BBA - General Subjects 1869 (2025) 130811

Contents lists available at ScienceDirect

BBA - General Subjects

journal homepage: www.elsevier.com/locate/bbagen

Factories without walls: The molecular architecture and functions of non-membrane organelles in small RNA-guided genome protection

Shinichi Kawaguchi *, Wakana Isshiki, Toshie Kai *

Graduate School of Frontier Biosciences, The University of Osaka, Osaka 565-0871, Japan

ARTICLE INFO

Keywords:
Non-membrane structure
Yb body
Nuage
piRNA
LLPS
Protein-protein interaction
AlphaFold3

ABSTRACT

Non-membrane organelles, Yb body and nuage, play an essential role in piRNA-guided genome defense in *Drosophila* gonad by mediating piRNA biogenesis and transposon silencing. Yb body, found in somatic follicle cells, is responsible for primary piRNA processing, while nuage, located in germline cells, facilitates the ping-pong cycle to amplify the piRNAs corresponding to both sense and antisense strands of the expressed transposons. These organelles are assembled by liquid-liquid phase separation (LLPS) and protein-protein interactions, integrating RNA helicases (Vasa, Armitage), Tudor domain-containing proteins (Krimper, Tejas, Qin/Kumo), and proteins containing both domains (Yb, SoYb, Spn-E). Within these condensates, we summarize the protein-protein interactions experimentally validated and predicted by AlphaFold3, providing new structural insights into the non-membrane organelle assembly. This review highlights how the dynamic organization of Yb body and nuage enables efficient RNA processing, ensuring transposon suppression and genome stability.

1. Introduction: non-membrane organelles in piRNA-mediated genome defense

Transposable elements (TEs), or "jumping genes," are mobile genetic elements that pose a significant threat to genome stability, particularly in germline cells where they can be inherited by the next generation. To counteract this threat, animals that reproduce sexually have evolved robust defense mechanisms to safeguard the genomic integrity of gametes by silencing TEs through specialized RNA pathways (for reviews, see [1,2]). Central to this defense system is the piRNA (PIWI-interacting RNA) pathway, which utilizes small RNAs with 24–29 nucleotide length to target and repress transposons at both transcriptional and post-transcriptional levels. The piRNA pathway has been identified in diverse animal species, and studies in *Drosophila melanogaster* and mice have provided pivotal models for molecular dissection of piRNA function and biogenesis [3–8].

Drosophila's genome contains over 200 distinct TEs, which are effectively silenced by the piRNA pathway through the production of complementary piRNAs. In *Drosophila*, two types of non-membrane organelles play crucial roles in piRNA biogenesis and transposon silencing: the Yb body in somatic gonadal cells and nuage in germline cells. Majority of antisense piRNA precursors are transcribed from distinct genomic loci, called piRNA clusters, that serve as reservoirs of transposon-derived sequences [3]. These precursors are exported to the cytoplasm, where they are processed within the nuage and Yb body. These non-membrane compartments act as cytoplasmic hubs, coordinating distinct steps of piRNA processing through compartmentalized and dynamic mechanisms. Yb body primarily facilitates the primary processing of piRNA precursors through phasing, generating piRNAs [9–12]. In contrast, nuage functions as the site for secondary piRNA amplification via the ping-pong cycle, a process that expands piRNA pools and reinforces transposon silencing through reciprocal cleavage of piRNA precursors and TE transcripts that are complementary to precursor-derived piRNA [3,13,14].

Rather than providing a detail of the molecular mechanisms of TE silencing by piRNAs—topics extensively covered in prior reviews [1,2]—this review focuses on the structural organization and assembly mechanisms of Yb bodies and nuage, emphasizing their roles as dynamic RNA processing hubs. We specifically examine how these non-membrane compartments, which lack lipid bilayers, assemble through dynamic interactions between RNA precursors and protein components involved in piRNA biogenesis. These structures are organized through modular protein domains, including RNA-binding motifs, Tudor domains, and scaffolding regions, which mediate protein-protein and protein-RNA interactions.

In addition to these domain-mediated interactions, liquid-liquid

* Corresponding authors.
E-mail addresses: kawaguchi.shinichi.fbs@osaka-u.ac.jp (S. Kawaguchi), kai.toshie.fbs@osaka-u.ac.jp (T. Kai).

https://doi.org/10.1016/j.bbagen.2025.130811
Received 15 February 2025; Received in revised form 10 April 2025; Accepted 16 April 2025
Available online 3 May 2025

phase separation (LLPS) plays a critical role in driving the assembly and function of these organelles. Key components of these structures, including the RNA helicases Yb and Vasa, (Vas) as well as Krimper (Krimp), a Tudor domain-containing protein, exhibit features associated with LLPS [9,13,15,16]. These proteins form dynamic condensates through multivalent interactions and intrinsically disordered regions (IDRs), contributing to the formation and stabilization of piRNA-processing hubs. The pioneer study about the biochemical LLPS properties have shown that the N-terminal IDR of Vas family/DDX4 proteins contribute to the LLPS, and its droplet formation is negatively regulated by the methylation on the side chain of arginine residue within the IDR [17]. Interestingly, arginine residues on N-terminal moiety of the PIWI family proteins are also known to be symmetrically di-methylated [13,18]. Post-translational modifications (PTMs) such as arginine methylation are important regulatory factors for the non-membrane structure and function (see also below).

Although this review primarily focuses on Yb body and nuage in *Drosophila*, we also briefly address related structures in other organisms

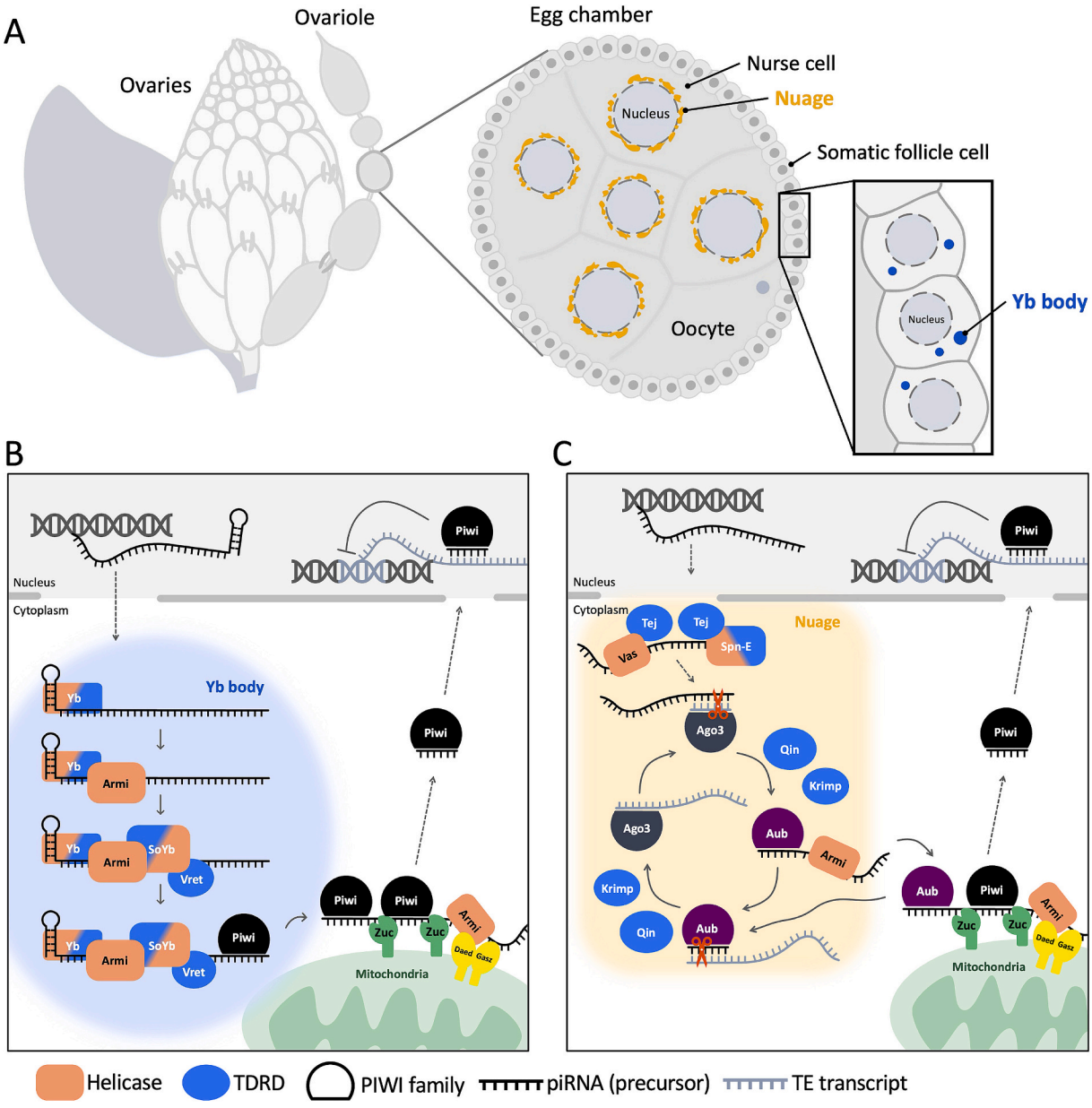

Fig. 1. Schematic representation of piRNA pathway-related structures and proteins in the *Drosophila* ovary.
(A) Schematic illustration showing the organization of *Drosophila* ovaries: whole ovaries containing 18-20 of ovarioles (left), and an individual egg chamber (middle). Each egg chamber contains germline cells (15 nurse cells and one oocyte) and a monolayer of somatic follicle cells. Amorphous nuage (orange) localizes around nurse cell nuclei. The inset (right) depicts somatic follicle cells containing Yb bodies (blue) in their cytoplasm. (B) piRNA processing at Yb body in somatic follicle cells. Yb protein catches piRNA precursors, Armi, SoYb/Vret are sequentially assembled, and finally Piwi binds to the precursor, forming functional Yb body. This pre-piRISC and Armi then translocate to mitochondria adjacent to Yb body, owing to Armi-Daed/Gasz binding. After cleaving and phasing of piRNA precursors by Zuc, Piwi-piRISC enters the nucleus and silences transposon transcription through downstream proteins. (C) piRNA processing in germline nurse cells. Vas_Tej and Spn-E_Tej complexes interact with piRNA precursor and localize it in nuage. Aub and Ago3 cooperate to generate piRNA and silence transposon post-transcriptionally by cleaving piRNA precursor and transposon transcript. Qin and Krimp facilitate the shuttling of RNA intermediates between Aub and Ago3, promoting the ping-pong cycle. Aub- and Armi-bound pre-piRNA translocate to mitochondria where they are phased and loaded onto Piwi, which then silences transposons transcriptionally in the nucleus, while Ago3 and Aub are engaged predominantly for pong-pong cycle, leading post-transcriptional silencing.

to provide additional context. For example, the chromatoid body in mouse spermatocytes performs comparable functions in piRNA biogenesis [19]. Similarly, perinuclear P granules in *C. elegans* produce piRNAs, albeit through distinct mechanisms specific to nematodes. Despite these differences, *C. elegans* piRNAs associate with PIWI family proteins and Tudor domain-containing proteins, underscoring conserved roles in transposon repression [4,5]. These comparisons highlight the functional diversity of non-membrane organelles while illustrating their shared roles in RNA-guided genome defense.

Here, we discuss the molecular architecture of Yb body and nuage, highlighting key protein components such as RNA helicases, Tudor domain proteins, Lotus domain-containing proteins, and scaffolding factors [15,20–24]. We examine their biochemical properties and domain structures, focusing on how these features drive LLPS assembly and RNA processing. To complement experimental findings, we integrate structural predictions using AlphaFold3 server (https://alphafoldserver.com/) to propose hypotheses about interaction networks and protein complexes within these organelles [25,26]. Through this approach, we aim to provide a framework for understanding how dynamic molecular condensates regulate piRNA pathways, protecting genome from transposon activity.

2. Yb body: The hub for primary piRNA processing in somatic gonadal cells

The piRNA pathway also operates to repress TEs in *Drosophila* somatic follicle cells, as several LTR family members, such as Zam and Gypsy, are known to often invade to oocytes via vitellogenin secretion pathway, posing a direct threat to genomic stability in the germline [27,28]. In somatic follicle cells, piRNAs are produced by a mechanism called “Primary Processing” (reviewed in [1,2]). First, long single-stranded precursor transcripts are transcribed from piRNA clusters, which are specialized genomic loci that contain sequences complementary to various transposon RNAs (Fig. 1A, B) [3]. For example, precursors are abundantly transcribed from *flamenco* (*flam*) locus, a canonical piRNA cluster, in somatic cells [3,29]. These transcripts accumulate in a non-membranous structure termed Flam body or Dot COM, which forms near the *flam* locus and is juxtaposed to the Yb body [30,31]. The Flam body was reported in the cytoplasm of ovarian somatic cells (OSCs) [30], while Dot COM was identified in the nucleus of in vivo follicle cells [31]. This difference in subcellular localization may reflect the distinct properties of cultured OSC cells versus native follicle cells in vivo. After reaching the cytoplasm, *flam* transcripts are processed at Yb body and mitochondrial surface to produce short piRNAs [11,32,33]. Upon their maturation, piRNA bound Piwi proteins translocate into the nucleus, and repress transposon transcriptions at their genomic loci [34,35].

2.1. Structural features of Yb body components

Yb body is a non-membrane organelle assembled via LLPS that plays a central role in the primary processing of piRNA precursors in somatic follicle cells of the *Drosophila* ovary (Fig. 1A, B) [9]. It serves as a dynamic platform for piRNA biogenesis, organizing RNA substrates and protein components into a phase-separated condensate, where substrates are selectively engaged in further processing. Yb body is composed of four major proteins: Yb, Armitage (Armi), Sister of Yb (SoYb), and Vreteno (Vret), three of which—Yb, Armi, and SoYb—are RNA helicases, while Vret acts as an assembly factor. Knockdown studies in OSCs have shown that Yb depletion disrupts granule formation, whereas knockdowns of the other proteins do not [9]. This suggests that Yb serves as the key organizer of Yb body, by recruiting protein components and RNAs to assemble into processing hubs.

Yb protein contains four well-defined domains: the Hel-C domain at its N-terminal region, an RNA helicase domain, another Hel-C domain, and an extended Tudor (eTud) domain at its C-terminus (Fig. 2A). Except for the N-terminal Hel-C domain in Yb, the following domains, helicase, Hel-C, ZnF motif, Tudor, are highly conserved among TDRD12 family members. Notably, there are two Yb paralogs, somatic SoYb and germline BoYb. SoYb is also a component of Yb body as described below. BoYb was shown to localize to nuage, a piRNA factory in *Drosophila* germline cells although its precise function remains elusive [36]. Yb appears to be an RNA helicase, but the typical DExD motif required for ATP hydrolysis is not conserved (DNLN instead of DExD), whereas the ATP-binding motif, known as motif I, is conserved [30]. Structural prediction of Yb monomer, registered in the AlphaFold Protein Structure Database (Q9W4W2) [37], suggests that the region between N-terminal Hel-C and the helicase domain is an IDR, which may enhance phase-separation properties. The previous studies have shown that all three structured domains are necessary for Yb body formation. Two Yb mutants in the helicase domain, Q399A and D537A, lost the binding affinity to RNA and could not form Yb body foci in OSCs [30]. Both Yb mutants lacking either Hel-C or eTud domain were also dispersed in the cytoplasm without forming a granule [9].

Armi is another highly conserved 5′ to 3′ RNA helicase belonging to the superfamily I helicases [38]. It is a major protein component of Yb body and is recruited by Yb protein, engaging substrate RNAs to process [10,33]. Studies have shown that Armi is essential for substrate specificity, as ATP hydrolysis drives selective binding to piRNA precursors while excluding degraded RNAs [33,39]. Loss-of-function mutations in its ATPase activity led to indiscriminate RNA binding, including degraded RNA fragments, and impair piRNA biogenesis [12]. Armi contains two helicase domains belonging to the Upf1 superfamily and an N-terminal oligonucleotide-binding (OB) fold domains that are predicted to facilitate RNA binding [40]. Armi also harbors an Ig-like domain, which resembles motifs in MOV10 helicases, a protein involved in RNA remodeling and silencing pathways [41]. This suggests that Armi may contribute to RNA structure remodeling and complex assembly, preparing substrates for downstream processing.

SoYb, a paralog of Yb, also contains DExD-box RNA helicase and Tudor domains but includes an additional eTud domain at the N-terminus and a CS domain at the C-terminus (Fig. 2A) [36]. The CS domains are also found near the C-terminal regions in the mouse and silkworm (*Bombyx mori*) orthologs, which belong to the TDRD12 family [36,42]. BmTdrd12 localizes in a non-membrane granule of germline derived BmN4 culture cells, whereas BmTdrd12 mutant lacking the CS domain lost the localization ability and diffused in the cytoplasm [42]. These results indicated that the CS domain is required for localization to the non-membrane granule in BmN4 cells, although the molecular mechanism remain elusive [42].

Vret lacks helicase activity but features two eTud domains and an RNA recognition motif (RRM) [36]. These domains are predicted to enhance RNA-binding affinity and scaffold interactions between Yb body components and piRNA precursors. Vret has been shown to bind with SoYb independently with Yb and Armi [9]. AlphaFold3 also predicted the SoYb_Vret heterodimer model suggesting interactions between their N- and C-terminal domains, respectively (Fig. 2D). The ipTM score is the confidence score (ranging from 0 to 1) for the interacting region of the complex and the SoYb_Vret dimer shows the moderate confidence score of 0.61. Furthermore, the Predicted Aligned Error (PAE) plot generated by AlfaFold3, which represents the reliability of the relative position of residues to identify rigid domains and evaluate inter-domain or inter-protein interactions, indicates that Vret-N region containing the RRM domain has a fixed position relative to the N- and M-regions of SoYb, while the Vret-C region containing the two eTud domains is defined relative to the SoYb-C region containing the CS domain (Fig. 2A, D, right panel). Thus, SoYb and Vret may act cooperatively and reinforcing RNA binding and protein-protein interactions essential for Yb body stability.

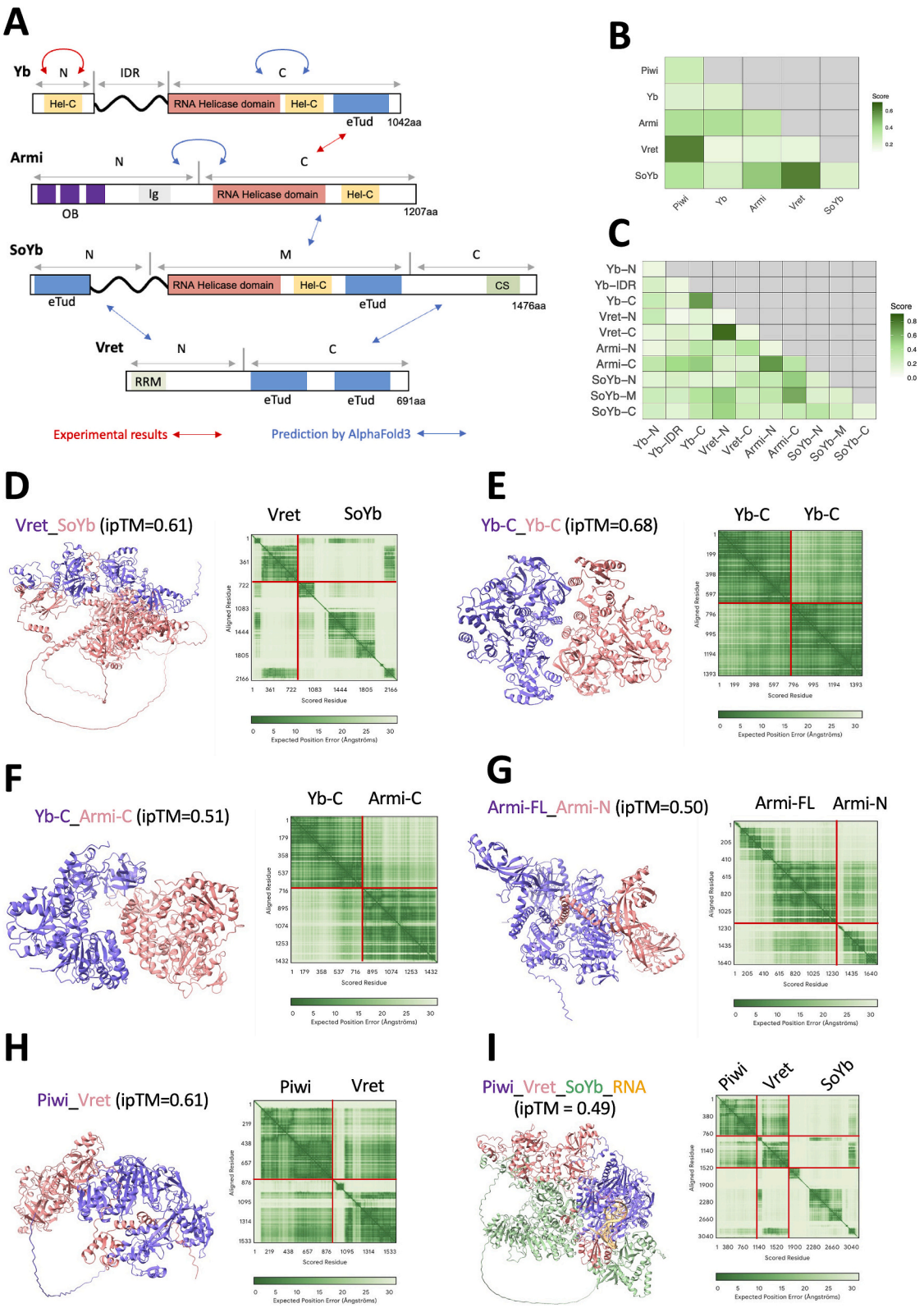

Fig. 2. The structural features and possible interactions among Yb body components.
(A) Schematic representation of the domains and IDR of Yb body components. Each protein is divided into two or three segments for domain-domain interaction analysis using AlphaFold3. Red arrows indicate experimentally validated interactions between domains, while blue arrows represent interactions predicted by AlphaFold3. Double-headed circular arrows denote the self-oligomerization. (B) The ipTM score for the protein-protein dimer model predicted by AlphaFold3. (C) The ipTM score for the domain-domain complex model predicted by AlphaFold3. (D~I) Three-dimensional structure models predicted by AlphaFold3. (D) Vret_SoYb, (E) Yb-C homodimer, (F) Yb-C_Armi-C, (G) Armi_Armi-N, (H) Piwi_Vret, and (I) Piwi_Vret_SoYb_RNA. PAE plots are also shown on the right for each structure model.

2.2. Formation and organization of Yb body

After being transcribed by RNA polymerase II and spliced, piRNA precursors are exported to the cytoplasm, where they accumulate in Yb body (Fig. 1A, B) [9,43]. Their recruitment to Yb body is mediated by specific sequence motifs and structural elements in their untranslated regions (UTRs). For instance, a stem-loop structure, called as T-hairpin, in the 3′ UTR of *traffic jam* (*tj*) mRNA, another piRNA precursor, has been identified as a cis-acting element for the piRNA production [44]. Similar structural features also exist in the major piRNA precursor, *flam* RNA, although this requires further investigation.

First, Yb body assembly is triggered by the binding of Yb protein to the cis-acting elements in the piRNA precursors. Then these RNA-protein complexes nucleate phase-separated granules. LLPS-driven granule formation often associated with the self-oligomerization of the components. In the case of Yb, N-terminal Hel-C domain facilitates its self-oligomerization [9]. However, Hel-C domain is not the sole determinant for the phase separation and Yb body formation relies on the most part of Yb protein including Hel-C, RNA helicase and eTud domains, suggesting that RNA binding and cooperative oligomerization via other domains are required to form Yb body [9]. Interestingly, structural predictions by AlphaFold3 did not support Yb's dimerization through the N-terminal Hel-C domain alone but instead predicted that dimerization via Yb-C region containing RNA helicase and eTud domains, which seemingly do not interfere its RNA binding ability (Fig. 2A, C, E).

Once Yb_RNA granules are formed, Armi is further localized there by the Yb protein, facilitating precursor RNA selection and processing. While Armi's RNA-binding ability is dispensable for its localization, its interaction with Yb's eTud domain is crucial [9,33]. AlphaFold3 predicts an interaction between the C-terminal helicase domain of Armi and the eTud domain of Yb (Fig. 2F), although the predicted model requires experimental validation. In addition, predicted Armi oligomerization predicted by AlphaFold3 suggests that Armi-C region interacts with Armi-N region of another Armi molecule and higher-order assemblies may stabilize its association with Yb and enhance RNA processing (Fig. 2G).

Subsequently, Armi further recruits the other two components, SoYb and Vret, completing Yb body assembly. While the precise roles of SoYb and Vret remain unclear, both contain RNA-binding motifs that likely retain RNA substrates in Yb body and enhance processing efficiency [36]. In addition, AlphaFold3 showed the rather high score for the interaction between Armi-C and SoYb-M regions (ipTM score = 0.63) while Vret domains were predicted in low scores with Armi domains (Fig. 2C). These predictions suggest Armi preferentially interact with SoYb to Vret. Although the detail binding model must be examined further, these protein-protein interactions, coupled with RNA-binding capabilities, stabilize the condensate and promote efficient processing of RNA precursors under phase-separated conditions.

Following RNA selection and processing in Yb body, Piwi binds to the 5′ ends of the cleaved piRNA precursors by unknown mechanism [45] and form pre-piRNA precursor RISC (pre-piRISCs) complexes [33]. Armi subsequently joins the complex and is exported to the mitochondrial surface. Notably, deletion of the N-terminal 34 residues of Armi prevents pre-piRISC from exiting the Yb body, suggesting that this region is essential for pre-piRISC translocation to mitochondria [33,46]. Further study indicated that the N-terminal region of Armi is required to bind to Daed on the mitochondria surface for the stable transfer of pre-piRISC [46]. Interestingly, AlphaFold3 predicted the additional interactions between Armi-C and SoYb-M and between Piwi and Vret (Fig. 2B, C, H). These interactions may help the multi-protein complex formation together with RNA precursors [9]. AlphaFold3 also predicted the Piwi_Vret_SoYb_RNA model (Fig. 2I), suggesting that the binding of Vret_SoYb to Piwi does not hinder the piRNA binding ability of Piwi. N-terminal region of Piwi is intrinsically disordered, contains nuclear localization signal, and undergo the methylation on Arg residues. These characteristics are important to regulate Piwi's function. In the Piwi_Vret_SoYb_RNA model (Fig. 2I), N-terminal region of Piwi protein is still exposed and seems flexible. The prediction of a multi-protein complex may be useful to speculate the functional model, although the precise molecular mechanisms underlying these interactions remain uncertain and warrant further investigation.

Yb body, therefore, acts as a dynamic hub that integrates RNA selection, protein recruitment, and RNA processing. Its phase-separated nature not only facilitates substrate recognition and piRNA precursor maturation but also coordinates downstream processing at mitochondria, ensuring the efficient generation of piRNAs required for transposon silencing [47].

3. piRNA processing at mitochondria: compartmentalization and evolution

Not only non-membrane structures such as Yb body and nuage, piRNA processing operates also on the mitochondrial outer membrane. This cellular compartmentalization plays an essential role in the precise regulation of the piRNA pathway. In *Drosophila melanogaster*, three proteins—Zuc, Daed, and Gasz—contain a single transmembrane domain to be anchored to the mitochondrial outer membrane (Fig. 1B, C) [32,46,48]. In addition, Armi possesses an N-terminal domain that interacts with Daed and Gasz, enabling it to shuttle between nuage/Yb body and the mitochondrial outer membrane [32,46]. This coordinated processing across multiple cellular compartments is a characteristic feature of both somatic and germline cells.

In *Drosophila* somatic follicle cells, Yb body is positioned near mitochondria, facilitating efficient processing. The processing occurs in a sequential manner: first, the Armi_Piwi-pre-piRNA precursor complex forms within Yb body [33]. This complex is then tethered to the mitochondrial surface through interactions between the N-terminal domains of Armi and Daed [46]. Subsequently, Zuc catalyzes the cleavage of the piRNA precursor, generating both 3′ and 5′ ends of the piRNA [49–51]. The resulting piRNA-Piwi complex dissociates from the mitochondrial surface and undergoes maturation through Hen1-mediated methylation of the piRNA ends [52]. This mature piRISC then translocate to the nucleus, where it suppresses transposons at the transcriptional level (reviewed in [2]). If the binding of Piwi and Armi to piRNA precursors is compromised, the complex remains trapped within Yb body. Interestingly, Armi remains associated with the cleaved piRNA precursor on the mitochondrial surface, facilitating the recruitment of additional Piwi molecules and subsequent Zuc-mediated cleavage events [32].

In *Drosophila* germline cells, the spatial arrangement of nuage and mitochondria differs significantly from that in follicle cells; while nuage localizes around the nuclear envelope, mitochondria are broadly distributed throughout the cytoplasm [30,53,54]. Despite this spatial separation, pre-piRISCs efficiently shuttle between these compartments. Specifically, Armi binds to the Aub-piRNA precursor complex in nuage and facilitates its recruitment to the mitochondrial surface [53]. However, the precise mechanism of this inter-compartmental transport remains elusive. A significant observation is that while Armi localizes to both nuage and mitochondria, Aub is detected only in nuage of germline cells [53]. The pre-piRISC, once tethered to mitochondria, undergoes Zuc-mediated cleavage like the somatic pathway. Subsequently, the Aub-piRNA complex returns to the nuage to participate in the "ping-pong cycle" described below. Current models suggest that Armi dissociates from the Aub-piRNA complex after cleavage and returns to the nuage to seek new partners [53]. In *Drosophila*, while the protein composition of germline nuage and somatic Yb body differs, the mitochondrial factors, Zuc, Daed and Gasz, and the shuttling factor Armi are commonly used. This suggests that the fundamental mechanism of primary processing is shared between both cell types.

Studies in mammals, particularly in mice, have revealed a structure called the inter-mitochondrial cement (IMC), a non-membrane organelle between mitochondrial outer membranes that serves as a site for piRNA processing (reviewed in [55,56]). A distinctive feature of the IMC, best

characterized in mouse spermatogenic cells, is that phase separation mediated by the coiled-coil domain of TDRD1 (a homolog of *Drosophila* Vret) is essential for both structural formation and piRNA processing [57]. Within this structure, MIWI (a mouse Piwi ortholog) is recruited by TDRKH on the surface of mitochondria outer membrane and binds to pre-piRNAs cleaved by mitoPLD/PLD6, the mammalian Zuc homolog [58–60]. The piRISC undergoes maturation through 3′ end trimming by PNLDC1 exonuclease and methylation by HENMT1, followed by MIWI arginine methylation-dependent translocation to the chromatoid body near the nuclear envelope [58,61,62]. The functional conservation of key factors like Zuc/mitoPLD and the post-translational modifications mentioned in Introduction suggests fundamental similarities in the molecular mechanisms of piRNA biogenesis across species.

4. Nuage: assembly and piRNA processing mechanisms in germline cells

Nuage is a non-membrane, perinuclear organelle found in germline cells across a wide range of animals, including *Drosophila melanogaster* and mice, and serves as a hub for piRNA biogenesis and transposon silencing (Fig. 1A, C) [6,63]. Similar structures, such as chromatoid body in mice and perinuclear germ granules in *C. elegans*, share functional parallels and molecular compositions; however, the latter exhibits fundamentally distinct mechanism of piRNA production [64]. In addition to Aub and Ago3, the two PIWI family proteins central to piRNA amplification, various nuage-localized factors have been identified which, similar to Yb body, assemble with piRNA precursors into dense granules [36,65]. These structures enable localized RNA processing, providing efficient spatial organization for enzymatic activity to amplify the mature piRNA bound with PIWI family proteins [66]. While LLPS contributes to their assembly, nuage organization also relies on additional mechanisms, including RNA-protein scaffolding and multivalent interactions [67].

4.1. The ping-pong amplification cycle

Nuage serves as the central hub for the ping-pong amplification cycle, a process critical for piRNA biogenesis and transposon silencing in germline cells (Fig. 1C). The cycle initiates when piRNA precursor transcripts from piRNA clusters are localized to the perinuclear nuage region through interaction with Vas_Tejas (Tej) and/or Spindle-E (Spn-E)_Tej complexes [21]. These precursors are loaded onto Aub to form pre-piRISC complexes, which associate with Armi and translocate to mitochondria for phasing-dependent processing [12,53]. The matured piRISCs subsequently return to nuage, where they cleave transposon transcripts, although the mechanisms governing this return remain to be elucidated. Krimp and Qin/Kumo facilitate the reciprocal RNA exchange between Aub and Ago3. Notably, Krimp's two eTud domains separately bind to Aub and Ago3, while Krimp forms homodimers, thereby enhancing the RNA transfer between these PIWI family proteins [13]. Qin specifically prevents homotypic Aub-Aub RNA transfer, thus promoting heterotypic Aub-Ago3 interactions, though the precise mechanism remains elusive [23]. Following 3′ end trimming by exonucleases and methylation, Ago3-bound transposon transcripts mature into piRISCs and cleave piRNA precursors to generate piRNA, which are complementary to transposon sequences and are retained in nuage by Tej_Spn-E and Tej_Vas complexes [3,21,52,68]. This sophisticated RNA-protein interaction network effectively suppresses transposons at the post-transcriptional level, a process enabled by nuage's unique properties as both a phase-separated compartment and a biomolecular condensate.

4.2. Domains and physical interactions of Nuage components

Given that Aub and Ago3 have been extensively reviewed elsewhere [1,2], they will not be the focus of this review. Instead, this section provides an overview of the major constituent proteins of nuage, discussing their structure and function from the perspective of their domains and interactions.

Vas is a germline-specific DEAD-box RNA helicase that has long served as a molecular marker for germline cells in many species [69]. The N-terminal region of Vas, spanning approximately 200 residues, is intrinsically disordered (Fig. 3A) [70]. Following this region, Vas contains a helicase core domain and a Hel-C domain responsible for RNA binding and remodeling activities. Structural analysis of Vas bound to single-stranded RNA (seven uridine residues) has revealed the detailed RNA-binding interface (PDB: 2DB3) [71]. Vas interacts with eLOTUS domain-containing proteins, such as Oskar (Osk) and Tej. Although eLOTUS of Osk, a protein critical for germ plasm assembly, binds to the Hel-C domain of Vas and enhances its helicase activity, whether Tej's eLOTUS binding affects Vas's activity remains unclear [72]. Computational prediction using AlphaFold3 also indicates a moderate confidence score for the interaction between Vas and Tej (Fig. 3B), consistent with experimental evidence [15,21].

Tej, a fly homolog of TDRD5, is a germline-specific protein localized to nuage [21,22]. Its domain architecture includes an eLOTUS domain at the N-terminus and an extended Tudor (eTud) domain at the C-terminus (Fig. 3A). These domains are connected by an IDR, which provides flexibility but does not appear to mediate direct interactions between two domains. Recent structural predictions and experimental data indicate that Tej's SRS (Spn-E Recruiting Sequence) adopts a helical conformation, serving as the binding interface for Spn-E helicase (Fig. 3A) [21]. Namely, Tej has binding abilities to both Vas and Spn-E, these two proteins, however, do not bind simultaneously to Tej, suggesting a regulated exchange mechanism although the dynamic control of such complexes within cells remains unclear [21]. Emerging studies have also implicated the LOTUS domain in recognizing specific G-quadruplex RNA structures [73], suggesting a broader role in RNA substrate selection.

Spn-E is a fly homolog of mammalian TDRD9 containing DEAH helicase motif. Unlike Vas, Spn-E lacks IDR but contains three domains, Hel-C, HA2 and eTud, at its C-terminal region (Fig. 3A). Its Hel-C domain mediate interactions with Tej [21]. Spn-E has nuclear localizing signal and is dynamically shuttles between the nucleus and cytoplasm, a process that may regulate precursor RNA transport [74]. Co-immunoprecipitation and mass spectrometry analysis with ovary lysate revealed that Spn-E interacts with Aub, Ago3, and other piRNA pathway proteins, including Qin and Squash (Squ), a less-characterized protein required for sufficient piRNA production [74]. AlphaFold3 predictions support direct 1:1 interactions between Spn-E and Qin, as well as Spn-E and Squ (Fig. 3B). The direct interaction between Spn-E_Squ has been characterized in S2 cells overexpressing these two proteins [26]. Possibly, the binding of Squ to Spn-E may regulate the Spn-E's helicase activity and RNA hand off.

Qin is a large nuage protein containing N-terminal RING and B-box domains followed by five Tudor domains at the C-terminus [23,24]. It is unique among nuage proteins in possessing a RING domain, typically associated with ubiquitin-like ligase activity, although its biochemical function in piRNA pathway remains unknown. Co-immunoprecipitation in S2 cells detected Qin's interactions with Spn-E and Aub, both mediated by its eTud domains [24]. AlphaFold3 predicted a moderate-confidence dimer structure for Qin_Spn-E, which Qin appears to surround the whole Spn-E (Fig. 3C).

Krimp localizes to nuage and facilitates piRNA transfer between Aub and Ago3 [13,75]. It dimerizes or oligomerizes through its N-terminal coiled-coil region, contributing to granule assembly (Fig. 3A). Krimp has two eTud domains (eTud1 and eTud2) and the eTud1 interact with AGO3 while eTud2 associates Aub [76].

4.3. Phase separation and dynamic assembly of Nuage

During these decades, the molecular functions of the piRNA-related

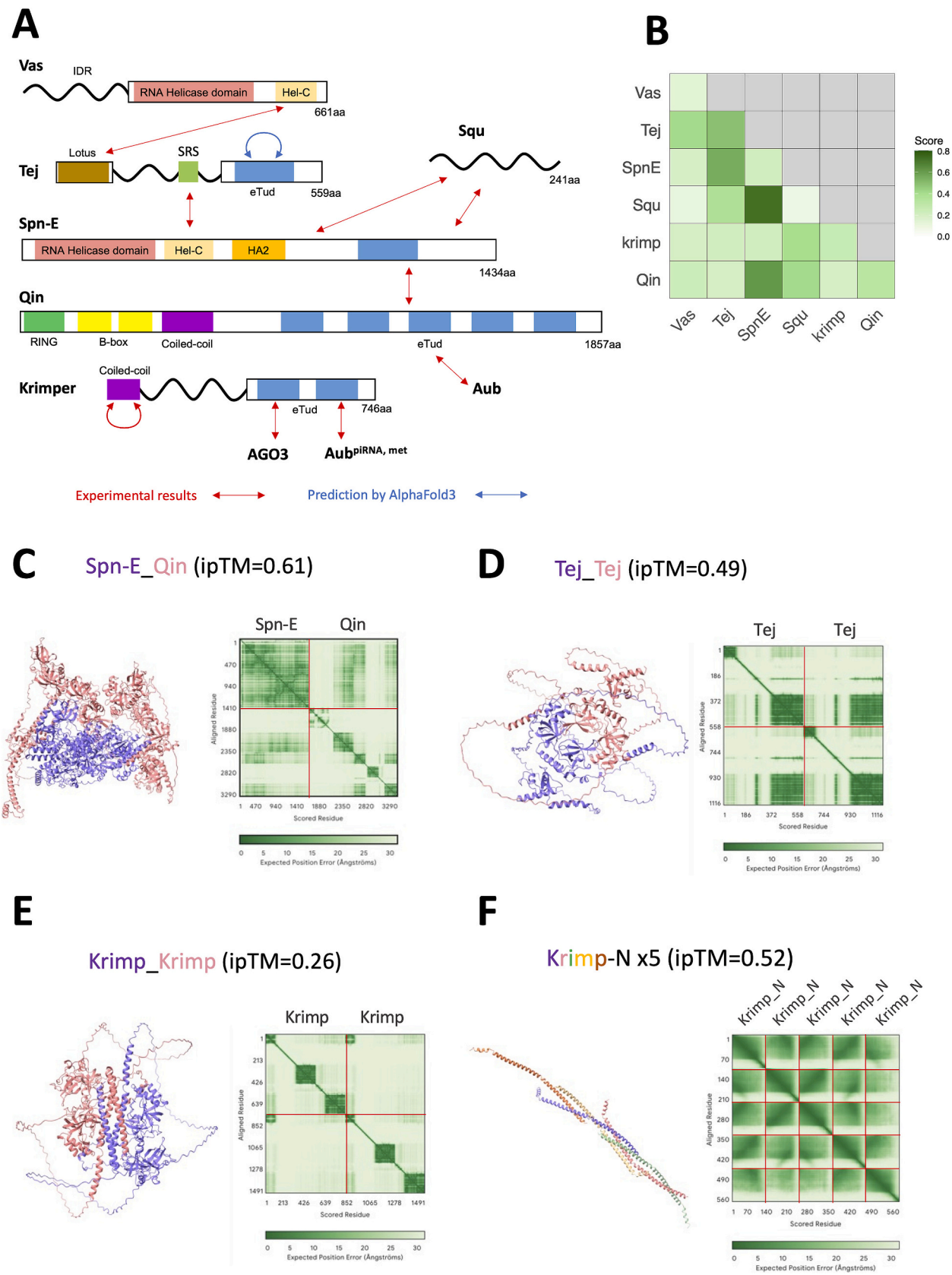

Fig. 3. The structural features and possible interactions among nuage components.
(A) Schematic representation of the domains and IDR of nuage proteins. Red arrows indicate experimentally validated interactions between domains, while blue arrows represent interactions predicted by AlphaFold3. Double-headed circular arrows denote the self-oligomerization. (B) The ipTM score for the protein-protein dimer model predicted by AlphaFold3. (C~F) Three-dimensional structure models predicted by AlphaFold3. (C) Spn-E_Qin, (D) Tej homodimer, (E) Krimp homodimer, (F) Homopentamer of Krimp-N containing coiled-coil region. PAE plots are also shown on the right for each structure model.

and nuage-localizing proteins has been revealed, but the assembly and their cooperative functions are poorly characterized. This section focuses the potential roles of the nuage-localizing protein in LLPS. Unlike spherical droplets commonly associated with LLPS, nuage exhibits irregular shapes, suggesting the coexistence of stable scaffolding structures and dynamic fluidity [66]. This structural complexity allows nuage to integrate rigid frameworks with dynamic compartments, optimizing its role in RNA processing and transposon silencing. Among proteins composing nuage, Vas has been extensively studied for its role in nuage assembly and piRNA processing [20,21,77]. Studies using S2 cells have shown that overexpression of Vas results in the formation of droplet-like granules, demonstrating its ability to undergo LLPS in the absence of other germline-specific proteins [15]. Importantly, both the IDR region and RNA binding are essential for Vas' droplet formation, supporting the hypothesis that Vas interacts directly with RNA to scaffold phase-separated condensates [70]. Studies in *Bombyx mori* (BmN4 cells) have further refined this model. Truncated Vas mutants lacking the N-terminal IDR fail to assemble granules and instead remain diffusely localized in the cytoplasm. Besides, FRAP (fluorescence recovery after photobleaching) assays in *Drosophila* suggest that Vas exhibits relatively high mobility within nuage, implying that its interactions with RNAs and proteins are transient and dynamic [21]. These findings support the idea that LLPS enhances RNA-binding and hand off efficiency, facilitating piRNA biogenesis.

Recent studies have highlighted Tej as a critical scaffold protein in nuage assembly and RNA trafficking. Its IDR regulates the mobility of nuage components and may contribute to LLPS of nuage [15,21]. In S2 culture cells, Tej forms spherical granules, suggesting that it can self-assemble independently with other germline specific proteins. AlphaFold3 predicts that Tej forms homodimer via its eTud domain, further supporting its role in scaffolding and condensate formation (Fig. 3B, D). Tej mutant lacking its eTud domain, however, fails to form granules, emphasizing the role of Tudor domain-mediated multivalent interactions in condensate formation. Conversely, constructs containing only the eTud domain form irregular aggregates, lacking the liquid-like properties of phase-separated droplets. This suggests that Tej's IDR endows flexibility to these assemblies, balancing structural integrity with dynamic exchange [21]. These observations suggest that Tej is a scaffold protein and employs a dual mechanism, using its eTudor domain for stable multivalent interactions and its IDR for dynamic fluidity. Functional studies in ovaries have further elucidated Tej's role. Loss of Tej disrupts the localization of Vas and Aub in nuage, leading to their dispersion into small, diffuse aggregates. Similarly, Spn-E becomes mislocalized, predominantly accumulating in the nucleus rather than in cytoplasmic nuage. These observations suggest that Tej anchors Spn-E and integrates it into nuage, stabilizing its interactions with RNA helicases and piRNA precursor [22].

Spn-E is another RNA helicase localized in nuage. Unlike with Vas RNA helicase, Spn-E does not independently undergo LLPS. When expressed alone in S2 cells, Spn-E localizes diffusely in the nucleus. Co-expression studies reveal that Tej recruits Spn-E into cytoplasmic granules by protein-protein interaction through Tej's SRS domain [21]. Furthermore, Spn-E co-localizes and bind to Squ, which forms cytoplasmic foci. This suggest that Squ contributes phase-separation properties to Spn-E, even though Squ itself does not readily form granules [26]. AlphaFold3 predicted no recognizable structured domains for Squ, but its IDR may contribute for the LLPS property in nuage.

Krimp plays a pivotal role in piRNA transfer and phase separation through its eTudor domains and coiled-coil regions [13]. AlphaFold3 also predicts the interaction through its N-terminal region although its ipTM score is low (Fig. 3E). When homo-oligomerization of the N-terminal alpha-helix region was predicted, the homo-pentamer showed the relatively high score (ipTM = 0.52) (Fig. 3F), suggesting the higher order homo-oligomerization of Krimp in the condensed granule, like a nuage. Interestingly, FRAP assays suggest that Krimp exhibits low exchange rates, implying reduced mobility compared to Tej and Spn-E [13]. This observation raises questions about whether Krimp functions as a rigid scaffold that stabilizes interactions within nuage rather than dynamically reorganizing like Vas [77]. Further investigation is required to determine whether Krimp undergoes partial LLPS or depends primarily on multivalent protein interactions.

4.4. Molecular coordination of Nuage assembly and piRNA biogenesis by PTMs and chaperones

Of note, nuage proteins exhibit hierarchical dependency relationships for their localization at perinuclear nuage. Genetic analyses revealed that deficiency in upstream factors involved in piRNA precursor recruitment to nuage, such as Spn-E, Vas, or Tej, causes delocalization of other nuage components from perinuclear aggregates, leading to either diffusion into the cytoplasm or cytoplasmic aggregation, ultimately disrupting functional nuage formation [21,22]. Conversely, loss of downstream factors in the ping-pong amplification cycle, such as Krimp and Ago3, does not affect the localization of these upstream components [13,77]. These observations suggest that nuage formation follows a stepwise, hierarchical process that reflects the sequential nature of the ping-pong amplification cycle of piRNA, rather than resulting from simple LLPS.

Similar to the *Drosophila* ovarian germline cells described above, testicular germline cells also possess perinuclear nuage, albeit with distinct spatiotemporal organization. This difference is primarily reflected in protein expression patterns during spermatogenesis. Notably, while one of the two key PIWI family protein, Aub is expressed from germline stem cells (GSCs) to spermatocytes and retain its localization at nuage, the other Ago3 can be observed only in GSCs and spermatogonia (SGs) but not in spermatocytes. Several studies suggest that even in the absence of Ago3, Aub can process piRNAs through homotypic Aub-Aub ping-pong cycles [78,79]. This observation indicates that while some proteins are sufficient for piRNA biogenesis, they may not all be strictly necessary.

As described in Introduction, nuage formation might also involve post-translational modifications (PTMs) such as arginine methylation, which have been shown to regulate PIWI family protein recruitment and RNA loading, predominantly through their binding to Tudor-domain proteins [18]. Another PTM, SUMOylation, has been reported for several piRNA pathway components [80]. Proteomics analysis of *Drosophila* ovaries detected SUMOylation of more than 1000 proteins, including piRNA-related proteins such as Piwi, Panoramix (Panx), Maelstrom (Mael), and Spn-E. Although SUMOylation of Panx is required for transposon silencing via interaction with Sov in heterochromatin formation [81], the molecular role of SUMOylation on other components, such as Spn-E, remains unclear. While SUMOylation is known to regulate LLPS in various contexts (reviewed in [82]), its function in nuage or Yb-body formation has not been established. Future research will clarify its biological significance in these non-membrane structures involved in piRNA production.

In addition to PTMs, chaperone complexes play a crucial role in piRNA biogenesis and nuage organization. Hsp90 and its co-chaperone Shutdown are required for proper PIWI protein function, facilitating piRNA loading and ensuring efficient RNA processing within nuage. Without chaperone assistance from co-chaperones, PIWI proteins fail to be loaded with piRNA precursors, leading to disrupted transposon silencing and defective nuage formation in *Drosophila* [83]. The function of Hsp90 and its cofactors is also required for piRNA production during the ping-pong cycle in nuage across different species. In mice, MIWI2-bound piRNAs and transposon repression depend on co-chaperone function. In the silkworm germline cell line BmN4, Hsp90 is essential for the removal of 16-nt ping-pong byproducts and for additional 5′-end trimming by a nuclease during the ping-pong cycle [84]. Taken together, these studies highlight a conserved role of molecular chaperones in piRNA biogenesis. The interaction of these chaperones with PIWI proteins may also contribute to the dynamic assembly of nuage, as loss of

Hsp70 or Hsp90 leads to altered nuage morphology and PIWI protein mislocalization. However, the precise molecular mechanisms by which chaperones coordinate with other piRNA pathway factors to regulate nuage assembly and function remain to be fully elucidated.

5. The landscape of non-membrane RNA processing granules

Various non-membrane RNA granules exist across cell types, facilitating RNA metabolism. While Yb body and nuage provide environments for piRNA processing as described above, other cytoplasmic RNA granules regulate mRNA translation, storage, and decay. Stress granules, formed in response to stress, sequester stalled translation complexes and contain translation factors, polyA-binding protein, and small ribosomal subunits, whereas P-bodies function as mRNA decay centers, containing decapping enzymes, the exonuclease XRN1, and Argonaute proteins (reviewed in [85]). P-bodies are often found to colocalize with piRNA/nuage/chromatoid bodies, forming cytoplasmic non-membrane structures called pi-bodies in *Drosophila* [86] piP-bodies in silkworm (*Bombyx mori*) [87] and in mice [88], where components of the piRNA pathway and mRNA decay machinery are spatially organized to facilitate piRNA biogenesis and transposon silencing. In the nucleus, specialized RNA-processing condensates, including Cajal bodies, nuclear speckles, paraspeckles, nucleolus and histone locus bodies (HLBs), organize enzymatic activities involved in RNA splicing, processing, modification, and transcription. Cajal bodies promote snRNP assembly and telomerase RNA processing by clustering RNA-processing enzymes via scaffold proteins such as Tudor-domain containing Coilin and SMN [89–91]. Nuclear speckles serve as hubs for splicing factors, including SC35 and SR proteins, facilitating their recruitment to active transcription sites (reviewed in [92]). Paraspeckles, in contrast, sequester specific transcripts to regulate their stability, assembling around NEAT1 lncRNA to retain hyperedited or unprocessed RNAs [93,94]. HLBs facilitate histone mRNA maturation through NPAT, FLASH, and U7 snRNA [95].

Despite their differences, these RNA granules share key organizational principles. Not only do they rely on LLPS, but they also utilize multivalent interactions and scaffold proteins to cluster enzymatic complexes, forming localized environments that regulate RNA metabolism. In other words, the RNA processing is effectively performed in the non-membrane organelles by increasing the local concentrations of RNA helicases/RNA-binding proteins and assembling them in the appropriate special arrangement. Among many components comprising such RNA granules, Tudor-domain proteins play a central role in condensate formation by binding symmetrically dimethylated (sDMA) or unmethylated arginine residues [96]. Notably, Tudor-domain proteins in nuage and Yb body contain additional RNA-binding motifs and enzymatic features, such as the Lotus domain (Tej/TDRD5), RNA helicase motifs (Spn-E/TDRD9, Yb, SoYb/TDRD12), RING domain (Qin/TDRD4), and RRM domain (Vret/TDRD1) [36]. In Cajal bodies, SMN, which contains a Gemin2-binding domain, binds Sm proteins and Coilin via its Tudor domain to mediate snRNP biogenesis and transport [97]. In HLBs, TDRD3, which contains an OB-fold domain, bridges chromatin modifiers (e.g., methylated histones H3R17me2) with RNA-processing complexes, influencing transcription and RNA stability [98]. Similarly, in Yb body and nuage, Tudor-domain proteins play essential roles in organizing piRNA pathway components. These findings highlight the conserved role of Tudor-domain proteins in scaffolding diverse non-membrane condensates while integrating RNA-binding and enzymatic functions.

Advancements in super-resolution microscopy, live-cell imaging, proteomics, and computational structure prediction continue to refine our understanding of RNA granules. While Yb bodies and nuage are well-characterized in piRNA pathways, the molecular mechanisms underlying non-membrane RNA granules remain an active area of research. Investigating how phase separation, post-translational modifications, and RNA-protein interactions drive condensate formation will provide deeper insights into RNA regulation and cellular dynamics.

Funding

This work was supported by Grant-in-Aid for Transformative Research Areas (A) [21H05275 to K.T.]; and the University of Osaka, Institute for Datability Science "Transdisciplinary Research Project" [Na22990007 to K.T. and K.S.]. I.W. was supported by Grant-in-Aid for JSPS Fellows.

CRediT authorship contribution statement

Shinichi Kawaguchi: Writing – review & editing, Writing – original draft, Visualization, Supervision, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Wakana Isshiki:** Writing – review & editing, Writing – original draft, Visualization. **Toshie Kai:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] B. Czech, M. Munafò, F. Ciabrelli, E.L. Eastwood, M.H. Fabry, E. Kneuss, G.J. Hannon, piRNA-guided genome defense: from biogenesis to silencing, Annu. Rev. Genet. 52 (2018) 131–157, https://doi.org/10.1146/annurev-genet-120417-031441.

[2] X. Wang, A. Ramat, M. Simonelig, M.-F. Liu, Emerging roles and functional mechanisms of PIWI-interacting RNAs, Nat. Rev. Mol. Cell Biol. 24 (2023) 123–141, https://doi.org/10.1038/s41580-022-00528-0.

[3] J. Brennecke, A.A. Aravin, A. Stark, M. Dus, M. Kellis, R. Sachidanandam, G. J. Hannon, Discrete small RNA-generating loci as master regulators of transposon activity in *Drosophila*, Cell 128 (2007) 1089–1103, https://doi.org/10.1016/j.cell.2007.01.043.

[4] G. Wang, V. Reinke, A C. elegans Piwi, PRG-1, Regulates 21U-RNAs during Spermatogenesis, Curr. Biol. 18 (2008) 861–867, https://doi.org/10.1016/j.cub.2008.05.009.

[5] P.J. Batista, J.G. Ruby, J.M. Claycomb, R. Chiang, N. Fahlgren, K.D. Kasschau, D. A. Chaves, W. Gu, J.J. Vasale, S. Duan, D. Conte, S. Luo, G.P. Schroth, J. C. Carrington, D.P. Bartel, C.C. Mello, PRG-1 and 21U-RNAs interact to form the piRNA complex required for fertility in C. Elegans, Mol. Cell 31 (2008) 67–78, https://doi.org/10.1016/j.molcel.2008.06.002.

[6] S.T. Grivna, E. Beyret, Z. Wang, H. Lin, A novel class of small RNAs in mouse spermatogenic cells, Genes Dev. 20 (2006) 1709–1714, https://doi.org/10.1101/gad.1434406.

[7] V.V. Vagin, A. Sigova, C. Li, H. Seitz, V. Gvozdev, P.D. Zamore, A distinct small RNA pathway silences selfish genetic elements in the germline, Science 313 (2006) 320–324, https://doi.org/10.1126/science.1129333.

[8] N.C. Lau, A.G. Seto, J. Kim, S. Kuramochi-Miyagawa, T. Nakano, D.P. Bartel, R. E. Kingston, Characterization of the piRNA complex from rat testes, Science 313 (2006) 363–367, https://doi.org/10.1126/science.1130164.

[9] S. Hirakata, H. Ishizu, A. Fujita, Y. Tomoe, M.C. Siomi, Requirements for multivalent Yb body assembly in transposon silencing in *Drosophila*, EMBO Rep. 20 (2019), https://doi.org/10.15252/embr.201947708.

[10] K. Saito, H. Ishizu, M. Komai, H. Kotani, Y. Kawamura, K.M. Nishida, H. Siomi, M. C. Siomi, Roles for the Yb body components Armitage and Yb in primary piRNA biogenesis in *Drosophila*, Genes Dev. 24 (2010) 2493–2498, https://doi.org/10.1101/gad.1989510.

[11] H. Qi, T. Watanabe, H.-Y. Ku, N. Liu, M. Zhong, H. Lin, The Yb body, a major site for Piwi-associated RNA biogenesis and a gateway for Piwi expression and transport to the nucleus in somatic cells, J. Biol. Chem. 286 (2011) 3789–3797, https://doi.org/10.1074/jbc.M110.193888.

[12] R.R. Pandey, D. Homolka, K.-M. Chen, R. Sachidanandam, M.-O. Fauvarque, R. S. Pillai, Recruitment of Armitage and Yb to a transcript triggers its phased processing into primary piRNAs in *Drosophila* ovaries, PLoS Genet. 13 (2017) e1006956, https://doi.org/10.1371/journal.pgen.1006956.

[13] A. Webster, S. Li, J.K. Hur, M. Wachsmuth, J.S. Bois, E.M. Perkins, D.J. Patel, A. A. Aravin, Aub and Ago3 are recruited to Nuage through two mechanisms to form a ping-pong complex assembled by Krimper, Mol. Cell 59 (2015) 564–575, https://doi.org/10.1016/j.molcel.2015.07.017.

[14] L.S. Gunawardane, K. Saito, K.M. Nishida, K. Miyoshi, Y. Kawamura, T. Nagami, H. Siomi, M.C. Siomi, A slicer-mediated mechanism for repeat-associated siRNA 5′ end formation in *Drosophila*, Science 315 (2007) 1587–1590, https://doi.org/10.1126/science.1140494.

[15] M. Jeske, C.W. Müller, A. Ephrussi, The LOTUS domain is a conserved DEAD-box RNA helicase regulator essential for the recruitment of vasa to the germ plasm and nuage, Genes Dev. 31 (2017) 939–952, https://doi.org/10.1101/gad.297051.117.
[16] Y. Kinoshita, R. Murakami, N. Muto, S. Kubo, R. Iizuka, S. Uemura, Heterogeneous dissociation process of truncated RNAs by oligomerized vasa helicase, Commun Biol 4 (2021) 1386, https://doi.org/10.1038/s42003-021-02918-0.
[17] T.J. Nott, E. Petsalaki, P. Farber, D. Jervis, E. Fussner, A. Plochowietz, T.D. Craggs, D.P. Bazett-Jones, T. Pawson, J.D. Forman-Kay, A.J. Baldwin, Phase transition of a disordered Nuage protein generates environmentally responsive Membraneless organelles, Mol. Cell 57 (2015) 936–947, https://doi.org/10.1016/j.molcel.2015.01.013.
[18] Y. Kirino, A. Vourekas, N. Sayed, F. de Lima Alves, T. Thomson, P. Lasko, J. Rappsilber, T.A. Jongens, Z. Mourelatos, Arginine methylation of Aubergine mediates Tudor binding and germ plasm localization, RNA 16 (2010) 70–78, https://doi.org/10.1261/rna.1869710.
[19] E. Beyret, H. Lin, Pinpointing the expression of piRNAs and function of the PIWI protein subfamily during spermatogenesis in the mouse, Dev. Biol. 355 (2011) 215–226, https://doi.org/10.1016/j.ydbio.2011.04.021.
[20] A.K. Lim, T. Kai, Unique germ-line organelle, nuage, functions to repress selfish genetic elements in *Drosophila melanogaster*, Proc. Natl. Acad. Sci. USA 104 (2007) 6714–6719, https://doi.org/10.1073/pnas.0701920104.
[21] Y. Lin, R. Suyama, S. Kawaguchi, T. Iki, T. Kai, Tejas functions as a core component in nuage assembly and precursor processing in *Drosophila* piRNA biogenesis, J. Cell Biol. 222 (2023), https://doi.org/10.1083/jcb.202303125.
[22] V.S. Patil, T. Kai, Repression of Retroelements in *Drosophila* germline via piRNA pathway by the Tudor domain protein Tejas, Curr. Biol. 20 (2010) 724–730, https://doi.org/10.1016/j.cub.2010.02.046.
[23] Z. Zhang, J. Xu, B.S. Koppetsch, J. Wang, C. Tipping, S. Ma, Z. Weng, W. E. Theurkauf, P.D. Zamore, Heterotypic piRNA ping-pong requires Qin, a protein with both E3 ligase and Tudor domains, Mol. Cell 44 (2011) 572–584, https://doi.org/10.1016/j.molcel.2011.10.011.
[24] A. Anand, T. Kai, The tudor domain protein Kumo is required to assemble the nuage and to generate germline piRNAs in *Drosophila*, EMBO J. 31 (2012) 870–882, https://doi.org/10.1038/emboj.2011.449.
[25] J. Abramson, J. Adler, J. Dunger, R. Evans, T. Green, A. Pritzel, O. Ronneberger, L. Willmore, A.J. Ballard, J. Bambrick, S.W. Bodenstein, D.A. Evans, C.-C. Hung, M. O'Neill, D. Reiman, K. Tunyasuvunakool, Z. Wu, A. Žemgulytė, E. Arvaniti, C. Beattie, O. Bertolli, A. Bridgland, A. Cherepanov, M. Congreve, A.I. Cowen-Rivers, A. Cowie, M. Figurnov, F.B. Fuchs, H. Gladman, R. Jain, Y.A. Khan, C.M. R. Low, K. Perlin, A. Potapenko, P. Savy, S. Singh, A. Stecula, A. Thillaisundaram, C. Tong, S. Yakneen, E.D. Zhong, M. Zielinski, A. Žídek, V. Bapst, P. Kohli, M. Jaderberg, D. Hassabis, J.M. Jumper, Accurate structure prediction of biomolecular interactions with AlphaFold 3, Nature 630 (2024) 493–500, https://doi.org/10.1038/s41586-024-07487-w.
[26] S. Kawaguchi, X. Xu, T. Soga, K. Yamaguchi, R. Kawasaki, R. Shimouchi, S. Date, T. Kai, In silico screening by AlphaFold2 program revealed the potential binding partners of nuage-localizing proteins and piRNA-related proteins, eLife 13 (2025) 101967, https://doi.org/10.7554/eLife.101967.3.
[27] E. Brasset, A. Taddei, F. Arnaud, B. Faye, A. Fausto, M. Mazzini, F. Giorgi, C. Vaury, Viral particles of the endogenous retrovirus ZAM from *Drosophila melanogaster* use a pre-existing endosome/exosome pathway for transfer to the oocyte, Retrovirology 3 (2006) 25, https://doi.org/10.1186/1742-4690-3-25.
[28] P. Leblanc, S. Desset, F. Giorgi, A.R. Taddei, A.M. Fausto, M. Mazzini, B. Dastugue, C. Vaury, Life cycle of an endogenous retrovirus, ZAM, in *Drosophila melanogaster*, J. Virol. 74 (2000) 10658–10669, https://doi.org/10.1128/JVI.74.22.10658-10669.2000.
[29] E. Sarot, G. Payen-Groschêne, A. Bucheton, A. Pélisson, Evidence for a *piwi* -dependent RNA silencing of the *gypsy* endogenous retrovirus by the *Drosophila melanogaster flamenco* gene, Genetics 166 (2004) 1313–1321, https://doi.org/10.1534/genetics.166.3.1313.
[30] Y. Murota, H. Ishizu, S. Nakagawa, Y.W. Iwasaki, S. Shibata, M.K. Kamatani, K. Saito, H. Okano, H. Siomi, M.C. Siomi, Yb integrates piRNA intermediates and processing factors into perinuclear bodies to enhance piRISC assembly, Cell Rep. 8 (2014) 103–113, https://doi.org/10.1016/j.celrep.2014.05.043.
[31] C. Dennis, V. Zanni, E. Brasset, A. Eymery, L. Zhang, R. Mteirek, S. Jensen, Y. S. Rong, C. Vaury, "Dot COM", a nuclear transit Center for the Primary piRNA pathway in *Drosophila*, PLoS One 8 (2013) e72752, https://doi.org/10.1371/journal.pone.0072752.
[32] M. Munafò, V. Manelli, F.A. Falconio, A. Sawle, E. Kneuss, E.L. Eastwood, J.W. E. Seah, B. Czech, G.J. Hannon, Daedalus and gasz recruit armitage to mitochondria, bringing piRNA precursors to the biogenesis machinery, Genes Dev. 33 (2019) 844–856, https://doi.org/10.1101/gad.325662.119.
[33] H. Yamashiro, M. Negishi, T. Kinoshita, H. Ishizu, H. Ohtani, M.C. Siomi, Armitage determines Piwi−piRISC processing from precursor formation and quality control to inter-organelle translocation, EMBO Rep. 21 (2020), https://doi.org/10.15252/embr.201948769.
[34] A. Le Thomas, A.K. Rogers, A. Webster, G.K. Marinov, S.E. Liao, E.M. Perkins, J. K. Hur, A.A. Aravin, K.F. Tóth, Piwi induces piRNA-guided transcriptional silencing and establishment of a repressive chromatin state, Genes Dev. 27 (2013) 390–399, https://doi.org/10.1101/gad.209841.112.
[35] X.A. Huang, H. Yin, S. Sweeney, D. Raha, M. Snyder, H. Lin, A major epigenetic programming mechanism guided by piRNAs, Dev. Cell 24 (2013) 502–516, https://doi.org/10.1016/j.devcel.2013.01.023.
[36] D. Handler, D. Olivieri, M. Novatchkova, F.S. Gruber, K. Meixner, K. Mechtler, A. Stark, R. Sachidanandam, J. Brennecke, A systematic analysis of *Drosophila* TUDOR domain-containing proteins identifies Vreteno and the Tdrd12 family as essential primary piRNA pathway factors, EMBO J. 30 (2011) 3977–3993, https://doi.org/10.1038/emboj.2011.308.
[37] M. Varadi, D. Bertoni, P. Magana, U. Paramval, I. Pidruchna, M. Radhakrishnan, M. Tsenkov, S. Nair, M. Mirdita, J. Yeo, O. Kovalevskiy, K. Tunyasuvunakool, A. Laydon, A. Žídek, H. Tomlinson, D. Hariharan, J. Abrahamson, T. Green, J. Jumper, E. Birney, M. Steinegger, D. Hassabis, S. Velankar, AlphaFold protein structure database in 2024: providing structure coverage for over 214 million protein sequences, Nucleic Acids Res. 52 (2024) D368–D375, https://doi.org/10.1093/nar/gkad1011.
[38] H.A. Cook, B.S. Koppetsch, J. Wu, W.E. Theurkauf, The *Drosophila* SDE3 homolog armitage is required for oskar mRNA silencing and embryonic Axis specification, Cell 116 (2004) 817–829, https://doi.org/10.1016/S0092-8674(04)00250-8.
[39] H. Ishizu, T. Kinoshita, S. Hirakata, C. Komatsuzaki, M.C. Siomi, Distinct and collaborative functions of Yb and Armitage in transposon-targeting piRNA biogenesis, Cell Rep. 27 (2019) 1822–1835.e8, https://doi.org/10.1016/j.celrep.2019.04.029.
[40] D.L. Theobald, R.M. Mitton-Fry, D.S. Wuttke, Nucleic acid recognition by OB-fold proteins, Annu. Rev. Biophys. Biomol. Struct. 32 (2003) 115–133, https://doi.org/10.1146/annurev.biophys.32.110601.142506.
[41] L.H. Gregersen, M. Schueler, M. Munschauer, G. Mastrobuoni, W. Chen, S. Kempa, C. Dieterich, M. Landthaler, MOV10 is a 5′ to 3′ RNA helicase contributing to UPF1 mRNA target degradation by translocation along 3′ UTRs, Mol. Cell 54 (2014) 573–585, https://doi.org/10.1016/j.molcel.2014.03.017.
[42] R.R. Pandey, Y. Tokuzawa, Z. Yang, E. Hayashi, T. Ichisaka, S. Kajita, Y. Asano, T. Kunieda, R. Sachidanandam, S. Chuma, S. Yamanaka, R.S. Pillai, Tudor domain containing 12 (TDRD12) is essential for secondary PIWI interacting RNA biogenesis in mice, Proc. Natl. Acad. Sci. USA 110 (2013) 16492–16497, https://doi.org/10.1073/pnas.1316316110.
[43] C. Dennis, E. Brasset, A. Sarkar, C. Vaury, Export of piRNA precursors by EJC triggers assembly of cytoplasmic Yb-body in *Drosophila*, Nat. Commun. 7 (2016) 1–12, https://doi.org/10.1038/ncomms13739.
[44] N. Takase, M. Otsu, S. Hirakata, H. Ishizu, M.C. Siomi, G. Kawai, T-hairpin structure found in the RNA element involved in piRNA biogenesis, RNA 28 (2022) 541–550, https://doi.org/10.1261/rna.078967.121.
[45] R. Onishi, S. Yamanaka, M.C., Siomi, piRNA- and siRNA-mediated transcriptional repression in *Drosophila*, mice, and yeast: new insights and biodiversity, EMBO Rep. 22 (2021), https://doi.org/10.15252/embr.202153062.
[46] Y. Koga, S. Hirakata, M. Negishi, H. Yamazaki, T. Fujisawa, M.C. Siomi, Dipteran-specific Daedalus controls zucchini endonucleolysis in piRNA biogenesis independent of exonucleases, Cell Rep. 43 (2024) 114923, https://doi.org/10.1016/j.celrep.2024.114923.
[47] S. Hirakata, M.C. Siomi, Assembly and function of gonad-specific non-membranous organelles in *Drosophila* piRNA biogenesis, noncoding, RNA 5 (2019) 52, https://doi.org/10.3390/ncrna5040052.
[48] S. Fukuhara, H. Nishimasu, L. Bonnefond, N. Matsumoto, R. Ishitani, O. Nureki, Expression, purification, crystallization and preliminary X-ray crystallographic analysis of Zucchini from *Drosophila melanogaster*, Acta Crystallogr Sect F Struct Biol Cryst Commun 68 (2012) 1346–1350, https://doi.org/10.1107/S1744309112038936.
[49] J.J. Ipsaro, A.D. Haase, S.R. Knott, L. Joshua-Tor, G.J. Hannon, The structural biochemistry of zucchini implicates it as a nuclease in piRNA biogenesis, Nature 491 (2012) 279–283, https://doi.org/10.1038/nature11502.
[50] B.W. Han, W. Wang, C. Li, Z. Weng, P.D., Zamore, piRNA-guided transposon cleavage initiates zucchini-dependent, phased piRNA production, Science 348 (2015) 817–821, https://doi.org/10.1126/science.aaa1264.
[51] F. Mohn, D. Handler, J., Brennecke, piRNA-guided slicing specifies transcripts for zucchini-dependent, phased piRNA biogenesis, Science 348 (2015) 812–817, https://doi.org/10.1126/science.aaa1039.
[52] M.D. Horwich, C. Li, C. Matranga, V. Vagin, G. Farley, P. Wang, P.D. Zamore, The *Drosophila* RNA methyltransferase, DmHen1, modifies germline piRNAs and single-stranded siRNAs in RISC, Curr. Biol. 17 (2007) 1265–1272, https://doi.org/10.1016/j.cub.2007.06.030.
[53] D.T. Ge, W. Wang, C. Tipping, I. Gainetdinov, Z. Weng, P.D. Zamore, The RNA-binding ATPase, Armitage, couples piRNA amplification in Nuage to phased piRNA production on mitochondria, Mol. Cell 74 (2019) 982–995.e6, https://doi.org/10.1016/j.molcel.2019.04.006.
[54] A. Szakmary, M. Reedy, H. Qi, H. Lin, The Yb protein defines a novel organelle and regulates male germline stem cell self-renewal in *Drosophila melanogaster*, J. Cell Biol. 185 (2009) 613–627, https://doi.org/10.1083/jcb.200903034.
[55] E.M. Eddy, Germ plasm and the differentiation of the germ cell line, Int. Rev. Cytol. (1976) 229–280, https://doi.org/10.1016/S0074-7696(08)60070-4.
[56] X. Wang, C. Lv, Y. Guo, S. Yuan, Mitochondria associated germinal structures in spermatogenesis: piRNA pathway regulation and beyond, Cells 9 (2020) 399, https://doi.org/10.3390/cells9020399.
[57] J. Gao, J. Jing, G. Shang, C. Chen, M. Duan, W. Yu, K. Wang, J. Luo, M. Song, K. Chen, C. Chen, T. Zhang, D. Ding, TDRD1 phase separation drives intermitochondrial cement assembly to promote piRNA biogenesis and fertility, Dev. Cell 59 (2024) 2704–2718.e6, https://doi.org/10.1016/j.devcel.2024.06.017.
[58] H. Wei, J. Gao, D.-H. Lin, R. Geng, J. Liao, T.-Y. Huang, G. Shang, J. Jing, Z.-W. Fan, D. Pan, Z.-Q. Yin, T. Li, X. Liu, S. Zhao, C. Chen, J. Li, X. Wang, D. Ding, M.-F., Liu, piRNA loading triggers MIWI translocation from the intermitochondrial cement to chromatoid body during mouse spermatogenesis, Nat. Commun. 15 (2024) 2343, https://doi.org/10.1038/s41467-024-46664-3.
[59] C. Chen, J. Jin, D.A. James, M.A. Adams-Cioaba, J.G. Park, Y. Guo, E. Tenaglia, C. Xu, G. Gish, J. Min, T. Pawson, Mouse Piwi interactome identifies binding

mechanism of Tdrkh Tudor domain to arginine methylated Miwi, Proc. Natl. Acad. Sci. USA 106 (2009) 20336–20341, https://doi.org/10.1073/pnas.0911640106.
[60] T. Watanabe, S. Chuma, Y. Yamamoto, S. Kuramochi-Miyagawa, Y. Totoki, A. Toyoda, Y. Hoki, A. Fujiyama, T. Shibata, T. Sado, T. Noce, T. Nakano, N. Nakatsuji, H. Lin, H. Sasaki, MITOPLD is a mitochondrial protein essential for Nuage formation and piRNA biogenesis in the mouse germline, Dev. Cell 20 (2011) 364–375, https://doi.org/10.1016/j.devcel.2011.01.005.
[61] Y. Zhang, R. Guo, Y. Cui, Z. Zhu, Y. Zhang, H. Wu, B. Zheng, Q. Yue, S. Bai, W. Zeng, X. Guo, Z. Zhou, B. Shen, K. Zheng, M. Liu, L. Ye, J. Sha, An essential role for PNLDC1 in piRNA 3′ end trimming and male fertility in mice, Cell Res. 27 (2017) 1392–1396, https://doi.org/10.1038/cr.2017.125.
[62] T. Ohara, Y. Sakaguchi, T. Suzuki, H. Ueda, K. Miyauchi, T. Suzuki, The 3′ termini of mouse Piwi-interacting RNAs are 2′-O-methylated, Nat. Struct. Mol. Biol. 14 (2007) 349–350, https://doi.org/10.1038/nsmb1220.
[63] C.D. Malone, J. Brennecke, M. Dus, A. Stark, W.R. McCombie, R. Sachidanandam, G.J. Hannon, Specialized piRNA pathways act in germline and somatic tissues of the *Drosophila* ovary, Cell 137 (2009) 522–535, https://doi.org/10.1016/j.cell.2009.03.040.
[64] E. Voronina, G. Seydoux, P. Sassone-Corsi, I. Nagamori, RNA Granules in Germ Cells, Cold Spring Harb. Perspect. Biol. 3 (2011) a002774, https://doi.org/10.1101/cshperspect.a002774.
[65] B. Czech, J.B. Preall, J. McGinn, G.J. Hannon, A transcriptome-wide RNAi screen in the *Drosophila* ovary reveals factors of the germline piRNA pathway, Mol. Cell 50 (2013) 749–761, https://doi.org/10.1016/j.molcel.2013.04.007.
[66] J.W. Pek, V.S. Patil, T., Kai, piRNA pathway and the potential processing site, the nuage, in the *Drosophila* germline, Develop. Growth Differ. 54 (2012) 66–77, https://doi.org/10.1111/j.1440-169X.2011.01316.x.
[67] S.F. Banani, A.M. Rice, W.B. Peeples, Y. Lin, S. Jain, R. Parker, M.K. Rosen, Compositional control of phase-separated cellular bodies, Cell 166 (2016) 651–663, https://doi.org/10.1016/j.cell.2016.06.010.
[68] H. Wang, Z. Ma, K. Niu, Y. Xiao, X. Wu, C. Pan, Y. Zhao, K. Wang, Y. Zhang, N. Liu, Antagonistic roles between nibbler and Hen1 modulate piRNA 3′ ends in *Drosophila*, Development 143 (2015) 530–539, https://doi.org/10.1242/dev.128116.
[69] E. Raz, The function and regulation of vasa-like genes in germ-cell development, Genome Biol. 1 (2000) 1–6, https://doi.org/10.1186/gb-2000-1-3-reviews1017.
[70] H. Yamazaki, Y. Namba, S. Kuriyama, K.M. Nishida, A. Kajiya, M.C. Siomi, *Bombyx* Vasa sequesters transposon mRNAs in nuage via phase separation requiring RNA binding and self-association, Nat. Commun. 14 (2023) 1942, https://doi.org/10.1038/s41467-023-37634-2.
[71] T. Sengoku, O. Nureki, A. Nakamura, S. Kobayashi, S. Yokoyama, Structural basis for RNA unwinding by the DEAD-box protein *Drosophila* vasa, Cell 125 (2006) 287–300, https://doi.org/10.1016/j.cell.2006.01.054.
[72] M. Jeske, M. Bordi, S. Glatt, S. Müller, V. Rybin, C.W. Müller, A. Ephrussi, The crystal structure of the *Drosophila* germline inducer Oskar identifies two domains with distinct vasa helicase- and RNA-binding activities, Cell Rep. 12 (2015) 587–598, https://doi.org/10.1016/j.celrep.2015.06.055.
[73] D. Ding, C. Wei, K. Dong, J. Liu, A. Stanton, C. Xu, J. Min, J. Hu, C. Chen, LOTUS domain is a novel class of G-rich and G-quadruplex RNA binding domain, Nucleic Acids Res. 48 (2020) 9262–9272, https://doi.org/10.1093/nar/gkaa652.
[74] A. Andress, Y. Bei, B.R. Fonslow, R. Giri, Y. Wu, J.R. Yates, R.W. Carthew, Spindle-E cycling between nuage and cytoplasm is controlled by Qin and PIWI proteins, J. Cell Biol. 213 (2016) 201–211, https://doi.org/10.1083/jcb.201411076.
[75] K. Sato, Y.W. Iwasaki, A. Shibuya, P. Carninci, Y. Tsuchizawa, H. Ishizu, M. C. Siomi, H. Siomi, Krimper enforces an antisense Bias on piRNA pools by binding AGO3 in the *Drosophila* germline, Mol. Cell 59 (2015) 553–563, https://doi.org/10.1016/j.molcel.2015.06.024.
[76] X. Huang, H. Hu, A. Webster, F. Zou, J. Du, D.J. Patel, R. Sachidanandam, K. F. Toth, A.A. Aravin, S. Li, Binding of guide piRNA triggers methylation of the unstructured N-terminal region of Aub leading to assembly of the piRNA amplification complex, Nat. Commun. 12 (2021) 4061, https://doi.org/10.1038/s41467-021-24351-x.
[77] J. Xiol, P. Spinelli, M.A. Laussmann, D. Homolka, Z. Yang, E. Cora, Y. Couté, S. Conn, J. Kadlec, R. Sachidanandam, M. Kaksonen, S. Cusack, A. Ephrussi, R. S. Pillai, RNA clamping by vasa assembles a piRNA amplifier complex on transposon transcripts, Cell 157 (2014) 1698–1711, https://doi.org/10.1016/j.cell.2014.05.018.
[78] E. Quénerch'du, A. Anand, T. Kai, The piRNA pathway is developmentally regulated during spermatogenesis in *Drosophila*, RNA 22 (2016) 1044–1054, https://doi.org/10.1261/rna.055996.116.
[79] A. Nagao, T. Mituyama, H. Huang, D. Chen, M.C. Siomi, H. Siomi, Biogenesis pathways of piRNAs loaded onto AGO3 in the *Drosophila* testis, RNA 16 (2010) 2503–2515, https://doi.org/10.1261/rna.2270710.
[80] M. Ninova, H. Holmes, B. Lomenick, K. Fejes Tóth, A.A. Aravin, Pervasive SUMOylation of heterochromatin and piRNA pathway proteins, Cell Genom. 3 (2023) 100329, https://doi.org/10.1016/j.xgen.2023.100329.
[81] V.I. Andreev, C. Yu, J. Wang, J. Schnabl, L. Tirian, M. Gehre, D. Handler, P. Duchek, M. Novatchkova, L. Baumgartner, K. Meixner, G. Sienski, D.J. Patel, J. Brennecke, Panoramix SUMOylation on chromatin connects the piRNA pathway to the cellular heterochromatin machinery, Nat. Struct. Mol. Biol. 29 (2022) 130–142, https://doi.org/10.1038/s41594-022-00721-x.
[82] X. Cheng, Protein SUMOylation and phase separation: partners in stress? Trends Biochem. Sci. 48 (2023) 417–419, https://doi.org/10.1016/j.tibs.2022.12.003.
[83] D. Olivieri, K.-A. Senti, S. Subramanian, R. Sachidanandam, J. Brennecke, The Cochaperone shutdown defines a Group of Biogenesis Factors Essential for all piRNA populations in *Drosophila*, Mol. Cell 47 (2012) 954–969, https://doi.org/10.1016/j.molcel.2012.07.021.
[84] N. Izumi, S. Kawaoka, S. Yasuhara, Y. Suzuki, S. Sugano, S. Katsuma, Y. Tomari, Hsp90 facilitates accurate loading of precursor piRNAs into PIWI proteins, RNA 19 (2013) 896–901, https://doi.org/10.1261/rna.037200.112.
[85] C.L. Riggs, N. Kedersha, P. Ivanov, P. Anderson, Mammalian stress granules and P bodies at a glance, J. Cell Sci. 133 (2020), https://doi.org/10.1242/jcs.242487.
[86] A.K. Lim, L. Tao, T., Kai, piRNAs mediate posttranscriptional retroelement silencing and localization to pi-bodies in the *Drosophila* germline, J. Cell Biol. 186 (2009) 333–342, https://doi.org/10.1083/jcb.200904063.
[87] P.Y. Chung, K. Shoji, N. Izumi, Y. Tomari, Dynamic subcellular compartmentalization ensures fidelity of piRNA biogenesis in silkworms, EMBO Rep. 22 (2021), https://doi.org/10.15252/embr.202051342.
[88] A.A. Aravin, G.W. van der Heijden, J. Castañeda, V.V. Vagin, G.J. Hannon, A. Bortvin, Cytoplasmic compartmentalization of the fetal piRNA pathway in mice, PLoS Genet. 5 (2009) e1000764, https://doi.org/10.1371/journal.pgen.1000764.
[89] J.-L. Liu, C. Murphy, M. Buszczak, S. Clatterbuck, R. Goodman, J.G. Gall, The *Drosophila melanogaster* Cajal body, J. Cell Biol. 172 (2006) 875–884, https://doi.org/10.1083/jcb.200511038.
[90] J.G. Gall, M. Bellini, Z. Wu, C. Murphy, Assembly of the nuclear transcription and processing machinery: Cajal bodies (coiled bodies) and Transcriptosomes, Mol. Biol. Cell 10 (1999) 4385–4402, https://doi.org/10.1091/mbc.10.12.4385.
[91] E. Courchaine, S. Gelles-Watnick, M. Machyna, K. Straube, S. Sauyet, J. Enright, K. M. Neugebauer, The coilin N-terminus mediates multivalent interactions between coilin and Nopp140 to form and maintain Cajal bodies, Nat. Commun. 13 (2022) 6005, https://doi.org/10.1038/s41467-022-33434-2.
[92] A.I. Lamond, D.L. Spector, Nuclear speckles: a model for nuclear organelles, Nat. Rev. Mol. Cell Biol. 4 (2003) 605–612, https://doi.org/10.1038/nrm1172.
[93] T. Yamazaki, S. Souquere, T. Chujo, S. Kobelke, Y.S. Chong, A.H. Fox, C.S. Bond, S. Nakagawa, G. Pierron, T. Hirose, Functional domains of NEAT1 architectural lncRNA induce Paraspeckle assembly through phase separation, Mol. Cell 70 (2018) 1038–1053.e7, https://doi.org/10.1016/j.molcel.2018.05.019.
[94] S. Nakagawa, T. Yamazaki, T. Hirose, Molecular dissection of nuclear paraspeckles: towards understanding the emerging world of the RNP milieu, Open Biol. 8 (2018), https://doi.org/10.1098/rsob.180150.
[95] A.E. White, B.D. Burch, X. Yang, P.Y. Gasdaska, Z. Dominski, W.F. Marzluff, R. J. Duronio, *Drosophila* histone locus bodies form by hierarchical recruitment of components, J. Cell Biol. 193 (2011) 677–694, https://doi.org/10.1083/jcb.201012077.
[96] J.W. Pek, A. Anand, T. Kai, Tudor domain proteins in development, Development 139 (2012) 2255–2266, https://doi.org/10.1242/dev.073304.
[97] O. Tapia, V. Lafarga, R. Bengoechea, A. Palanca, M. Lafarga, M.T. Berciano, The SMN Tudor SIM-like domain is key to SmD1 and coilin interactions and to Cajal body biogenesis, J. Cell Sci. 127 (2014) 939–946, https://doi.org/10.1242/jcs.138537.
[98] Y. Yang, Y. Lu, A. Espejo, J. Wu, W. Xu, S. Liang, M.T. Bedford, TDRD3 is an effector molecule for arginine-methylated histone Marks, Mol. Cell 40 (2010) 1016–1023, https://doi.org/10.1016/j.molcel.2010.11.024.