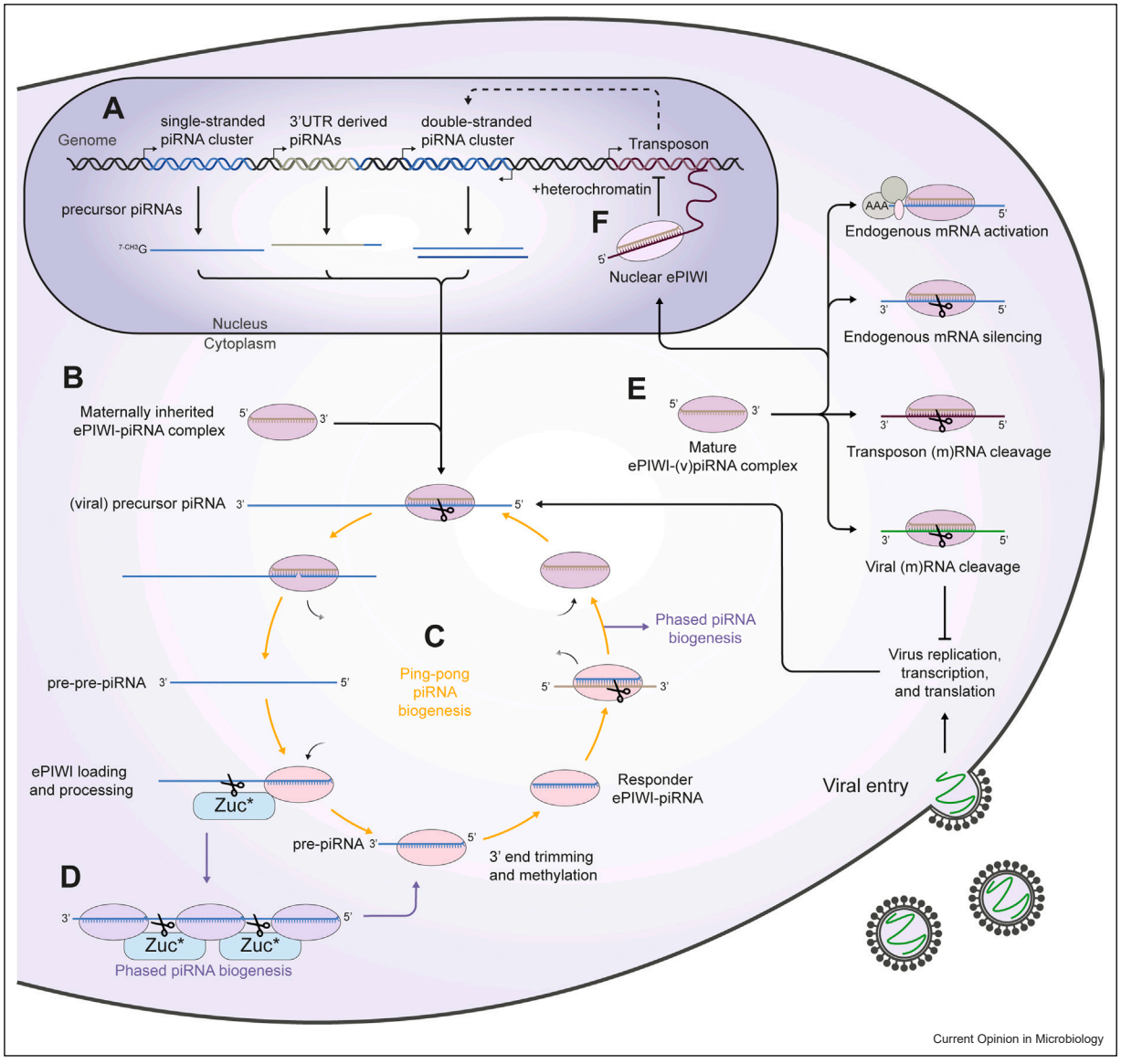
Proteins typically involved in eukaryotic RNA silencing pathways (e.g. Drosha, Dicer, and RdRP) are absent in prokaryotes. Instead of regulating gene expression, emerging insights show that pAgos from all clades interfere with invading plasmid and bacteriophage DNA in a guide-dependent manner [6–8,14–17,24,57]. The mechanisms underlying anti-MGE activities vary for pAgos from distinct phylogenetic clades.

Most long-A pAgos have a complete DEDX catalytic tetrad, while all long-B pAgos are catalytically inactive. Long-A pAgos have been broadly characterized *in vitro* and can use ssDNA and/or ssRNA guides to cleave ssDNA and/or ssRNA targets [23,24,58–64]. While *in vivo* characterization of long-A pAgos is limited to a handful of studies, they show long-A pAgos mediate small-interfering (si)DNA-guided DNA cleavage to interfere with plasmid DNA transformation and propagation [7,16,17] as well as with bacteriophage infection and proliferation [17,65]. Interestingly, the catalytic activity of long-A pAgos is not essential to provide protection against MGEs [17]. This suggests that, similar to eAGOS, pAgos can silence MGEs by guide-mediated target binding, possibly by silencing transcription/replication and/or by recruiting host-encoded auxiliary proteins.

How siDNAs are generated and loaded onto pAgos, and how pAgos distinguish self (genomic DNA) from nonself (invader DNA) remains poorly understood. Some pAgos can ‘chop’ dsDNA substrates in a guide-independent manner, albeit with low efficiency, to generate small dsDNA fragments that can be loaded onto pAgos akin to miRNA/miRNA* or siRNA duplexes in eAGO [7,24,57,66]. While long-A pAgos preferentially acquire siDNAs targeting invader DNA, they also load siDNAs targeting the host genome [7,17,65,67]. siDNAs associated with catalytically inactive pAgos no longer preferentially contain sequences from invader DNA [17]. This suggests that chopping might contribute to the generation of invader-targeting siDNAs. The host DNA repair machinery (RecBCD or AddAB) also contributes to the generation of siDNAs from Chi sites at dsDNA breaks and genomic replication termination (Ter) sites [17,68,69]. Finally, akin to the piRNA ping-pong pathway, pAgos might use initiator siDNAs to cleave DNA targets, after which the generated 5′-end can be loaded as a responder siDNA [70].

From the long-B pAgo clade, only *Rhodobacter sphaeroides* pAgo (RsAgo) has been functionally characterized [8]. In contrast to long-A pAgos, RsAgo uses guide RNAs to interfere with invading DNA. Although it is catalytically inactive, RsAgo silences plasmid-encoded gene expression and induces plasmid degradation, possibly by recruiting auxiliary nucleases. RsAgo-associated guide RNA sequence abundance correlates with the abundance of cellular mRNAs, suggesting it randomly samples the RNA degradome for guide RNAs. Yet, DNA targets associated with RsAgo–RNA complexes are enriched in invader sequences, which suggests that it preferentially targets extrachromosomal DNA sequences [8].

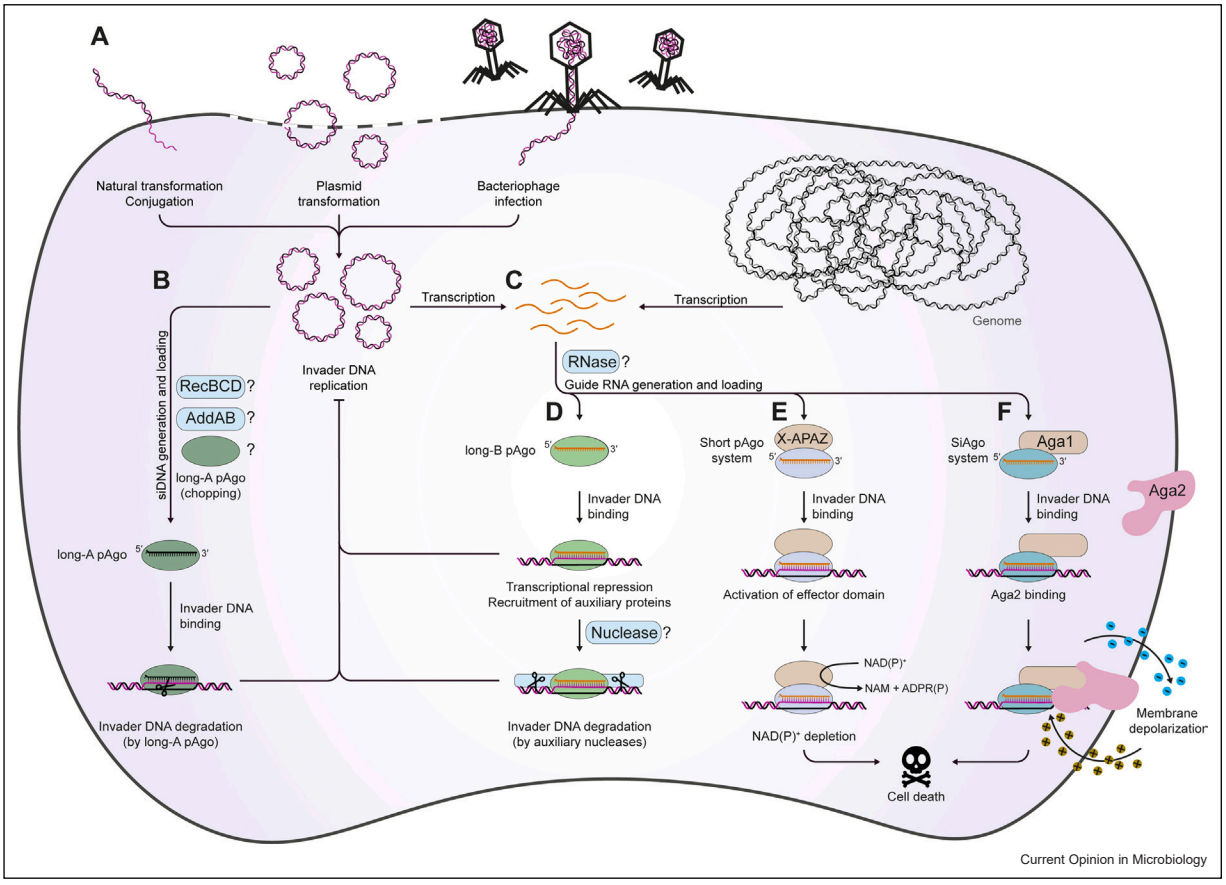
Figure 3



General pathways mediated by ePIWI-clade eAgos. **(A)** piRNA precursors are transcribed from ss piRNA clusters, from heterochromatin-induced noncanonical transcription of ds piRNA clusters, or are derived from the 3′-untranslated region (UTR) of mRNAs. **(B)** Maternally inherited ePIWI–piRNA complexes initiate piRNA processing in the cytoplasm by targeting piRNA precursors. **(C)** piRNA generation in the ping-pong cycle starts by ePIWI-mediated cleavage of a piRNA precursor. Generated pre–pre-piRNAs are loaded onto another ePIWI and further processed by a nuclease (*: Zucchini in flies). Pre-piRNAs are 3′-end-trimmed and methylated, resulting in the generation of responder ePIWI–piRNA complexes, which also target piRNA precursors completing the ping-pong cycle. **(D)** In the phased piRNA biogenesis pathway, trailing piRNAs are generated from a cleaved pre–pre-piRNA. **(E)** Cytoplasmic ePIWIs bound to piRNAs or virus-derived piRNAs (vpiRNAs) cleaves or silences endogenous mRNA transcripts, transposon transcripts, and viral RNAs. Certain ePIWI–piRNA complexes can recruit auxiliary proteins to increase translation. **(F)** Nuclear ePIWI–piRNA complexes target transcripts and induce heterochromatin formation in adjacent DNA.

It should be noted that the mechanistic and functional diversity of long pAgos is much larger than the handful of long pAgos that are currently characterized *in vivo*: *in vitro*, some long-A pAgos are guided by RNAs [23,58,59,62] or mediate DNA-guided RNA targeting [58,60,61]. Furthermore, long-A and long-B pAgos are regularly coencoded in operons with putative enzymes, which suggests they are functionally linked [1,2]. Finally, long-A pAgos that are known to interfere with MGEs have also been implicated in functions beyond immunity, including DNA replication [69,71] and DNA repair [17,72,73]. Therefore, insights obtained about

Figure 4



General pathways mediated by long-A, long-B, and (pseudo-)short pAgos. **(A)** Invading DNA enters the cell by conjugation, (natural) transformation, or bacteriophage infection and propagates in the cell. **(B)** In the long-A pAgo pathway, siDNA guides are generated in a poorly understood process by the host DNA repair machinery and/or pAgo chopping activity. Long-A pAgo–siDNA complexes bind unwound dsDNA targets and cleave the strand complementary to the siDNA, inhibiting invader propagation. **(C)** Long-B and (pseudo-)short pAgos are guided by ssRNA guides generated from mRNA transcripts. **(D)** Long-B pAgo-mediated binding of unwound dsDNA targets results in translational repression and recruitment of auxiliary nucleases that degrade the target. **(E)** Short pAgos form heterodimeric complexes with associated X-APAZ proteins and mediate RNA-guided binding of unwound dsDNA targets. This results in catalytic activation of the 'X' domain. In SPARTA and SPARSA systems, 'X' is a TIR or SIR2 NADase domain, respectively, and activation results in NAD(P)$^+$ depletion causing cell death. **(F)** Pseudoshort pAgos form a heterodimeric complex with Aga1. Upon detection of invader DNA, Aga2 is activated, which results in membrane depolarization and triggers cell death.

specific long pAgos cannot freely be extended to other pAgos even if they reside in the same phylogenetic clade (Figure 4).

Short prokaryotic Argonautes induce abortive infection

Short pAgos are defined by the presence of the MID and PIWI domain, absence of the N and PAZ domains, L1 and L2 L segments, and the lack of the DEDX tetrad in the PIWI domain [1–3,20]. Short pAgos are fused to or encoded in the same operon as an 'X'-APAZ (analog of PAZ) domain [2,3], where 'X' can be a Toll/interleukin-1 receptor (TIR)-like, SIR2, DUF4365, Mrr, or one of various other domains (Figure 1a) [1–3,20]. Short pAgos that associate with (TIR)–APAZ (SPARTA systems) or SIR2–APAZ (SPARSA systems) form heterodimeric complexes that facilitate guide RNA-mediated detection of invading plasmid and/or bacteriophage DNA [6,14,74]. Upon target binding by short pAgos, NAD(P)ase activity of the TIR or SIR2 domains is unleashed, resulting in lower cell fitness and cell death through NAD$^+$ depletion [6,14]. Other short pAgos associate with Mrr–APAZ proteins that act as random nucleases [59]. However, it is unknown if and how these and other types of short pAgos confer immunity to MGEs. The mechanisms by which short pAgo RNA guides are generated are not fully understood. Similar to long-B RsAgo [8], SPARTA randomly samples the transcriptome for guide RNAs [6]. In contrast, SPARSA-associated guide RNAs are enriched in 5′-AU sequences

expressed from genome-encoded genes and, to a lesser extent, from plasmid-encoded origin of replication (*ori*) regions [14].

Truncated pAgos comprising only the MID and PIWI domain can also be found within the long-A and long-B clades [20]. As these pAgos do not phylogenetically cluster with short pAgos and do not co-occur with X-APAZ, they are referred to as pseudoshort pAgos [20]. The pseudoshort pAgo from *Sulfolobus islandicus* (SiAgo) and its homologs forms a phylogenetic clade that is distinct from long and short pAgos [20] (Figure 1a). SiAgo does not associate with X-APAZ but is encoded in an operon with two other proteins, Aga1 and Aga2 [15]. SiAgo and Aga1 form a heterodimeric complex that *in vitro* preferentially interacts with RNA guides. Although it is unknown what guide/target combinations activate the SiAgo system, upon phage infection, SiAgo–Aga1 forms a complex with the transmembrane effector Aga2. This results in membrane depolarization by Aga2 and consequentially cell death, preventing viral proliferation [15].

Unlike eAgos and long pAgos that directly target MGEs or MGE-encoded transcripts, (pseudo-)short pAgos and their associated proteins function as abortive infection systems: upon sequence-specific detection of MGEs by short pAgos, the associated effector proteins get activated and kill their host. This limits the propagation of the MGEs, and as such, (pseudo-)short pAgos provide population-based immunity [6,14,15,20].

Discussion

Argonautes interfere with mobile genetic elements

Given the wide conservation of Ago-mediated immunity in all domains of life, we propose that the primordial function of Agos is to protect the host against invading MGEs. While both eAgos and pAgos interfere with viruses, it is not clear whether pAgos also interfere with transposable elements. As such, elements can be present in multiple copies in the cell and can have extrachromosomal life phases, they are eligible pAgo targets. Indeed, some long-A and long-B pAgo-associated guides are enriched in sequences targeting transposons [8,12]. However, in a long-A pAgo knockout, transposase expression is not increased [75]. Furthermore, in sequenced genomes encoding pAgos, there is no evidence of decreased transposon presence [2,75].

Origin of guides and distinguishing self from nonself

In eukaryotes, specific guide biogenesis pathways and dedicated proteins that generate and load functional guides have evolved. In contrast, no dedicated guide-generating pathway or protein has been identified in prokaryotes. DNA-guided pAgos rely on 'chopping' activity of the pAgo itself [17,24,57,66] or on activity of the host DNA repair machinery [17,69,71,73,76], while RNA-guided pAgos appear to randomly sample the RNA degradome for guides [6,8]. It should be noted, however, that these guides are often purified from pAgos (over)expressed in heterologous hosts (with some exceptions [66,68]). Such analyses might not accurately represent the guides that would associate with pAgos under native expression levels in their natural hosts. However, without exception, pAgos are loaded with self-targeting guides. In the absence of a dedicated guide-generation pathway, it remains unclear how pAgos achieve MGE interference without targeting the genome and becoming a toxic burden to the host. It has been described that archaeal genomic DNA is protected from chopping by pAgo by histones [57], but it remains to be determined if this mechanism is conserved in other prokaryotes.

Inhibition of Argonaute-mediated immunity

To escape eAGO-mediated immunity, viruses infecting plants [25], insects [26], and mammals [27] encode viral suppressors of RNA interference (VSRs, Figure 2). VSRs have evolved independently and act during distinct stages of eAGO-mediated immunity: inhibition strategies include masking or degradation of dsRNA to prevent vsiRNA generation, binding to vsiRNAs to prevent loading onto eAGO, inhibition of DICER or eAGO activity, triggering eAGO degradation, inhibiting RdRP-mediated generation of secondary vsiRNAs, and inhibition of AGO-mediated DNA methylation. In turn, plants and insects evolved mechanisms to counter VSR suppression [25,26]. Finally, certain viruses encode miRNA to counteract antiviral immunity and promote virus replication [77].

The coevolution of prokaryotes and their viruses also resulted in the emergence of viral anti-immune system proteins (e.g. anti-CRISPR (Acr) [78]). Yet, no pAgo inhibitors have been identified to date, and it is unknown if eAGO-targeting VSRs also inhibit pAgo activity. If future studies uncover pAgo inhibitors, it would be interesting to learn if they are homologous to VSRs. As Acrs can be used to regulate the activity of CRISPR-based tools [78], pAgo inhibitors might allow to regulate activity of pAgo-based tools [79].

Argonaute functions beyond immunity

Beyond their function in immunity, eAgos have evolved to become key components of complex pathways that regulate endogenous gene expression both before and after transcription. While it has previously been proposed that the last eukaryotic common ancestor encoded both ePIWI and eAGO-clade eAgos [80,81], to date, no pAgo-mediated RNA-guided RNA silencing has been described *in vivo*. As pAgos closely related to eAgos mediate DNA-guided DNA cleavage, the exact origin of RNA silencing pathways remains elusive. Nevertheless,

pAgos generally do associate with ssDNA or ssRNA guides targeting native genes [7,17,65,67]. However, self-targeting guides do not appear to affect cell growth [68], but might play a role in DNA replication [69,71] and repair [17]. A recently identified clade of DNA-guided long-A pAgos strictly targets RNA [60,61], suggesting that also pAgos silence genes post transcription. On the eAgo side, direct interactions with DNA might be an underexposed aspect: hAgo2 and yeast *Kluyveromyces polysporus* eAgo catalyze ssDNA-guided RNA-cleavage activity *in vitro* [82–84], but eAgo–DNA interactions are otherwise hardly studied. Much is still to be discovered to fully understand the different function and mechanisms of the highly diverse Agos protein family.

Data Availability

No new data were used for the research described in the article.

Declaration of Competing Interest

D.C.S. has submitted several patent applications regarding the utilization of pAgos for nucleic acid detection and modification.

Acknowledgements

Research in the Argonaute field is highly extensive and diverse — here, we only describe general pathways to which many exceptions exist. We apologize to the colleagues whose relevant work cannot be described or cited here due to space limitations. We thank members of the Swarts lab for discussion. This work was supported by a Grant from the Mexican National Council of Science and Technology (CONACyT, CVU No. 682509) to P.B.U., and Grants from the Netherlands Organization for Scientific Research (NWO, 016. Veni.192.072) and the European Research Council (ERC, ERC-2020-STG 948783) to D.C.S.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. • Swarts DC, Makarova K, Wang Y, Nakanishi K, Ketting RF, Koonin EV, Patel DJ, van der Oost J: **The evolutionary journey of Argonaute proteins**. *Nat Struct Mol Biol* 2014, **21**:743-753, https://doi.org/10.1038/nsmb.2879.
This review provides a detailed overview of the different Ago domains and their functions, including the catalytic mechanism of guide-mediated target cleavage.

2. •• Ryazansky S, Kulbachinskiy A, Aravin AA: **The expanded universe of prokaryotic argonaute proteins**. *MBio* 2018, **9**:1-20, https://doi.org/10.1128/mBio.01935-18.
This bioinformatics study represents the most extensive bioinformatics study of pAgos to date.

3. Makarova KS, Wolf YI, van der Oost J, Koonin EV: **Prokaryotic homologs of Argonaute proteins are predicted to function as key components of a novel system of defense against mobile genetic elements**. *Biol Direct* 2009, **4**:29, https://doi.org/10.1186/1745-6150-4-29

4. Ozata DM, Gainetdinov I, Zoch A, O'Carroll D, Zamore PD: **PIWI-interacting RNAs: small RNAs with big functions**. *Nat Rev Genet* 2018, **20**:89-108, https://doi.org/10.1038/s41576-018-0073-3

5. Park MS, Sim GY, Kehling AC, Nakanishi K: **Human Argonaute2 and Argonaute3 are catalytically activated by different lengths of guide RNA**. *Proc Natl Acad Sci USA* 2020, **117**:28576-28578, https://doi.org/10.1073/pnas.2015026117

6. Koopal B, Potocnik A, Mutte SK, Aparicio-Maldonado C, Lindhoud S, Vervoort JJM, Brouns SJJ, Swarts DC: **Short prokaryotic Argonaute systems trigger cell death upon detection of invading DNA**. *Cell* 2022, **185**:1471-1486, https://doi.org/10.1016/j.cell.2022.03.012

7. • Swarts DC, Jore MM, Westra ER, Zhu Y, Janssen JH, Snijders AP, Wang Y, Patel DJ, Berenguer J, Brouns SJJ, van der Oost J: **DNA-guided DNA interference by a prokaryotic Argonaute**. *Nature* 2014,258-261, https://doi.org/10.1038/nature12971.
This study provided the first insights into DNA-guided DNA interference by the long-A pAgo from *Thermus thermophilus*.

8. • Olovnikov I, Chan K, Sachidanandam R, Newman DK, Aravin AA: **Bacterial Argonaute Samples the Transcriptome to Identify Foreign DNA**. *Mol Cell* 2013, **51**:594-605, https://doi.org/10.1016/J.MOLCEL.2013.08.014.
This study provided the first insights into RNA-guided DNA interference by the long-B pAgo from *Rhodobacter sphaeroides*.

9. Wang Y, Sheng G, Juranek S, Tuschl T, Patel DJ: **Structure of the guide-strand-containing argonaute silencing complex**. *Nature* 2008, **456**:209-213, https://doi.org/10.1038/nature07315

10. Wang Y, Juranek S, Li H, Sheng G, Wardle GS, Tuschl T, Patel DJ: **Nucleation, propagation and cleavage of target RNAs in Ago silencing complexes**. *Nature* 2009, **461**:754-761, https://doi.org/10.1038/nature08434

11. Wang Y, Juranek S, Li H, Sheng G, Tuschl T, Patel DJ: **Structure of an argonaute silencing complex with a seed-containing guide DNA and target RNA duplex**. *Nature* 2008, **456**:921-926, https://doi.org/10.1038/nature07666

12. Nakanishi K, Weinberg DE, Bartel DP, Patel DJ: **Structure of yeast Argonaute with guide RNA**. *Nature* 2012, **486**:368-374, https://doi.org/10.1038/nature11211

13. Schirle NT, MacRae IJ: **The crystal structure of human argonaute2**. *Science* 2012, **336**:1037-1040, https://doi.org/10.1126/science.1221551

14. Zaremba M, Dakineviciene D, Golovinas E, Zagorskaitė E, Stankunas E, Lopatina A, Sorek R, Manakova E, Ruksenaite A, Silanskas A, Asmontas S, Grybauskas A, Tylenyte U, Jurgelaitis E, Grigaitis R, Timinskas K, Venclovas Č, Siksnys V: **Short prokaryotic Argonautes provide defence against incoming mobile genetic elements through NAD+ depletion**. *Nat Microbiol* 2022, **7**:1857-1869, https://doi.org/10.1038/s41564-022-01239-0

15. Zeng Z, Chen Y, Pinilla-Redondo R, Shah SA, Zhao F, Wang C, Hu Z, Wu C, Zhang C, Whitaker RJ, She Q, Han W: **A short prokaryotic Argonaute activates membrane effector to confer antiviral defense**. *Cell Host Microbe* 2022, **30**:930-943.e6, https://doi.org/10.1016/J.CHOM.2022.04.015

16. Swarts DC, Hegge JW, Hinojo I, Shiimori M, Ellis MA, Dumrongkulraksa J, Terns RM, Terns MP, van der Oost J, van der Oost J: **Argonaute of the archaeon *Pyrococcus furiosus* is a DNA-guided nuclease that targets cognate DNA**. *Nucleic Acids Res* 2015, **43**:5120-5129, https://doi.org/10.1093/nar/gkv415

17. • Kuzmenko A, Oguienko A, Esyunina D, Yudin D, Petrova M, Kudinova A, Maslova O, Ninova M, Ryazansky S, Leach D, Aravin AA, Kulbachinskiy A: **DNA targeting and interference by a bacterial Argonaute nuclease**. *Nature* 2020, **587**:632-637, https://doi.org/10.1038/s41586-020-2605-1.
This study provided the first proof that long-A pAgos interfere with bacteriophages and gives insights into guide generation by the DNA repair complex RecBCD.

18. Wilson RC, Doudna JA: **Molecular mechanisms of RNA interference**. *Annu Rev Biophys* 2013, **42**:217, https://doi.org/10.1146/ANNUREV-BIOPHYS-083012-130404

19. Sheng G, Zhao H, Wang J, Rao Y, Tian W, Swarts DC, Van Der Oost J, Patel DJ, Wang Y: **Structure-based cleavage mechanism of Thermusthermophilus argonaute DNA guide strand-mediated DNA target cleavage**. *Proc Natl Acad Sci USA* 2014, **111**:652-657, https://doi.org/10.1073/pnas.1321032111

20. •• Koopal B, Mutte SK, Swarts DC: **A long look at short prokaryotic Argonautes**. *Trends Cell Biol* 2022, https://doi.org/10.1016/J.TCB.2022.10.005.
This review provides a comprehensive overview of all recently characterized short pAgo systems.

21. Jonas S, Izaurralde E: **Towards a molecular understanding of microRNA-mediated gene silencing**. *Nat Rev Genet* 2015, **16**:421-433, https://doi.org/10.1038/nrg3965

22. Kwak PB, Tomari Y: **The N domain of Argonaute drives duplex unwinding during RISC assembly**. *Nat Struct Mol Biol* 2012, **19**:145-151, https://doi.org/10.1038/nsmb.2232

23. Doxzen KW, Doudna JA: **DNA recognition by an RNA-guided bacterial Argonaute**. *PLoS One* 2017, **12**:1-14, https://doi.org/10.1371/journal.pone.0177097

24. Kuzmenko A, Yudin D, Ryazansky S, Kulbachinskiy A, Aravin AA: **Programmable DNA cleavage by Ago nucleases from mesophilic bacteria *Clostridium butyricum* and *Limnothrix rosea***. *Nucleic Acids Res* 2019, **47**:5822-5836, https://doi.org/10.1093/nar/gkz379

25. • Li F, Wang A: **RNA-targeted antiviral immunity: more than just RNA silencing**. *Trends Microbiol* 2019, **27**:792-805, https://doi.org/10.1016/j.tim.2019.05.007.
This review provides a comprehensive overview of antiviral eAGO pathways in plants.

26. • Bonning BC, Saleh MC: **The interplay between viruses and RNAi pathways in insects**. *Annu Rev Entomol* 2021, **66**:61-79, https://doi.org/10.1146/annurev-ento-033020-090410.
This review provides a comprehensive overview of antiviral eAGO pathways in insects.

27. • Takahashi T, Heaton SM, Parrish NF: **Mammalian antiviral systems directed by small RNA**. *PLoS Pathog* 2021, **17**:e1010091, https://doi.org/10.1371/JOURNAL.PPAT.1010091.
This review provides a comprehensive overview of antiviral eAGO pathways in animals.

28. • Gammon DB: ***Caenorhabditis elegans* as an emerging model for virus-host interactions**. *J Virol* 2017, **91**:1-7, https://doi.org/10.1128/jvi.00509-17.
This review provides a comprehensive overview of antiviral eAGO pathways in worms.

29. Claycomb JM: **Ancient endo-siRNA pathways reveal new tricks**. *Curr Biol* 2014, **24**:R703-R715, https://doi.org/10.1016/j.cub.2014.06.009

30. Bartel DP: **Metazoan microRNAs**. *Cell* 2018, **173**:20-51, https://doi.org/10.1016/J.CELL.2018.03.006

31. Wang J, Mei J, Ren G: **Plant microRNAs: biogenesis, homeostasis, and degradation**. *Front Plant Sci* 2019, **10**:360, https://doi.org/10.3389/FPLS.2019.00360

32. Tomari Y, Zamore PD: **Perspective: machines for RNAi**. *Genes Dev* 2005, **19**:517-529, https://doi.org/10.1101/gad.1284105

33. Sala L, Chandrasekhar S, Vidigal JA: **AGO unchained: canonical and non-canonical roles of Argonaute proteins in mammals**. *Front Biosci* 2020, **25**:1-42, https://doi.org/10.2741/4793

34. Lee CH, Carroll BJ: **Evolution and diversification of small RNA pathways in flowering plants**. *Plant Cell Physiol* 2018, **59**:2169-2187, https://doi.org/10.1093/PCP/PCY167

35. Golden DE, Gerbasi VR, Sontheimer EJ: **An inside job for siRNAs**. *Mol Cell* 2008, **31**:309-312, https://doi.org/10.1016/j.molcel.2008.07.008

36. Drinnenberg IA, Weinberg DE, Xie KT, Mower JP, Wolfe KH, Fink GR, Bartel DP: **RNAi in budding yeast**. *Science* 2009, **326**:544, https://doi.org/10.1126/SCIENCE.1176945

37. Wang J, Jia ST, Jia S: **New insights into the regulation of heterochromatin**. *Trends Genet* 2016, **32**:284-294, https://doi.org/10.1016/J.TIG.2016.02.005

38. Castel SE, Martienssen RA: **RNA interference in the nucleus: roles for small RNAs in transcription, epigenetics and beyond**. *Nat Rev Genet* 2013, **14**:100-112, https://doi.org/10.1038/nrg3355

39. Sijen T, Fleenor J, Simmer F, Thijssen KL, Parrish S, Timmons L, Plasterk RHA, Fire A: **On the role of RNA amplification in dsRNA-triggered gene silencing**. *Cell* 2001, **107**:465-476, https://doi.org/10.1016/S0092-8674(01)00576-1

40. Smialowska A, Djupedal I, Wang J, Kylsten P, Swoboda P, Ekwall K: **RNAi mediates post-transcriptional repression of gene expression in fission yeast *Schizosaccharomyces pombe***. *Biochem Biophys Res Commun* 2014, **444**:254-259, https://doi.org/10.1016/J.BBRC.2014.01.057

41. Andika IB, Kondo H, Suzuki N: **Dicer functions transcriptionally and posttranscriptionally in a multilayer antiviral defense**. *Proc Natl Acad Sci USA* 2019, **116**:2274-2281, https://doi.org/10.1073/PNAS.1812407116

42. Shapiro JS, Schmi S, Aguado LC, Sabin LR, Yasunaga A, Shim JV, Sachs D, Cherry S, Oever BRT: **Drosha as an interferon-independent antiviral factor**. *Proc Natl Acad Sci USA* 2014, **111**:7108-7113, https://doi.org/10.1073/pnas.1319635111

43. Vagin VV, Sigova A, Li C, Seitz H, Gvozdev V, Zamore PD: **A distinct small RNA pathway silences selfish genetic elements in the germline**. *Science* 2006, **313**:320-324, https://doi.org/10.1126/science.1129333

44. Lewis SH, Quarles KA, Yang Y, Tanguy M, Frézal L, Smith SA, Sharma PP, Cordaux R, Gilbert C, Giraud I, Collins DH, Zamore PD, Miska EA, Sarkies P, Jiggins FM: **Pan-arthropod analysis reveals somatic piRNAs as an ancestral defence against transposable elements**. *Nat Ecol Evol* 2017, **2**:174-181, https://doi.org/10.1038/s41559-017-0403-4

45. Brennecke J, Aravin AA, Stark A, Dus M, Kellis M, Sachidanandam R, Hannon GJ: **Discrete small RNA-generating Loci as master regulators of transposon activity in Drosophila**. *Cell* 2007, **128**:1089-1103, https://doi.org/10.1016/J.CELL.2007.01.043

46. •• Wang X, Ramat A, Simonelig M, Liu MF: **Emerging roles and functional mechanisms of PIWI-interacting RNAs**. *Nat Rev Mol Cell Biol* 2022, **24**:1-19, https://doi.org/10.1038/s41580-022-00528-0.
This review provides a comprehensive overview of ePIWI pathways.

47. Gainetdinov I, Colpan C, Arif A, Cecchini K, Zamore PD: **A single mechanism of biogenesis, initiated and directed by PIWI proteins, explains piRNA production in most animals**. *Mol Cell* 2018, **71**:775-790.e5, https://doi.org/10.1016/j.molcel.2018.08.007

48. Reuter M, Berninger P, Chuma S, Shah H, Hosokawa M, Funaya C, Antony C, Sachidanandam R, Pillai RS: **Miwi catalysis is required for piRNA amplification-independent LINE1 transposon silencing**. *Nature* 2011, **480**:264-267, https://doi.org/10.1038/nature10672

49. Aravin AA, Sachidanandam R, Bourc'his D, Schaefer C, Pezic D, Toth KF, Bestor T, Hannon GJ: **A piRNA pathway primed by individual transposons is linked to de novo DNA methylation in mice**. *Mol Cell* 2008, **31**:785-799, https://doi.org/10.1016/j.molcel.2008.09.003

50. Sienski G, Dönertas D, Brennecke J: **Transcriptional silencing of transposons by Piwi and maelstrom and its impact on chromatin state and gene expression**. *Cell* 2012, **151**:964-980, https://doi.org/10.1016/J.CELL.2012.10.040

51. Santos D, Verdonckt TW, Mingels L, van den Brande S, Geens B, van Nieuwerburgh F, Kolliopoulou A, Swevers L, Wynant N, vanden Broeck J: **PIWI proteins play an antiviral role in Lepidopteran cell lines**. *Viruses* 2022, **14**:1442, https://doi.org/10.3390/V14071442/S1

52. Miesen P, Girardi E, van Rij RP: **Distinct sets of PIWI proteins produce arbovirus and transposon-derived piRNAs in *Aedes aegypti* mosquito cells**. *Nucleic Acids Res* 2015, **43**:6545-6556, https://doi.org/10.1093/NAR/GKV590

53. Wu PH, Fu Y, Cecchini K, Özata DM, Arif A, Yu T, Colpan C, Gainetdinov I, Weng Z, Zamore PD: **The evolutionarily conserved piRNA-producing locus pi6 is required for male mouse fertility**. *Nat Genet* 2020, **52**:728-739, https://doi.org/10.1038/s41588-020-0657-7

54. Rouget C, Papin C, Boureux A, Meunier AC, Franco B, Robine N, Lai EC, Pelisson A, Simonelig M: **Maternal mRNA deadenylation**

and decay by the piRNA pathway in the early Drosophila embryo. *Nature* 2010, **467**:1128-1132, https://doi.org/10.1038/nature09465

55. Dufourt J, Bontonou G, Chartier A, Jahan C, Meunier AC, Pierson S, Harrison PF, Papin C, Beilharz TH, Simonelig M: **PiRNAs and Aubergine cooperate with Wispy poly(A) polymerase to stabilize mRNAs in the germ plasm**. *Nat Commun* 2017, **8**:1-12, https://doi.org/10.1038/s41467-017-01431-5

56. Dai P, Wang X, Gou LT, Li ZT, Wen Z, Chen ZG, Hua MM, Zhong A, Wang L, Su H, Wan H, Qian K, Liao L, Li J, Tian B, Li D, Fu XD, Shi HJ, Zhou Y, Liu MF: **A translation-activating function of MIWI/piRNA during mouse spermiogenesis**. *Cell* 2019, **179**:1566-1581.e16, https://doi.org/10.1016/j.cell.2019.11.022

57. Zander A, Willkomm S, Ofer S, van Wolferen M, Egert L, Buchmeier S, Stöckl S, Tinnefeld P, Schneider S, Klingl A, Albers SV, Werner F, Grohmann D: **Guide-independent DNA cleavage by archaeal Argonaute from *Methanocaldococcus jannaschii***. *Nat Microbiol* 2017, **2**:1-10, https://doi.org/10.1038/nmicrobiol.2017.34

58. Liu Y, Li W, Jiang X, Wang Y, Zhang Z, Liu Q, He R, Chen Q, Yang J, Wang L, Wang F, Ma L: **A programmable omnipotent Argonaute nuclease from mesophilic bacteria *Kurthia massiliensis***. *Nucleic Acids Res* 2021, **49**:1597-1608, https://doi.org/10.1093/nar/gkaa1278

59. Kim S-Y, Jung Y, Lim D: **Argonaute system of *Kordia jejudonensis* is a heterodimeric nucleic acid-guided nuclease**. *Biochem Biophys Res Commun* 2020, **525**:755-758, https://doi.org/10.1016/J.BBRC.2020.02.145

60. Lisitskaya L, Shin Y, Agapov A, Olina A, Kropocheva E, Ryazansky S, Aravin AA, Esyunina D, Murakami KS, Kulbachinskiy A: **Programmable RNA targeting by bacterial Argonaute nucleases with unconventional guide binding and cleavage specificity**. *Nat Commun* 2022, **13**:4624, https://doi.org/10.1038/s41467-022-32079-5

61. Li W, Liu Y, He R, Wang L, Wang Y, Zeng W, Zhang Z, Wang F, Ma L: **A programmable pAgo nuclease with RNA target preference from the psychrotolerant bacterium *Mucilaginibacter paludis***. *Nucleic Acids Res* 2022, **50**:5226, https://doi.org/10.1093/NAR/GKAC315

62. Kaya E, Doxzen KW, Knoll KR, Wilson RC, Strutt SC, Kranzusch PJ, Doudna JA: **A bacterial Argonaute with noncanonical guide RNA specificity**. *Proc Natl Acad Sci USA* 2016, **113**:4057-4062, https://doi.org/10.1073/pnas.1524385113

63. Ma JB, Yuan YR, Meister G, Pei Y, Tuschl T, Patel DJ: **Structural basis for 5′ -end-specific recognition of guide RNA by the *A. fulgidus* Piwi protein**. *Nature* 2005, **434**:666-670, https://doi.org/10.1038/nature03514

64. Yuan YR, Pei Y, Ma JB, Kuryavyi V, Zhadina M, Meister G, Chen HY, Dauter Z, Tuschl T, Patel DJ: **Crystal structure of *A. aeolicus* argonaute, a site-specific DNA-guided endoribonuclease, provides insights into RISC-mediated mRNA cleavage**. *Mol Cell* 2005, **19**:405-419, https://doi.org/10.1016/j.molcel.2005.07.011

65. Lisitskaya L, Kropocheva E, Agapov A, Prostova M, Panteleev V, Yudin D, Ryazansky S, Kuzmenko A, Aravin AA, Esyunina D, Kulbachinskiy A: **Bacterial Argonaute nucleases reveal different modes of DNA targeting in vitro and in vivo**. *BioRxiv* 2022, https://doi.org/10.1101/2022.09.09.507302

66. Swarts DC, Szczepaniak M, Sheng G, Chandradoss SD, Zhu Y, Timmers EM, Zhang Y, Zhao H, Lou J, Wang Y, Joo C, van der Oost J: **Autonomous generation and loading of DNA guides by bacterial Argonaute**. *Mol Cell* 2017, **65**:985-998.e6, https://doi.org/10.1016/j.molcel.2017.01.033

67. Hegge JW, Swarts DC, Chandradoss SD, Cui TJ, Kneppers J, Jinek M, Joo C, van der Oost J: **DNA-guided DNA cleavage at moderate temperatures by *Clostridium butyricum* Argonaute**. *Nucleic Acids Res* 2019, **47**:5809-5821, https://doi.org/10.1093/nar/gkz306

68. Olina A, Kuzmenko A, Ninova M, Aravin AA, Kulbachinskiy A, Esyunina D: **Genome-wide DNA sampling by Ago nuclease from the cyanobacterium *Synechococcus elongatus***. *RNA Biol* 2020, **17**:677-688, https://doi.org/10.1080/15476286.2020.1724716

69. Jolly SM, Gainetdinov I, Jouravleva K, Zhang H, Strittmatter L, Bailey SM, Hendricks GM, Dhabaria A, Ueberheide B, Zamore PD: ***Thermus thermophilus* Argonaute functions in the completion of DNA replication**. *Cell* 2020, **182**:1545-1559.e18, https://doi.org/10.1016/j.cell.2020.07.036

70. Xun G, Liu Q, Chong Y, Guo X, Li Z, Li Y, Fei H, Li K, Feng Y: **Argonaute with stepwise endonuclease activity promotes specific and multiplex nucleic acid detection**. *Bioresour Bioprocess* 2021, **8**:1-12, https://doi.org/10.1186/s40643-021-00401-6

71. Olina A, Agapov A, Yudin D, Kuzmenko A, Aravin AA, Kulbachinskiy A: **Bacterial Argonaute proteins aid cell division in the presence of topoisomerase inhibitors in *Escherichia coli***. *BioRxiv* 2022, https://doi.org/10.1101/2022.09.13.507849

72. Fu L, Xie C, Jin Z, Tu Z, Han L, Jin M, Xiang Y, Zhang A: **The prokaryotic Argonaute proteins enhance homology sequence-directed recombination in bacteria**. *Nucleic Acids Res* 2019, **47**:3568-3579, https://doi.org/10.1093/nar/gkz040

73. Huang S, Wang K, Mayo SL: **Genome manipulation by guide-directed Argonaute cleavage**. *Nucleic Acids Research* 2023,1-8, https://doi.org/10.1093/nar/gkad188

74. Garb J, Lopatina A, Bernheim A, Zaremba M, Siksnys V, Melamed S, Leavitt A, Millman A, Amitai G, Sorek R: **Multiple phage resistance systems inhibit infection via SIR2-dependent NAD+ depletion**. *Nat Microbiol* 2022, **7**:1849-1856, https://doi.org/10.1038/s41564-022-01207-8

75. Swarts DC, Koehorst JJ, Westra ER, Schaap PJ, van der Oost J: **Effects of Argonaute on gene expression in *Thermus thermophilus***. *PloS one* 10; 2015,1-13, https://doi.org/10.1371/journal.pone.0124880

76. Esyunina D, Okhtienko A, Olina A, Panteleev V, Prostova M: **AA Aravin and A Kulbachinskiy, Specific targeting of plasmids with Argonaute enables genome editing**. *Nucleic Acids Research* 2023,1-14, https://doi.org/10.1101/2022.04.14.488398

77. Cullen BR: **MicroRNAs as mediators of viral evasion of the immune system**. *Nat Immunol* 2013, **14**:205-210, https://doi.org/10.1038/ni.2537

78. Jia N, Patel DJ: **Structure-based functional mechanisms and biotechnology applications of anti-CRISPR proteins**. *Nat Rev Mol Cell Biol* 2021, **22**:563-579, https://doi.org/10.1038/s41580-021-00371-9

79. Kropocheva EV, Lisitskaya LA, Agapov AA, Musabirov AA, Kulbachinskiy AV, Esyunina DM: **Prokaryotic Argonaute proteins as a tool for biotechnology**. *Mol Biol* 2022, **56**:854-873, https://doi.org/10.1134/S0026893322060103

80. Shabalina SA, Koonin EV: **Origins and evolution of eukaryotic RNA interference**. *Trends Ecol Evol* 2008, **23**:578-587, https://doi.org/10.1016/J.TREE.2008.06.005

81. Koonin EV: **Evolution of RNA- and DNA-guided antivirus defense systems in prokaryotes and eukaryotes: common ancestry vs convergence**. *Biol Direct* 2017, **12**:5, https://doi.org/10.1186/s13062-017-0177-2

82. Willkomm S, Zander A, Grohmann D, Restle T: **Mechanistic insights into archaeal and human argonaute substrate binding and cleavage properties**. *PLoS One* 2016, **11**:e0164695, https://doi.org/10.1371/JOURNAL.PONE.0164695

83. Lima WF, Wu H, Nichols JG, Sun H, Murray HM, Crooke ST: **Binding and cleavage specificities of human Argonaute2**. *J Biol Chem* 2009, **284**:26017-26028, https://doi.org/10.1074/JBC.M109.010835

84. Dayeh DM, Cantara WA, Kitzrow JP, Musier-Forsyth K, Nakanishi K: **Argonaute-based programmable RNase as a tool for cleavage of highly-structured RNA**. *Nucleic Acids Res* 2018, **46**:e98-e98, https://doi.org/10.1093/NAR/GKY496