REVIEW

A Small-RNA Perspective on Gametogenesis, Fertilization, and Early Zygotic Development

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Transient populations of cis- and trans-acting small RNAs have recently emerged as key regulators of extensive epigenetic changes taking place during periconception, which encompasses gametogenesis, fertilization, and early zygotic development. These small RNAs are not only important to maintain genome integrity in the gametes and zygote, but they also actively contribute to assessing the compatibility of parental genomes at fertilization and to promoting long-term memory of the zygotic epigenetic landscape by affecting chromatin. Striking parallels exist in the biogenesis and modus operandi of these molecules among diverse taxa, unraveling universal themes of small-RNA–mediated epigenetic reprogramming during sexual reproduction.

Protecting the sperm and oocyte, assessing their compatibility at fertilization, and safeguarding the progeny are key stages of sexual reproduction during which genome integrity must be monitored and preserved from the effects of potentially harmful genomic parasites including transposons. A major, pan-eukaryotic safeguard mechanism during this important time window is RNA silencing, whereby 20- to 35-nt-long RNA species guide transcriptional (TGS) or posttranscriptional gene silencing (PTGS) of complementary DNA or RNA, respectively. Silencing small RNAs fall into three categories [reviewed in (*1*)]. Small interfering RNA (siRNA) populations are produced from long double-stranded RNAs (dsRNAs) arising from converging or overlapping transcription; inverted gene duplication; or, in some organisms, through RNA-dependent RNA polymerases (RDRs) acting on single-stranded RNA. Discrete micro-RNA (miRNA) species, by contrast, are excised from stem-loop precursors transcribed from genetically defined loci. siRNAs and miRNAs accumulate in both soma and germline, are processed as 20- to 24-nt species by ribonuclease III–like Dicer enzymes, and are loaded into Argonaute (AGO) effector proteins that often display endonucleolytic ("slicer") activity on target RNA. A third small-RNA class, about 30 nt long, is made up of PIWI-associated RNAs (piRNAs), which are mostly germline-specific and Dicer independent, and are effected through AGO-like PIWI proteins (Fig. 1A).

This review examines recent evidence supporting central roles for transposon-derived siRNAs and piRNAs during pre- and postzygotic reproduction stages. The amplified nature, noncell autonomy and trans-targeting capacity of these molecules not only allow protection of gametes' genomes (preparation phase) but also might actively contribute to assessing parental compatibility at fertilization (confrontation phase) in *Drosophila*, *Arabidopsis*, and ciliates. Remarkably, when they are translocated into the nucleus, these small RNAs may also guide retrograde mechanisms, which allow chromatin-based reinforcement and potential memory of these events in the zygote and developing embryo (consolidation phase). We discuss further the possible impact of potentially related phenomena in mammalian reproduction.

Preparation

During preparation, small-RNA–based mechanisms monitor and enforce gametic genome integrity, which is required for successful fertilization. A double-layer piRNA system protects the *Drosophila* female germline against two distinct threats (Fig. 2A). First, Gypsy-like long terminal repeat (LTR) retroviruses proliferating in surrounding, somatic follicle cells can infect the oocyte as follicle cells feed the growing germline. Follicle cells are able to capture de novo integrating transposons (*2*, *3*) through genomic clusters epitomized by the FLAMENCO locus, whose transcription produces antisense, PIWI-bound piRNAs that guide degradation of complementary Gypsy-like mRNAs (Fig. 2A, top). Second, the female germline itself, composed of the haploid oocyte and polyploid nurse cells, faces the threat of many endogenous transposon types. These are silenced through the amplified "ping-pong" mechanism initiated in piRNA clusters, such as the 42AB locus, which display a wider transposon-trapping spectrum than FLAMENCO (Fig. 1A and Fig. 2A, bottom) (*4*). Ping-pong piRNAs are likely produced in nurse cells and fed to the quiescent meiotic oocyte through cytoplasmic bridges; their biogenesis within the germline-specific "Nuage" compartment requires, in addition to PIWI, the PIWI-like proteins Ago3 and Aubergine (*4*). Mutations in any of these components cause transposon mobilization and female sterility. Comparatively, maintenance of gametic genome integrity is more enigmatic in the male germline, where the piRNA pathway seems less specialized for transposon targeting (*5*). It notably controls the male germ cell–specific *Stellate* gene amplified on the X chromosome; Stellate protein aggregation into crystals is thought to compromise male gamete integrity (*6*).

Plant genomes lack PIWI proteins and adaptive piRNA genomic clusters. Consequently, revealing transposons' presence in gametophytes paradoxically entails their reactivation in accessory or nursing cells by overcoming two independent, yet often overlapping, mechanisms for transposon silencing (*7*). A first, innate TGS process involves the activities of MET1, which maintains DNA methylation at symmetric CG sites, and of DDM1, a SWI-SNF chromatin-remodeling factor with intrinsic affinity for transposons and/or repeats (Fig. 1B, top). Second, a plant-specific polymerase, PolIV (p4), can back up MET1-DDM1 action at some loci, owing to a presumed affinity for methylated or heterochromatic DNA. RDR2 converts PolIV transcripts into dsRNAs, which are further processed by Dicer-like protein DCL3 into 24-nt-long "p4" siRNAs. The p4 siRNAs guide AGO4 back onto the DNA, in cis, by recruiting a second plant-specific polymerase, PolV. AGO4-PolV complexes then attract de novo DNA methyltransferases, which target cytosines in all sequence contexts (CG, CHG, and CHH) (H = A, T, or C) (Fig. 1B, middle). Non-CG methylation, notably, provides a diagnostic signature of this back-up system. Only its combined loss with that of MET1-DDM1 allows PolII-dependent transcription and mobilization of transposons (*8*, *9*). As an ultimate back-up process, highly accumulating transposons may undergo posttranscriptional gene silencing, presumably by means of the RDR6-DCL4-AGO1–dependent pathway that normally silences transgenes through 21-nt-long siRNAs (Fig. 1B, bottom) (*7*, *10*). Both p4 and 21-nt siRNAs are mobile in *Arabidopsis*, which allows TGS and PTGS transmission not only between neighboring cells—for instance, those found in gametophytes (see below)—but also between distant organs, through the vascular system (*11*, *12*).

During female gametogenesis in angiosperm plants, such as *Arabidopsis*, the haploid egg cell and the homodiploid central cell—respectively precursors of the diploid embryo and triploid endosperm—are formed. Owing to MET1 downregulation during gametogenesis (*13*), a first wave of p4 siRNA production uncovers the presence and identity of transposons. Strictly restricted to the central cell is the additional and coincident induction of DEMETER (DME), a DNA glycosylase that erases cytosine methylation in all

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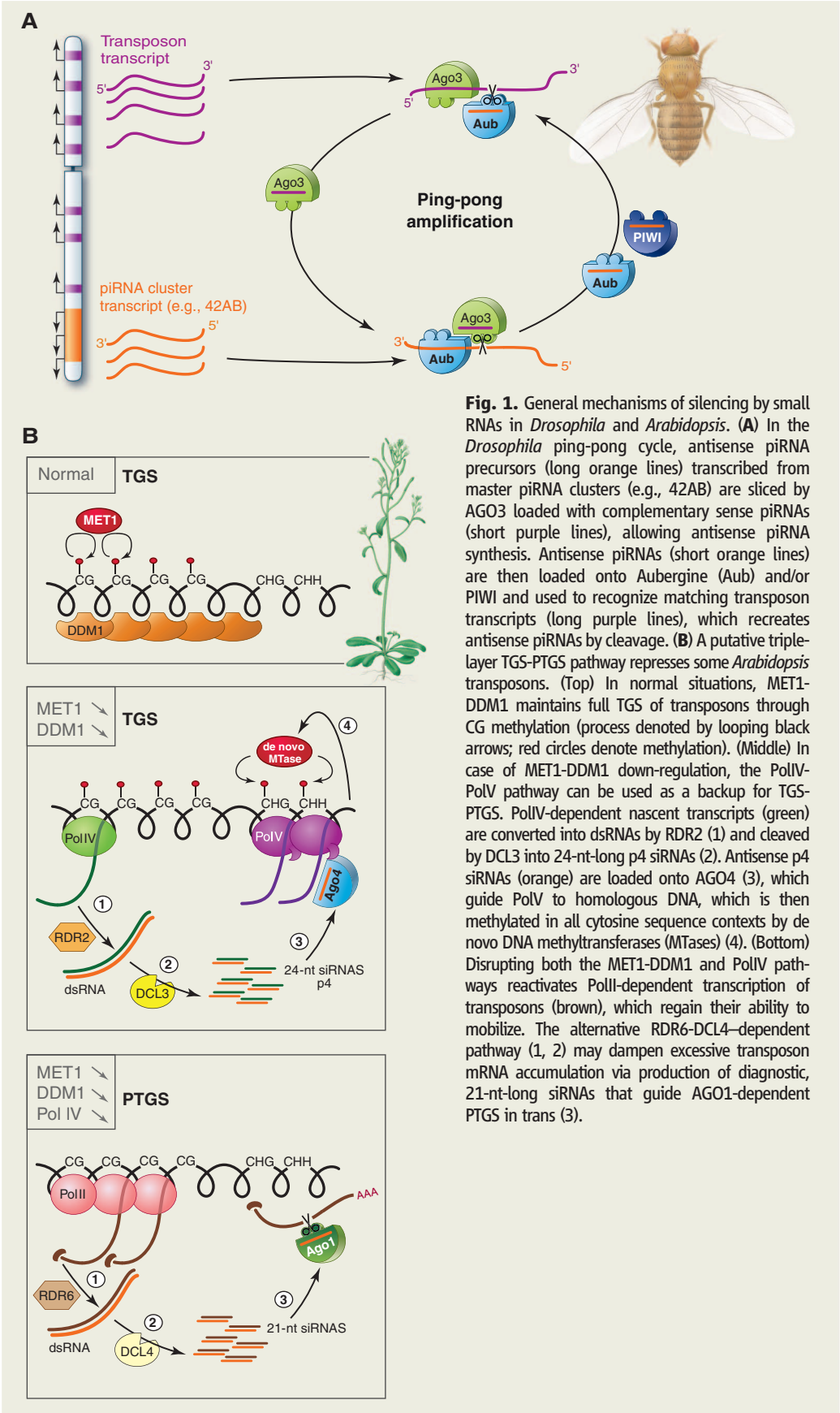

Fig. 1. General mechanisms of silencing by small RNAs in *Drosophila* and *Arabidopsis*. (**A**) In the *Drosophila* ping-pong cycle, antisense piRNA precursors (long orange lines) transcribed from master piRNA clusters (e.g., 42AB) are sliced by AGO3 loaded with complementary sense piRNAs (short purple lines), allowing antisense piRNA synthesis. Antisense piRNAs (short orange lines) are then loaded onto Aubergine (Aub) and/or PIWI and used to recognize matching transposon transcripts (long purple lines), which recreates antisense piRNAs by cleavage. (**B**) A putative triple-layer TGS-PTGS pathway represses some *Arabidopsis* transposons. (Top) In normal situations, MET1-DDM1 maintains full TGS of transposons through CG methylation (process denoted by looping black arrows; red circles denote methylation). (Middle) In case of MET1-DDM1 down-regulation, the PolIV-PolV pathway can be used as a backup for TGS-PTGS. PolIV-dependent nascent transcripts (green) are converted into dsRNAs by RDR2 (1) and cleaved by DCL3 into 24-nt-long p4 siRNAs (2). Antisense p4 siRNAs (orange) are loaded onto AGO4 (3), which guide PolV to homologous DNA, which is then methylated in all cytosine sequence contexts by de novo DNA methyltransferases (MTases) (4). (Bottom) Disrupting both the MET1-DDM1 and PolIV pathways reactivates PolII-dependent transcription of transposons (brown), which regain their ability to mobilize. The alternative RDR6-DCL4–dependent pathway (1, 2) may dampen excessive transposon mRNA accumulation via production of diagnostic, 21-nt-long siRNAs that guide AGO1-dependent PTGS in trans (3).

sequence contexts (Fig. 2B) (*14*). ROS3, a protein required for DME-like protein action, binds small RNAs in vitro and in vivo (*15*); it is therefore tempting to speculate that the first wave of p4 siRNAs might direct the DME-dependent genome-wide demethylation typically observed in the central cell and, after fertilization, in the endosperm (*16*, *17*). The ensuing transposon reactivation is likely to stimulate strongly the PolIV back-up pathway (*8*, *9*), and this probably explains the massive central cell– and endosperm-specific accumulation of p4 siRNAs seen in female gametophytes (*18*). These abundant p4 siRNAs are then potentially available to enforce transposon silencing through non-CG methylation in the egg cell and future embryo (Fig. 2B). Similarly, in the male gametophyte (pollen), which is composed of two haploid sperm cells and one accessory vegetative cell, only the vegetative cell relaxes transcriptional gene silencing of transposons, through the simultaneous down-regulation of DDM1 and dampening of the PolIV pathway (Fig. 2C) (*19*). As a backup, the vegetative cell uses PTGS to degrade the now highly abundant transcripts from mobilizing transposons, generating 21-nt-long siRNAs with intrinsic trans-activity (*19*). Should transposons escape the robust TGS set in sperm cells, these mobile 21-nt siRNAs can potentially provide an additional protection layer through PTGS and, perhaps, TGS via non-CG methylation (Fig. 2C). Therefore, accessory or nursing cells of plant gametophytes apparently lose their genome integrity in order to supply gametes with protective siRNAs.

In unicellular ciliates such as *Tetrahymena* and *Paramecium*, the diploid micronucleus (mic) fulfils germline functions, whereas the polyploid macronucleus (mac) ensures gene expression during vegetative growth. Although they occur post-zygotically, mechanisms for maintenance of genome stability in ciliates are conceptually similar to those observed in pollen, except that the germline equivalent (mic) is here involved in ensuring the integrity of the somatic genome (mac) (*20*). In the preparation phase, the meiotic mic exits quiescence to produce

DICER-dependent "scan" RNAs (scnRNAs), which map to all germline sequences, including transposons, whereas the mac transcribes longer, noncoding RNAs (ncRNAs), which represent an RNA "cache" of the somatic genome (Fig. 2D). After loading into meiosis-specific PIWI-like proteins (*21*), trans-acting scnRNAs then confront the mac-derived ncRNAs, as detailed below.

Confrontation

During confrontation, the genomic compatibility of gametes and/or nuclei is "assessed" through RNA-RNA interactions, preventing parental genomes' toxicity to the zygote. Confrontation in *Drosophila* primarily relies on ping-pong piRNAs that have accumulated in the oocyte (Fig. 3A). In the embryo, maternally deposited antisense piRNAs can initiate novel ping-pong cycles against any matching, sperm-delivered sense transposon (*22*). Although not formally demonstrated, a similar scenario of parental RNA confrontation may apply in plants. Double fertilization of the egg and the central cells by each sperm cell produces the embryo and the nurturing endosperm, respectively. Abundant maternal p4 siRNAs that have accumulated in the central cell could predictably direct non-CG methylation of matching, paternal transposons brought by the sperm into the endosperm. Conversely, sperm-derived 21-nt siRNAs could prevent excessive maternal transposition in the hypomethylated endosperm. In the embryo, these paternal 21-nt siRNAs could degrade transposon RNAs that have potentially escaped TGS (Fig. 3B). In ciliates, confrontation proceeds through a subtractive hybridization, where mic-derived scnRNAs are neutralized by annealing to complementary, mac-derived ncRNAs (Fig. 3C) (*20*). Noncomplementary scnRNAs originating from the mic genome escape this filtering and are thus singularized as nonself sequences,

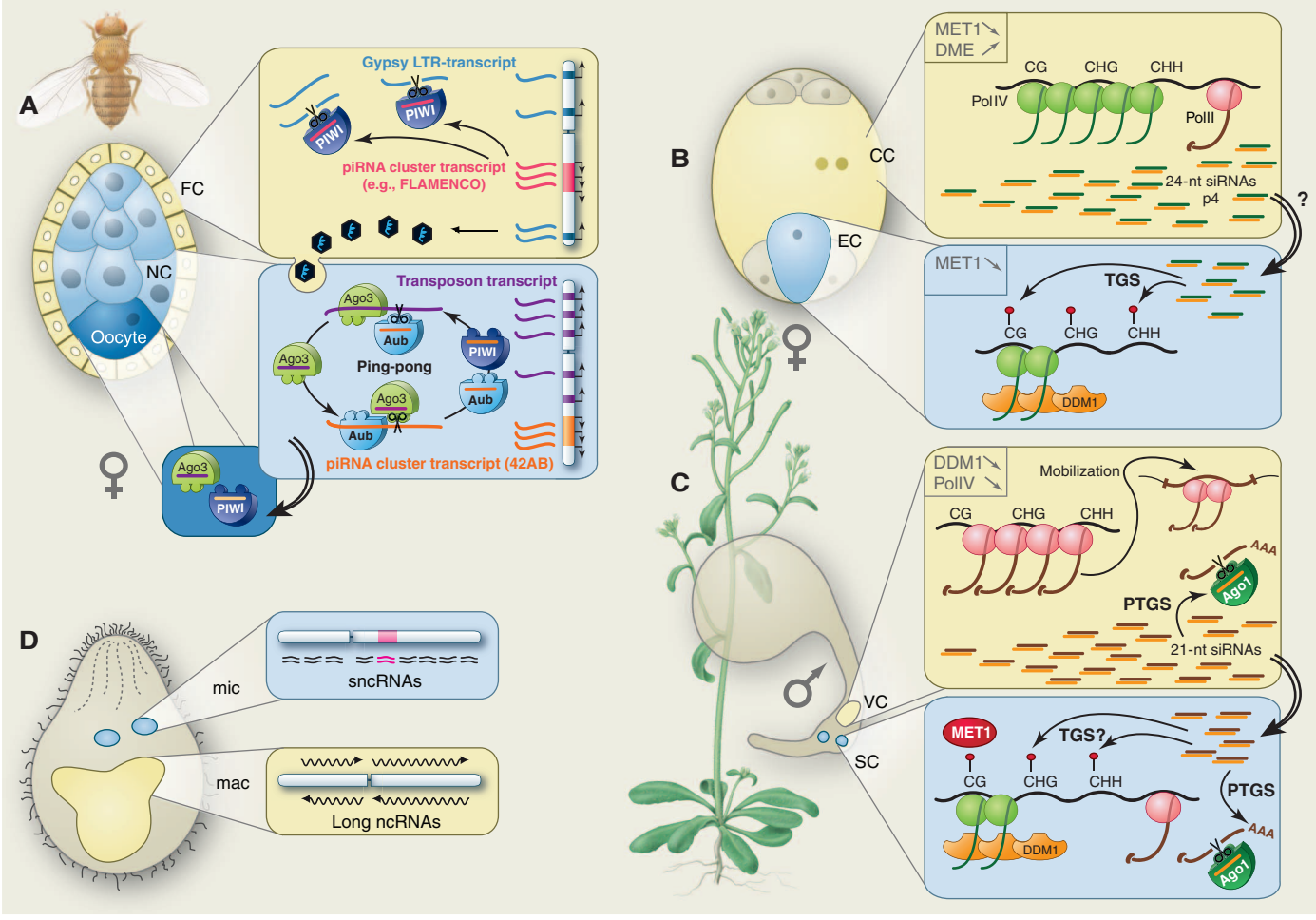

Fig. 2. Preparation phase in *Drosophila*, *Arabidopsis*, and ciliates. Accessory or nursing cells are depicted in yellow; germline cells, in blue. Molecular components are represented with the same color codes as in Fig. 1. (**A**) In *Drosophila* ovarioles, FLAMENCO-like piRNA clusters (red) in follicle cells (FC) feed a PIWI-dependent linear piRNA pathway that silences Gypsy-like retroviruses (blue), which can otherwise form infectious particles and invade the germline. A ping-pong–based piRNA pathway (Fig. 1A) operates in the germline nurse cells (NC) surrounding the haploid oocyte (darker blue), in which PIWI-Aub– and AGO3-bound piRNAs are likely deposited (double-line arrow). NC polyploidy may further enhance the ping-pong potency against transposons. (**B**) In the *Arabidopsis* female gametophyte, loss of MET1 occurs both in the diploid central cell (CC) and the haploid egg cell (EC). Simultaneous DME activation in the CC causes genome-wide demethylation only in this cell type, incurring strong production of PolIV-dependent, 24-nt-long p4 siRNAs (green and orange lines as in Fig. 1B) and, potentially, some PolII-dependent transcription. p4 siRNAs may move into the EC (double-line arrow) to enforce TGS through CG and non-CG methylation (CHG and CHH). The repressive state is potentially maintained by DDM1. (**C**) In the *Arabidopsis* male gametophyte, combined loss of DDM1 and PolIIV strongly reactivates PolII-dependent transcription and mobilization of transposons in the vegetative cell (VC). Excessive transposon levels promote massive production of 21-nt siRNAs (brown and orange lines) and PTGS via AGO1 (as in Fig. 1B). In the two sperm cells (SC), MET1 and DDM1 are present to provide full TGS of transposons. Nonetheless, in case of relaxation, 21-nt siRNAs delivered by the VC (double-line arrow) can enforce transposon silencing by PTGS, and maybe TGS through CG and non-CG methylation. (**D**) In *Paramecium*, scnRNAs and long ncRNAs are produced from the entire genome of the germline-like mic and somatic mac nuclei, respectively. The two nuclei may differ in their DNA content (pink chromosomal region in the mic nucleus and pink lines denoting scnRNAs from this region).

which will require elimination in the new zygotic mac (*23*).

Consolidation

To consolidate the outcome of preparation and confrontation, RNA-based information, labile by nature, is converted into chromatin-based information, which allows long-term perpetuation of embryonic and adult epigenetic landscapes. In *Drosophila*, piRNAs guide PTGS in the cytoplasmic Nuage structure, but PIWI also interacts in the nucleus with a variant of heterochromatin protein–1 (HP1α) (*24*). Disruption of piRNA biogenesis or function promotes a shift in transposon chromatin states from inactive to active (*25*), which suggests that PIWI-bound piRNAs guide the process of heterochromatinization and long-term TGS of at least some target loci (Fig. 3A). This process could, in principle, unfavorably silence master ping-pong piRNA clusters (e.g., 42AB) and ultimately compromise the pathway's adaptive potential, but a distinct HP1 homolog, Rhino, apparently prevents this by competing with HP1α at the 42AB locus (Fig. 3A) (*26*). In plants, the endosperm feeds the embryo with nutrients, and so p4 siRNAs may conceivably also use this path to reinforce silencing in the embryo, which indeed displays characteristic non-CG methylation at transposons or repeats (Fig. 3B) (*16*). The ultimate consolidation scheme appears to function in ciliates, where unwanted DNA is physically eliminated at once. Hence, mic-derived, PIWI-bound scnRNAs that have previously escaped subtractive hybridization guide repressive histone marks at homologous loci in the zygotic mac (*27*), from which DNA is excised (Fig. 3C).

When the System Fails: Hybrid Incompatibilities

Postfertilization barriers between distant strains or species often cause sterility or lethality in hybrid offspring. Given the processes discussed above, hybrid incompatibility might sometimes entail qualitative and/or quantitative inadequacies between transposon RNAs and transposon-derived small RNAs contributed by each confronting gamete or nucleus. In support of this view, transposons are reliable markers of genetic distance and speciation (*28*). In *Drosophila*, maternal ping-pong piRNAs can, in principle, accommodate infinite quantities of matching transposon RNAs delivered by the sperm (Figs. 1A and 3A). The system fails, however, if paternal transposons are too sequence-divergent to be recognized by the maternal piRNA reservoir (Fig. 4A). This incurs "hybrid dysgenesis," where transposons invading the developing embryonic germline cause genome instability and, ultimately, sterility in the offspring (*22*, *29*). A related mechanism may partly explain phenotypes of hybrid seed abortion observed in interspecific *Arabidopsis* crosses, which were indeed correlated with the massive activation of paternal ATHILA transposons (*30*). Here, hybrid failure could result

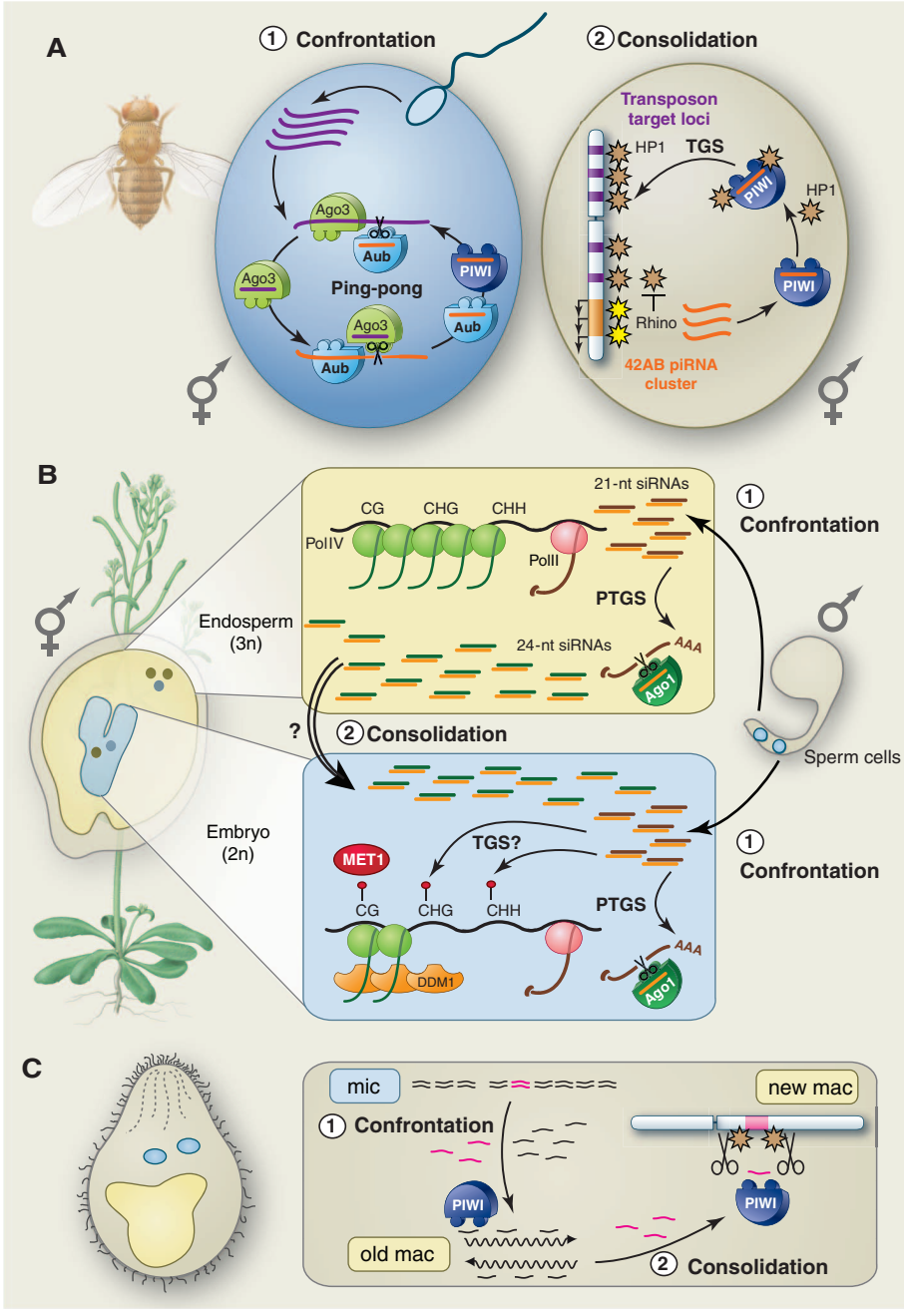

Fig. 3. Mechanisms for confrontation and consolidation. (**A**) In the *Drosophila* confrontation phase (1), Aub-PIWI- and Ago3-bound piRNAs deposited in the oocyte before fertilization protect the zygote against any matching, sperm-delivered transposon (purple), through the adaptive ping-pong pathway. During consolidation (2), PIWI-bound piRNAs can further direct heterochromatinization and TGS of target transposon loci (purple) via HP1 (brown stars). The HP1 homolog Rhino (yellow stars) prevents TGS of master ping-pong piRNA clusters of the 42AB type (orange), thereby preserving primary piRNA production. (**B**) In *Arabidopsis*, confrontation (1) might occur at fertilization through putative delivery of 21-nt siRNAs by the sperm cells into the CC (yellow) and the EC (blue), giving rise, respectively, to the triploid endosperm and diploid embryo. These 21-nt siRNAs can potentially direct PTGS (via AGO1) of complementary transposon RNAs escaping silencing, and they may also trigger transposon TGS by recruiting the DNA methylation machinery (as in Fig. 1B). TGS in the embryo (blue) can be potentially consolidated (2) by delivery of p4 siRNAs previously produced in the CC and fed by the endosperm (double-line arrow). (**C**) In ciliates, confrontation (1) consists in the filtering of most PIWI-bound mic-derived scnRNAs by long mac-derived ncRNAs (as in Fig. 2D). In the consolidation phase (2), remaining scnRNAs (pink) target heterochromatinization (brown stars) and excision of complementary sequences in the new zygotic mac.

either from excessive divergence between sequences of paternal transposon RNAs and maternal transposon-derived siRNAs or from an inadequate dosage of each protagonist at fertilization (Fig. 4A) (*31*). Consistent with the latter proposal, increasing maternal ploidy can override parental incompatibility in *Arabidopsis* (*30*). Similarly, one could envision that precise stoichiometry between confronting RNAs from the mic and the mac nuclei might influence ciliates' reproductive success (Fig. 4A). Hence, crucial portions of the mac genome may be erroneously eliminated in case of overrepresentation of mic-derived scnRNAs or underrepresentation of mac-derived ncRNAs. Conversely, overaccumulating ncRNAs may prevent elimination of unwanted sequences in the mac genome.

What About Mammals?

Are some of the above concepts applicable to mammals? As in *Drosophila*, the mammalian germline produces amplifiable piRNAs (*32*), and similarly to plants, genome-wide demethylation unmasks transposons (*33*), although mammalian gametes do not rely on accessory or nursing cells for this. Although impairing piRNA biogenesis or function reactivates transposons and compromises fertility in males (*33*), any similar function in oocytes is less evident. Nonetheless, dysgenesis-related phenomena are common among hybrid mammals, and we anticipate that some may involve inadequacies between transposon RNAs and silencing small RNAs brought by one or both parents into the zygote. In support of this view, transposon RNA can be carried over from parents to embryo in LINE1 transgenic mice (*34*), and amplification of endogenous retroelements occurs in hybrid progenies of distant kangaroo species (*35*). Moreover, crosses between females of the DDK inbred mouse strain and non-DDK males incur early embryonic lethality (DDK syndrome), owing to incompatibility between a maternally deposited factor and a paternal locus (*36*). Remarkably, the DDK susceptibility locus is strongly enriched in LTR and long interspersed nuclear element (LINE) repeats (*37*). Perhaps the DDK syndrome results from paternal transposon expansions that cannot be qualitatively or quantitatively matched by the female DDK factor, which might be small RNAs. Consistent with this idea, siRNAs and moderate amounts of piRNAs are detected in mammalian oocytes (*38*). Recent evidence suggests that the quiescent mammalian sperm also contains small RNAs (*39*), although whether they can be transmitted during fertilization is unknown. Nonetheless, ectopic miRNAs delivered by mouse sperm were shown to promote gene expression changes that, remarkably, persist in progenies (*40*).

Conclusions and Perspectives

RNA cross talk between nuclei or cells might thus represent widely used mechanisms for gametic or zygotic genome integrity and hybrid compatibility. Although presented merely from the standpoint of genome protection, the processes evoked here may also implicate non-transposon small RNAs with regulatory rather than defensive functions, such as miRNAs and trans-acting siRNAs. In *Brassica*, small RNAs produced during gamete preparation can overcome inbreeding barriers and therefore expand mating possibilities (*41*); specific to the diploid phase of heterozygous pollens, a dominant allele produces small RNAs that mediate in trans DNA methylation and repression of the recessive allele of a key locus involved in pollen or pistil recognition. Expression or sequence polymorphisms between regulatory small RNAs in one parent and their targets in the other could also influence hybrid compatibility during the confrontation phase. How hypothetical, maternal mRNAs encoding toxic products could act as fertilization gateways through their specific inactivation by matching paternal siRNAs or miRNAs is depicted in Fig. 4B. Excessive phylogenetic distance between the two parents would, this way, be strongly counterselected. Recent evidence for miRNA-directed DNA methylation in plants (*42*) further suggests a possible zygotic or embryonic consolidation of such putative events. Finally, in *Arabidopsis*, stress-responsive loci produce mobile small RNAs with the potential to invade the germline (*43*), whereas in *Drosophila*, aging and temperature influence female sterility in nonmendelian ways reminiscent of piRNA action (*44*). It is thus conceivable that environmental cues perceived in parents might not only affect epigenetic transgenerational memory (*43*), but also the mere capacity to pass their genome to subsequent generations.

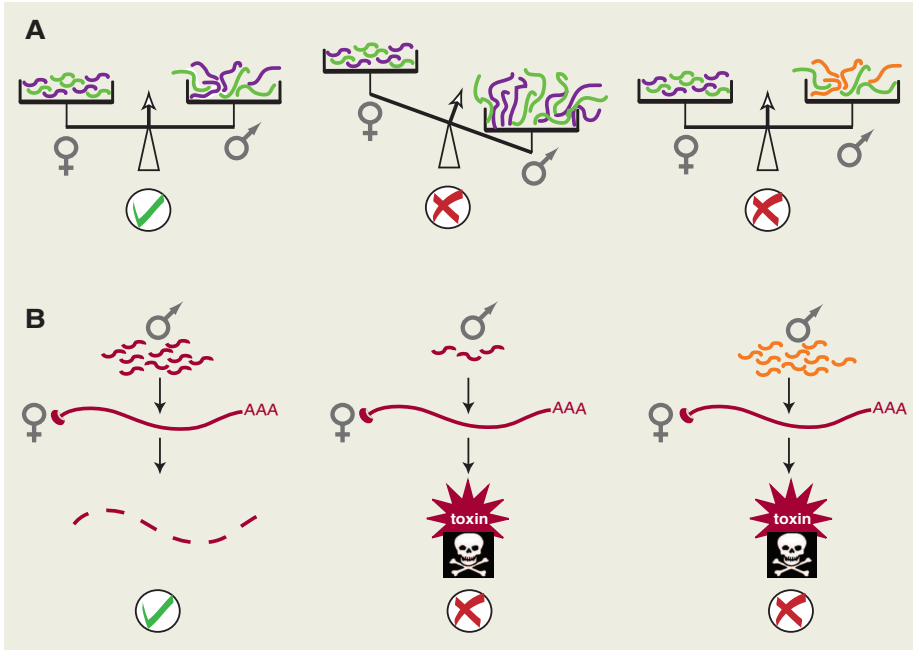

Fig. 4. Possible mechanisms underlying hybrid incompatibility. (**A**) Compatibility is fulfilled when appropriate levels of female small-RNA pools match transposon RNAs delivered by the male (left). Incompatibility may occur because of quantitative (middle) or qualitative (right) inadequacies between these two parental RNA populations. (**B**) Compatibility checking might also hypothetically involve maternal mRNAs (capped and polyadenylated), for instance, encoding a lethal toxin, that act as sentinels of features of sperm-delivered siRNAs or miRNAs. Degradation of maternal toxic mRNAs by paternal small RNAs would allow further development (left), while quantitative (middle) or qualitative (right) inadequacies would incur incompatibility. Schemes in (A) to (B) could apply to crosses in both parental directions.

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REVIEW

Epigenetic Reprogramming in Plant and Animal Development

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Epigenetic modifications of the genome are generally stable in somatic cells of multicellular organisms. In germ cells and early embryos, however, epigenetic reprogramming occurs on a genome-wide scale, which includes demethylation of DNA and remodeling of histones and their modifications. The mechanisms of genome-wide erasure of DNA methylation, which involve modifications to 5-methylcytosine and DNA repair, are being unraveled. Epigenetic reprogramming has important roles in imprinting, the natural as well as experimental acquisition of totipotency and pluripotency, control of transposons, and epigenetic inheritance across generations. Small RNAs and the inheritance of histone marks may also contribute to epigenetic inheritance and reprogramming. Reprogramming occurs in flowering plants and in mammals, and the similarities and differences illuminate developmental and reproductive strategies.

Epigenetic marks are enzyme-mediated chemical modifications of DNA and of its associated chromatin proteins. Although they do not alter the primary sequence of DNA, they also contain heritable information and play key roles in regulating genome function. Such modifications—including cytosine methylation, posttranslational modifications of histone tails and the histone core, and the positioning of nucleosomes (histone octamers wrapped with DNA)—influence the transcriptional state and other functional aspects of chromatin. For example, methylation of DNA and certain residues on the histone H3 N-terminal tail [e.g., H3 lysine 9 (H3K9)] are important for transcriptional gene silencing and the formation of heterochromatin. Such marks are essential for the silencing of nongenic sequences—including transposons, pseudogenes, repetitive sequences, and integrated viruses—that could become deleterious to cells if expressed and hence activated. Epigenetic gene silencing is also important in developmental phenomena such as imprinting in both plants and mammals, as well as in cell differentiation and reprogramming.

DNA methylation occurs in three different nucleotide sequence contexts: CG, CHG, and CHH (where H = C, T, or A). In both mammals and plants, CG methylation is maintained by the maintenance DNA methyltransferase, termed DNMT1 [DNA (cytosine-5)-methyltransferase 1] in mammals and MET1 (DNA METHYLTRANSFERASE 1) in *Arabidopsis*, and by a cofactor that recognizes hemimethylated DNA at replication foci, called UHRF1 (ubiquitin-like containing PHD and RING finger domains 1) in mammals and VIM (VARIATION IN METHYLATION) family proteins in *Arabidopsis* (*1*). In addition, the mammalian de novo DNA methyltransferases DNMT3A and Dnmt3b are required for the maintenance of CG methylation at some loci (*2*). CHG methylation is common in *Arabidopsis* and other plant genomes and is maintained by a feedforward loop that is formed by a plant-specific DNA methyltransferase, CMT3 (CHROMOMETHYLASE 3), and a histone methyltransferase, KYP (KRYPTONITE) (*1*, *3*, *4*). CHH methylation is also abundant in plants and is maintained by the RNA-directed DNA methylation (RdDM) pathway, which actively targets the DNA methyltransferase DRM2 (DOMAINS REARRANGED METHYLTRANSFERASE 2; a homolog of Dnmt3) to DNA by means of 24-nucleotide (nt) small interfering RNAs (siRNAs) bound by AGO4 (ARGONAUTE 4) (*1*) (Fig. 1). CHG and CHH methylation are also present at detectable levels in mammals, especially in stem cells, and this methylation is likely introduced by DNMT3A and DNMT3B (*5*, *6*). De novo methylation of DNA in all of these sequence contexts is generally established by the DNMT3 (mammals) and DRM2 (*Arabidopsis*) methyltransferases. Mammals do not have an *Arabidopsis*-like RNA-directed DNA methylation pathway, but in germ cells, PIWI-associated RNAs (piRNAs) are thought to guide DNMT3 activity (*7*). Mammals have a noncatalytic paralog of de novo methyltransferase, DNMT3L, which interacts with DNMT3A and unmethylated H3K4 (as does DNMT3A and DNMT3B) (*8*–*10*); these findings imply a targeting mechanism of these methyltransferases to chromatin. Unmethylated CpG islands are specifically bound by CFP1 (CXXC finger protein–1), which in turn recruits the histone H3K4 methyltransferase SETD1 (SET domain containing–1) (*11*); this suggests that H3K4 methylation, and therefore exclusion of DNMT3 from CpG islands, could help to explain how promoters remain unmethylated. Consistent with this idea, demethylation of H3K4 has been shown to be important for the acquisition of DNA methylation in imprinted genes in oocytes (*12*). Additionally, transcription can also help to establish de novo DNA methylation at imprinted regions (*13*). Earlier this year, it was shown that the nucleosome landscape also influences the methylation patterning in both plant and animal genomes (*14*).

Some histone modifications are also thought to be actively maintained during DNA replication, in part facilitated by the association of the histone modification enzymes with the DNA replication machinery. For example, the mammalian histone H3K9 methyltransferases G9A and SETDB1 (SET domain bifurcated–1), the mammalian H4K20 methyltransferase SETD8 (SET domain containing–8), and the plant histone H3K27 monomethyltransferases ATXR5 (ARABIDOPSIS TRITHORAX-RELATED PROTEIN 5) and ATXR6 interact with the replication protein PCNA (proliferating cell nuclear antigen) (*15*, *16*). However, histone methylation can also be very dynamic and is controlled

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A Small-RNA Perspective on Gametogenesis, Fertilization, and Early Zygotic Development

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