Me31B, A Key Repressor in Germline Regulation and Beyond

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Abstract

Me31B, an evolutionarily conserved ATP-dependent RNA helicase, plays an important role in the development of the germline across diverse animal species. Its cellular functionality has been posited as a translational repressor, participating in various RNA metabolism pathways to intricately regulate the spatiotemporal expression of RNAs. Despite its evident significance, the precise role and mechanistic underpinnings of Me31B remain insufficiently understood. This article endeavors to comprehensively review historic and recent research on Me31B, distill the major findings, discern generalizable patterns in Me31B's functions across different research contexts, and provide insights into its fundamental role and mechanism of action. The primary focus of this article centers on elucidating the role of *Drosophila* Me31B within the germline, while concurrently delving into pertinent research on its orthologs within other species and cellular systems.

1. Drosophila Germline and Maternal Effect Gene *me31B*

Drosophila melanogaster germline is a useful model for exploring the genetic factors involved in animal germline development (1-7). Within the female germline, the ovaries consist of a series of tubular structures named ovarioles, each of which houses a chain of developing egg chambers at various stages of oogenesis (which range from 1 to 14, Figure 1). This process begins with the division of germline stem cells in the germarium at the tip of each ovariole, resulting in interconnected cyst cells. Each cyst includes 15 nurse cells and one oocyte, surrounded by a layer of somatic follicle cells, forming an egg chamber. Within each egg chamber, the nurse cells synthesize nutrient molecules - including maternally expressed RNAs and proteins - and deposit them into the developing egg, supporting growth and maturation. These maternal RNAs and proteins form a complex gene-expression-regulation program, guiding the germline through oogenesis, embryogenesis, and eventual development into the next generation (4, 8-14). The protein **M**aternally **E**xpressed at **31B** (Me31B) is a crucial component among the maternal gene products for the development of germline (15). Its orthologs in other animals also appear to possess this same significance (16-18).

2. Historic Background of *me31B* Gene

The discovery of the second maternally expressed *Drosophila* gene, *me31B*, was made by De Valoir et al. (19). Similar to the first maternal gene *vas* (encodes another RNA helicase Vas), *me31B* transcripts are predominantly found in *Drosophila* female germline cells: nurse cells, oocytes, and early embryos. As a result, *me31B* was named after this maternal expression pattern and its genomic location at the 31B region of the

2nd chromosome (19). In the early study, *me31B* expression was only detected in the female germline due to the limited detection sensitivity. However, recent studies have discovered *me31B*'s expression and roles in the male germline and various somatic tissues.

3. The Me31B Helicase Family and Me31B's Domain/Motifs

Me31B belongs to the DEAD-box RNA helicase family, a large group of proteins that use the energy from ATP hydrolysis to unwind RNAs during various RNA metabolism processes (20-27). This is indicated by the presence of the signature Asp-Glu-Ala-Asp (DEAD) sequence and other structural features (19). Me31B and its orthologs are classified under the evolutionarily conserved subfamily of Me31B/DDX6-like, ATP-dependent, DEAD-box, RNA helicases. This subfamily includes various members, including human DDX6/RCK, mouse p54, frog Xp54, worm CGH-1, yeast Dhh1p, and others, with a sequence similarity of 60% to 80% between each other (15, 18, 28). For simplicity, we commonly use the term Me31B as a generic reference to the aforementioned proteins, unless there is a need to specify.

The protein Me31B contains several conserved domains, motifs, and amino acids important for its functions. Table 1 summarizes these functional domains and motifs' locations, known/hypothetical functions, and level of conservation. Me31B domains and motifs have been categorized into six groups based on functions and sequence characteristics. Group One consists of two large RecA-like domains (N-terminal RecA-like and C-terminal RecA-like domains) that potentially cooperate to unwind RNAs, along with four motifs (DVLARAK, DEAD-box, SAT, and HRIGR motifs)

that are critical for ATPase/helicase activities. Group Two contains 11 highly conserved motifs (Q, I, Ia, GG, Ib, II, III, IV, QxxR, V, and VI motifs) with poorly understood functions. Group Three contains four amino acids (H333, R334, C351, and S352) that are crucial for human development as deduced from human ortholog DDX6. Group Four has two intrinsically disordered regions (IDRs) that could affect Me31B protein self-aggregation. Group Five includes two hypothetical nuclear localization and export signal sequences (NLS and NES, both deduced from homologous sequences of DDX6). Group Six contains 16 additional motifs that facilitate Me31B's interaction with other proteins. The above domains and motifs are believed to play important roles in Me31B functions for regulating germline RNA stability, localization, and translation, which, in turn, contributes to proper germline development.

Table 1. *Drosophila* Me31B Domains and Motifs

Me31B Domains and Motifs	Extent of Conservation*	Known or Hypothetical Functions	Reference
Group One: Large functional domains and motifs participating in ATPase and RNA helicase activity			
N-terminal domain (1 – 267)	Conserved RecA-like domain in humans and other species	N-terminal RecA-like domain; ATP binding; Helicase activity; P-body assembly; Female fertility	(18, 29, 30)
C-terminal domain (268 – 459)	Conserved RecA-like domain in humans and other species	C-terminal RecA-like domain; RNA translational repression; Protein binding; P-body assembly; Female fertility	(29-31)
DEAD box (207 – 210, within II motif)	100% in humans and other species	ATP-binding, RNA binding, RNA translational control, protein binding; Female fertility	(28-30, 32)
SAT (238 – 240, same as III motif)	100% in humans and other species	Helicase activity	(18, 29)
HRIGR (381 – 385, within VI motif)	100% in humans and other species	ATP binding, RNA binding, helicase activity, RNA translational repression, p-body accumulation, and P-body assembly; Female fertility	(28-30)
DVLARAK (97 – 103, partial overlap with I motif)	93% of human and other species	RNA binding, translational control, P-body assembly; Female fertility	(29, 30)
Group Two: Motifs highly conserved in the Me31B/DDX6 family in humans and other species with mostly unknown functions			
Q (77 – 85)	94% in humans and 89% - 94% in other species	Unknown	(18)
I (102 – 109)	94% in humans and 94% - 100% in other species	Unknown	(18)
I_a (134 – 141)	100% in humans and other species	Unknown	(18)
GG (162 – 163)	100% in humans and other species	Unknown	(33)
I_b (180 – 186)	94% in humans and 94% - 100% other species	Unknown	(18)

II (204 – 210, contains DEAD box)	94% in humans and 94% - 100% in other species	Containing the DEAD box	(18)
III (238 – 240, same as SAT motif)	100% in humans and other species	Helicase activity	(18, 29)
IV (299 – 301)	100% in humans and other species	Unknown	(18)
QxxR (331 – 334)	100% in humans and other species (for Q and R)	Unknown	(16)
V (356 – 360)	100% in humans and other species	Unknown	(18)
VI (380 – 390, contains HRIGR motif)	100% in humans and 95% - 100% in other species	Unknown	(18)
Group Three: Motifs conserved in humans and their mutations result in human developmental defects			
H333 (within QxxR motif)	100% in humans and other species	The mutation causes cognitive and developmental delays and defects (in DDX6)	(16)
R334 (within QxxR motif)	100% in humans and other species	The mutation causes cognitive and developmental delays and defects (in DDX6)	(16)
C351	100% in humans and other species	Mutation causes cognitive and developmental delays and defects (in DDX6)	(16)
S352	50% in humans and 50% - 100% in other species	Mutation causes cognitive and developmental delays and defects (in DDX6)	(16)
Group Four: Intrinsically disordered regions (IDR)			
N-terminal IDR (1 – 53)	Presence, length, and sequence vary among species	Protein aggregation regulation	(34)
C-terminal IDR (431 – 459)			
Group Five: Hypothetical signal peptides involved in nuclear import/export			
Nuclear localization signal (NLS, 35 – 48, hypothetical)	Presence, length, and sequence vary among species	Nuclear localization signal (in DDX6)	(35)
Nuclear export signal (NES, 113 – 122, hypothetical)		Nuclear export signal (in DDX6)	
Group Six: Other protein- and RNA-binding motifs			
FDF motif-binding (285 – 289, part of EDC3 and Tral binding region motif and other motifs)	100% in humans and other species	FDF-domain binding motif; Aggregation status	(30, 36)
W pocket (310 – 314)	100% in humans and other species	4E-T, EDC3, and LSM14A binding site (in DDX6)	(36)
Protein binding patch 1 (281 – 294, partially overlap with EDC3 and Tral binding region motif and other motifs)	100% in humans and 94% - 100% in other species	Potential FDF-binding site for binding Pat1 and EDC3 (in DDX6)	(35, 37)
Protein binding patch 2 (405 – 412, partially overlaps with Y401-L407 motif)	63% in humans and 50% - 63% in other species	Interaction with EDC3 (in DDX6)	(33, 35)
Protein binding patch 3 (304 – 307)	75% in humans and 50% - 75% in other species	4E-T and PATL1 binding site (in DDX6)	(35)
NOT1 Binding Specificity motif (290 – 296, partially overlaps with EDC3 and Tral binding region motif and other motifs)	93% in humans and 86% - 93% in other species	Provides binding specificity to NOT1; RNA translational repression (in DDX6)	(38)
EDC3 and Tral binding motif (274 – 292, partially overlaps with Protein binding patch 1 and other motifs)	95% in humans and 92% - 95% in other species	EDC3/Tral binding; Localization to P-body; RNA translational repression	(29, 33)
F63 – L70	87% in humans and 81% to 87% in other species	Interaction with eIF4E-3 isoform	(39)
R347	100% in humans and other species	Needed for binding to CNOT1 (in DDX6)	(35, 37, 38)
R80	50% in humans and 0% - 50% in other species	Potential symmetrically demethylated arginine (SDMA) interacting with Tud-domain proteins	(40)

R156	50% in humans and other species	Potential SDMA interacting with Tud-domain proteins	(40)
R254	0% in humans and 0% - 100% in other species	Potential SDMA interacting with Tud-domain proteins	(40)
RG (R357 and G358)	100% in humans and other species	Involved in de-capping (in Dhh1p)	(41)
K108	100% in humans and other species	ATP-binding and RNA translational control (in Bel helicase)	(42)
Y273	100% in humans and other species	Potential Phosphorylation site; function unknown (in DDX6)	(35)
Y401-L407 (partially overlaps with Protein binding patch 2 motif)	79% in humans and 71% - 79% in other species	Interaction with eIF4E-1	(39)

***** Extent of Conservation is calculated with 6 species: *C. elegans*, planarian, *Drosophila*, *Xenopus*, mouse, and human. We counted a mismatch between two chemically different amino acids like arginine (R) and Valine (V) as a mismatch, while a mismatch between two chemically similar amino acids like arginine (R) and lysine (K) was counted as half a match.
* Note that certain domains and motifs' sequences and functions are deductions from Me31B orthologs in other species.

4. Analysis of Me31B's Roles in The Germline

To investigate the role of Me31B in the germline, a range of different *me31B* alleles have been generated using traditional and targeted-motif-mutation approaches.

4.1 Loss-of-function alleles

Nakamura et al. initially produced three loss-of-function alleles of *me31B*, specifically, $me31B^{\Delta 1}$, $me31B^{\Delta 2,}$ and $me31B^{\Delta 3}$, which resulted from partial deletions of the *me31B* gene (15). The three alleles all caused recessive lethality, with homozygous mutants failing to develop during the second or third-larval stages, despite no visible morphological defects. These findings demonstrate that *me31B* is an essential gene for *Drosophila*. To gain a deeper understanding of its roles in the germline, the researchers used the $me31B^{\Delta}$ allele to create germline clone strains, where animals carried homozygous $me31B^{\Delta}$ mutation in the germline but otherwise wild type. In the germline clone ovaries, egg chambers rarely developed beyond Stage 10, with various developmental defects such as egg chamber degeneration and germline cell membrane collapse. Strong *me31B* germline knockdown strains showed similar oogenesis defects (40), further emphasizing the importance of *me31B* in oogenesis. Crucially, the loss of

me31B in the germline led to the abnormal translational activation of key germline-development RNAs, *osk,* and *BicD*, suggesting that Me31B acts as a translation repressor.

4.2 Loss of one *me31B* gene copy

Although the loss of one *me31B* gene copy (m*e31B*$^{\Delta}$ heterozygotes) does not seem to affect growth in *Drosophila*, the presence of heterozygosity for both m*e31B*$^{\Delta}$ and other crucial germline or neuron development genes could result in observable phenotypes in the corresponding tissues. In the germline, a reduction in germ cell number phenotype was observed in early embryos when one *me31B* gene copy was lost. Additionally, when one *me31B* gene copy and one copy of germ-plasm-protein genes like *vas* or *tud* were lost simultaneously, a more severe reduction was observed (43). This implies that *me31B* has a genetic interaction with germ plasm genes, and the interaction is crucial for proper germ cell formation. The effects of *me31B*$^{\Delta}$ heterozygosity in neurons will be discussed further below (see Me31B in The Soma).

4.3 Targeted-domain mutations of Me31B

Target-motif-mutations of *me31B* were generated to investigate the functions of specific Me31B motifs. To investigate the role of Me31B helicase activity, three helicase-activity-mutation alleles (*me31B*^{E208A}, *me31B*^{DVLAAAA,} and *me31B*^{R385Q}) were generated using CRISPR through point mutations in those motifs: D**E**AD→D**A**AD, DVLA**R**A**K**→DVLA**A**A**A**, and HRIG**R**→HRIG**Q** (30). These three alleles cause female sterility dominantly. The sterility is associated with various oogenesis and

embryogenesis defects such as egg chamber degenerations, dorsal appendage abnormalities, and unhatchable eggs. This underscores the crucial role of Me31B's helicase activity in germline development and fertility. At the molecular level, the dominant helicase-activity mutations reduce Me31B protein levels and alter the germ plasm localization pattern of germline mRNA *nos* (encoding protein Nos, a key and conserved regulator involved in embryo patterning and germ cell development (1, 44-46)). This suggests that Me31B's helicase activity is involved in stabilizing the protein and modulating substrate RNAs.

To investigate the functions of Me31B's N-ter domain, C-ter domain, and FDF-binding motif (a partner protein binding motif), three additional target-motif-mutation alleles have been created. These are $me31B^{N\text{-}ter}$ (containing N-terminal AA 1 – 276), $me31B^{C\text{-}ter}$ (containing C-terminal AA 277 – 459), and $me31B^{FDF}$ (point mutations in the FDF-binding motif which mediates Me31B's interaction with partner proteins like Tral and Edc3) (30). Homozygous *Drosophila* carrying the three alleles ($me31B^{N\text{-}ter}$, $me31B^{C\text{-}ter}$, and $me31B^{FDF}$) are viable and can complete oogenesis, indicating that these are weak alleles of *me31B*. However, homozygotes of the three alleles display varying degrees of fertility reduction, with $me31B^{C\text{-}ter}$ causing complete sterility in a recessive manner (associated with embryo patterning defects). At the molecular level, the $me31B^{N\text{-}ter}$ and $me31B^{C\text{-}ter}$ mutations reduce Me31B protein levels to varying degrees, while all three mutations ($me31B^{N\text{-}ter}$, $me31B^{C\text{-}ter}$, and $me31B^{FDF}$) alter Me31B intracellular localizations in different ways. This suggests that Me31B's N-ter domain, C-ter domain, and FDF-binding motif all contribute distinct functions to Me31B stability,

subcellular localization, and ultimately, fertility.

5. Me31B Expression and Me31B RNP Granules

me31B transcripts are present throughout all stages of oogenesis from early to late egg development. The transcripts are found in all types of germline cells, including nurse cells (with a burst of *me31B* transcription at early-to-mid oogenesis, around stage 6 - 7), developing oocytes, and early embryos (0 – 4 hours). The transcripts exhibit a uniform, cytoplasmic distribution in these cells. However, the transcripts are no longer detectable in later embryos (after 4 hours) (19).

The expression level of Me31B protein parallels that of the *me31B* mRNA. Me31B is detected in early oogenesis in the germarium (the part of the ovary that contains undifferentiated germline stem cells) and remains at a relatively low level during the early stages of oogenesis (stages 1 – 5). However, there is a significant increase in Me31B around stages 6 – 7, which is consistent with elevated transcription. Me31B remains at a high level throughout the rest of oogenesis and declines to non-detectable levels in early embryogenesis (15). Unlike mRNA, Me31B proteins have specific subcellular localization and aggregation patterns (15, 40, 47) (Figure 1, bottom). They are observed in the cytoplasm, not the nuclei, of both nurse cells and developing oocytes. Me31B is concentrated in oocytes, possibly transported from nurse cells via ring canals (channel-like structures that connect the cytoplasm between the nurse cells and oocytes). In nurse cells and oocytes, Me31B often takes the form of aggregated granules. These granules accumulate in specific regions, such as the peri-nuclear regions of nurse cells (nuage), the cortex of mid-stage oocytes, and the germ plasm

area at the posterior pole of mid-to-late-stage oocytes (germ plasm is the special cytoplasm that contains germ-cell-formation determinants). The granules disperse and become uniformly distributed in late-stage eggs and early embryos before disappearing by the cellular blastoderm stage of embryos.

The Me31B-containing granules are germline ribonucleoproteins (RNPs), which are membraneless and liquid-like condensates composed of germline RNAs and proteins (1, 4, 11, 12, 48-51). These granules are classified into three major groups based on their molecular compositions, subcellular locations, and morphologies: nuage granules found around nurse cell nuclei, P-body/sponge body granules dispersed in the cytoplasm of nurse cells, developing oocytes, and early embryos, and germ plasm granules at the posterior pole of developing oocytes/embryos. Me31B plays an important role in regulating the fate of RNAs in the RNPs by interacting with the RNA and protein constituents, which is an essential process for germline development (9, 47, 52-55).

6. Functions of Me31B in RNP granules

6.1 Physical properties and dynamics of the granules

RNP granules containing Me31B, such as P-bodies, sponge bodies, nuage granules, and germ plasm granules, display physical properties similar to sticky, liquid droplets and are referred to as biomolecular condensates (1, 11, 34, 48, 55-58). A recent study used Me31B-labeled P-bodies during late oogenesis to early embryogenesis to illustrate the general physical properties of germline RNPs and how the properties change with different cellular contexts (34).

In mature oocytes, P-bodies labeled with Me31B are widespread and universally distributed in the bulk oocyte cytoplasm. These micro-sized structures vary in morphology and are highly dynamic, constantly rearranging through fusion and fission. The P-body structure is maintained by a variety of physicochemical and biological forces, including hydrophobic interactions, salt concentrations, RNAs, the cytoskeleton, and protein-protein interactions (particularly the protein-protein interactions between Me31B and Tral). Moreover, recombinant Me31B proteins can form spherical condensates in the presence of a crowding agent, and Me31B's Intrinsically Disordered Regions (IDRs) play a role in modulating the self-aggregation process, suggesting that the IDRs may regulate Me31B aggregation within the P-bodies.

As the developmental stage progresses from mature oocyte to early embryo, the physical properties of Me31B-labeled P-bodies change. P-bodies in mature oocytes maintain typical morphologies and undergo slow rearrangement, with very limited exchange between Me31B in the P-bodies and the cytoplasm. This limited exchange suggests that the P-bodies function as storage units for oocyte RNAs. However, in early embryos after egg activation, the P-bodies become much more dynamic. They disperse into smaller condensates, contain more highly mobile Me31B, and dissociate from stored maternal RNAs like *bicoid (bcd)*. The change from the arrested state in mature oocytes to the dynamic state in early embryos is believed to occur upon egg activation, facilitating the disintegration of the P-bodies and the release of the stored RNAs for translation, enabling further embryogenesis.

6.2 Me31B interactome in the RNPs.

To gain insight into the functions of Me31B complexes in RNP granules, a proteomics approach was implemented to isolate Me31B complexes from *Drosophila* ovaries. Through this approach, the Me31B protein interactome was comprehensively identified (40), revealing the presence of proteins from four functional groups: RNA regulators (repressors like Cup and Tral; degraders like PCM and EDC3), cytoskeleton and motor proteins (like dynein, kinesin, and tubulin components), glycolytic enzymes, and core germ plasm proteins involved in germ plasm assembly and germ cell formation (like Vas, Aub, and Tud). This led to the development of a model for a Me31B-centered functional unit in the RNP granules, with Me31B serving as the central hub (Figure 2). The proposed model outlines the various components that work in conjunction with Me31B, highlighting their individual purposes and relationships. Firstly, Me31B collaborates with RNA repressor partners to enable post-transcriptional regulations over the RNAs. Secondly, cytoskeleton/motor proteins act as moving tracks and energy sources for the transportation of the granules. Thirdly, glycolytic enzymes provide ATP to support the functioning of RNA helicases and motor proteins. Fourthly, Me31B interacts with core germ plasm proteins, which hints at its possible roles in the assembly and function of nuage and germ plasm granules.

In a subsequent investigation utilizing a comparable proteomics approach, a Me31B early-embryo interactome was acquired, which corroborated the suggested model above by revealing the same four protein groups. In addition, when compared with its ovary interactome counterpart, the Me31B early-embryo interactome showed a

significant reduction of core germ plasm proteins, supporting the notion that Me31B RNP granules differ in composition in different cellular contexts (47).

To understand Me31B's RNA interactome, a study utilized an RNA immunoprecipitation + sequencing (RIP-seq) approach to comprehensively identify Me31B-bound mRNAs in *Drosophila* early embryos. The results revealed that transcripts from almost all expressed genes were enriched in the Me31B precipitates (59). This suggests that Me31B may bind most if not all, expressed germline mRNAs without specificity. This interpretation was further supported by a Me31B-germline mRNA co-staining experiment, which found seven representative mRNAs (*osk*, *BicD*, *bcd, nos*, *orb*, *Pgc*, and *gcl*) to colocalize with Me31B granules in various stages of egg chambers (15). Additionally, the RIP-Seq study has revealed that Me31B has different effects on its bound mRNAs depending on the cellular contexts (59). To elaborate, during the early stages of embryonic development (0-1 hour, before the Maternal to Zygotic Transition (MZT), an event that marks the shift in developmental control from maternal factors to zygotic gene expression), Me31B binds to nearly all transcripts and inhibit their translation. However, as the embryos go through MZT (1-5 hours), the transcripts bound by Me31B undergo progressive changes, and the binding shifts from repressing translation to destabilizing the mRNAs. This highlights the dynamic RNA interactome and functions of Me31B throughout germline development.

6.3 Me31B repressor complex on *nos* mRNA

To explain how a Me31B-containing repressor complex can suppress and break down mRNAs during germline development, we reference the *nanos (nos)* mRNA

repression complex model proposed by Gotze et al (54) and its associated sources. The intricate suppression and degradation of *nos* mRNA is crucial for the proper timing and location of Nos protein production, which ultimately contributes to *Drosophila* embryo body patterning and germ cell formation (10, 60-62). This repression mechanism relies on the collaboration of Me31B and other RNA repression and degradation factors, such as Smaug (Smg), Belle (Bel), Cup, Tral, the CCR4-NOT complex, and more (Figure 3) (31, 42, 54, 63-66). The model begins with Smaug (Smg), an RNA repressor/degrader during the MTZ of embryogenesis, binding to the two Smaug Recognition Elements (SREs) in the 3' UTR of *nos*. Smg then recruits Cup, which further binds eIF4E, Tral, and Me31B directly or indirectly. Me31B interacts with Tral via its FDF-binding motif to Tral's FDF motif, and the Me31B-Tral complex along the length of *nos* RNA, coating it and inhibiting its translation. Me31B or eIF4E may also bind Belle (Bel), another repressor helicase, to cooperatively repress *nos* RNA. Finally, Me31B and/or Smaug recruits the deadenylation complex CCR4-NOT to digest the *nos* from its 3' poly-A tail and destabilize the RNA for degradation. It is important to note that different repression models may be at work on *nos* mRNA at different developmental stages. For example, in late oogenesis rather than early embryogenesis, *nos* repression relies on Glorund (Glo). It directly binds to *nos* mRNA at a 3'-UTR sequence different from the SREs and recruits another repressor dFMRP to inhibit *nos* translation (46, 67-69). Despite this, the example of *nos* repressor complex suggests that Me31B may play repressor roles on germline mRNAs by directly coating the mRNA to shield it from translational machinery and facilitating the communication/recruitment of other mRNA-specific (like Smg) or

non-specific (like Cup and Tral) repressors.

6.4 Interaction between Me31B-containing P-bodies and U bodies

Recent research revealed physical and genetic connections between Me31B-containing P-bodies and other RNP structures like U bodies. U bodies, named after their Uridine-rich small nuclear ribonucleoproteins (U snRNPs), are believed to be the cytoplasmic sites of snRNP biogenesis, assembly, and storage before they are transported into the nucleus for mRNA processing (70). While distinct entities within *Drosophila* germline cells (70), P-bodies and U bodies exhibit evidence of mutual interaction. For instance, although not all P-bodies are linked with U bodies, U bodies consistently associate with P bodies (70, 71). Additionally, mutations affecting key components of both structures (such as SMN in U bodies and Cup in P-bodies) result in similar nuclear morphology phenotypes, disrupting their typical morphology and distribution pattern (72). These findings suggest a dependence of Me31B-containing P-bodies on other RNP structures like U bodies in RNP assembly and hint at potential collaboration in the intracellular trafficking of snRNPs and mRNPs (72).

7. Me31B in the Male Germline

Research on Me31B's role in the male germline is limited compared to that in females. However, recent studies have shown that this maternal gene also functions in the male germline cells. In one study, Me31B was found to be expressed in male testis cells like germline stem cells (GSCs), spermatogonia (SG), and spermatocytes (73). Me31B was observed in cytoplasmic P-bodies, where it co-localizes with Decapping

protein 1 (Dcp1) and exoribonuclease Pacman (PCM), both of which are involved in RNA degradation pathways. The study suggested that Me31B and Dcp1 work together to bind mRNAs and present them for degradation by PCM, which is required for male *Drosophila* spermatogenesis and fertility. Interestingly, the co-localization pattern of Me31B-Dcp1-PCM in male cells was similar to that seen in P-bodies in female nurse cells (32, 74), indicating a potentially common mRNA regulation pathway mediated by Me31B-containing P-bodies in both sexes.

In a second study, Me31B's role in maintaining *Drosophila* male germline stem cell (GSC) homeostasis was revealed (75). The study indicated that Me31B is vital in preventing excessive de-differentiation of spermatogonia back into GSC, which may result from the failure in downregulating the expression of the GSC-maintenance and germ cell identity regulator *nos*. The study proposed a model explaining Me31B's role in GSC homeostasis. The model suggests that *nos* mRNA is initially translated in GSCs and their immediate daughter cells, which helps maintain the GSC's stem cell status. Then, as GSCs give rise to SGs, Me31B binds and translationally represses *nos* mRNA, causing a Nos protein level decrease in the SGs, thereby promoting SG differentiation. This mechanism of action bears resemblance to the function of Me31B in suppressing *nos* mRNA translation during early embryo development, hinting at a comparable role of Me31B in regulating germline mRNAs in both sexes.

According to a third study, Me31B is associated with eIF4E-3, a specific isoform of Eukaryotic translation initiation factor 4E that is expressed in *Drosophila* testis and essential for spermatogenesis (76). The research demonstrated that the two proteins co-express, co-immunoprecipitate, and co-localize in male germline cells such as

spermatocytes. The Me31B-eIF4E-3 interaction connects the repressive function of Me31B to the regulation of translation.

8. Me31B in The Soma

Beyond the germline, it is noteworthy that Me31B also plays pivotal biological roles in somatic cells, with a particular emphasis on its involvement in neuronal functions. In fact, both *Drosophila* and mammalian neurons contain dynamic, cytoplasmic RNP granules known as “neuronal granules” (77-81) that share many similarities with germline RNPs in terms of morphology, composition, and function. These granules often contain RNA regulators such as Me31B, FMRP (dFMR1 in *Drosophila*), Staufen (Stau), and ATX-2 (32, 82), and appear to be involved in the storage and regulation of translationally repressed mRNAs (83-88). In the following paragraphs, we will examine a range of studies (conducted both in cell cultures and brains) that explore Me31B’s impact on neuron physiology, development, and neuronal mRNA metabolism.

In primary culture *Drosophila* neurons, the overexpression of Me31B leads to abnormal down-regulation of high-order dendritic complexity. Conversely, the knockdown of Me31B yielded opposite effects, with all phenotypes dependent on Me31B’s DEAD-box motif. These results suggest Me31B’s important role in dendrite morphogenesis (32).

In adult *Drosophila* brains, Me31B is present in several types of neuronal cells, including olfactory projection neurons, local interneurons, pacemaker neurons, mushroom-body neurons, and glial cells (89). In olfactory projection neurons (PNs),

Me31B is essential for the translational repression of CaMKII reporter mRNA, which is a miRNA-regulated, synapse-localizing mRNA. Additionally, the *me31B* gene and its genetic interaction with *atx2* and *dFMR1* are necessary for long-term olfactory habituation (LTH), a behavior trait involving olfactory PNs, and LTH-associated neuron structural plasticity (90-92). In circadian pacemaker neurons, Me31B associates with and genetically interacts with *atx2* to maintain rhythmic circadian behaviors. This process is hypothetically linked to the repression functions of an Atx2-Me31B-NOT1 complex (93). In mushroom body γ neurons (neurons involved in learning and memory), Me31B-containing neuronal granules condense, recruit repressor protein Imp and its target mRNA *profilin*, and inhibit *profilin* translation during neuron aging (94). Conversely, the granules de-condense, release Imp and *profilin* mRNA, and de-repress *profilin* translation upon neuron stimulation by a biogenic peptide (95).

The Me31B ortholog, DDX6, can be located in the hippocampal neurons within mammals. As the neurons mature, DDX6-containing neuronal granules will disassemble into smaller-sized granules, but they will re-assemble into large granules if synaptic activities are inhibited. Additionally, DDX6 plays an important role in synaptic functions such as the appropriate clustering of a postsynaptic marker protein in neuronal dendrites and the frequency of Ca^{2+} peaking (96).

Me31B is not only found in neurons but also non-neuron soma. Me31B plays a crucial role in the cytoplasmic foci formation of core P-body components Dcp1 and Pcm in *Drosophila* wing imaginal discs (undifferentiated precursor cells of *Drosophila* wing). It also helps in the recruitment of repressor dFMR1 to P-bodies, and *bantam* and *miR2* miRNA- mediated translational repression (82). In *Drosophila* eyes, Me31B, along with

its partner repressor Tral, is needed for a *dFMR1* gene-mediated “rough eye” phenotype (32).

Numerous studies examined in this section have provided convincing evidence that Me31B, or its orthologs like DDX6, plays a critical role in regulating neuron physiology, development, and synaptic activities. Me31B likely accomplishes this by forming and modifying neuronal RNP granules, which in turn regulates of the translation of neuronal mRNAs in a spatiotemporal manner. Storage, localization, repression, activation, and decay of these transcripts enable the neurons to respond appropriately to intracellular and extracellular signals (96-98). This mode of action of Me31B in neuronal (and other somatic) granules is similar to what was observed in the germline, indicating that Me31B may be a necessary common component for the repressing RNPs in various cell types and species.

9. How Does Me31B Affect Target mRNAs?

Many studies have identified Me31B as a general repressor in various RNPs, yet the exact means by which the protein affects target mRNA remains elusive. A growing body of evidence suggests Me31B promotes RNA storage, repression, and degradation through a network of interrelated mechanisms, including direct RNA coating, interaction with RNA-induced silencing complex (RISC), decapping factors, deadenylation complexes, and ribonucleases, as well as interfering with ribosomes (a schematic illustration showed in Figure 4). Given the limited research on *Drosophila* Me31B, we also draw upon studies of its orthologs (such as DDX6/RCK in humans, p54 in mice, Xp54 in frogs, CGH-1 in worms, Dhh1p in yeast) to shed light how this conserved family

of helicases affects target mRNAs.

9.1 RNA coating.

According to the following studies, it is believed that Me31B functions as a repressor of target mRNAs by forming oligomers on them, coating them, and potentially shielding them from translation machinery. One study proposed that Me31B-Tral repressor dimers polymerize along *nos* mRNA (54). Another study utilizing an Xp54 RNA-tethering assay found that Xp54 oligomerizes on mRNA in an RNA- and helicase activity-dependent manner (99). Similarly, in a binding assay with recombinant DDX6 proteins, DDX6 polymerized and bound to single-stranded RNA targets (100).

9.2 Participation in RISC-mediated pathways

RISCs are cellular complexes that play a vital role in the mRNA regulation pathway. Equipped with small RNAs, these complexes guide themselves to complementary or partially complementary target mRNAs, using effector proteins like Argonaute (AGO) to achieve translational repression or degradation (101-104). RISC-mediated RNA regulation pathways rely on Me31B, or its equivalents in other organisms (105-112), to facilitate relevant mRNA degradation events such as P-body assembly (29, 106, 110, 113-115), mRNA decapping (41, 116-119), deadenylation (38, 117, 120-122), and nuclease digestion (47, 89, 108, 123, 124). While human DDX6 and fly Me31B act as repressors for RISC-targeted mRNAs, they do not cleave the mRNAs (which is usually done by nucleases like AGO) (105). This is consistent with Me31B's role as a general repressor, rather than a degrader. When working with decapping factors, yeast

Dhh1p promotes efficient mRNA decapping and directly interacts with main decapping enzymes Dcp1/Dcp2 and other decapping activators like Pat1 and Edc3 (116, 117). However, Dhh1p is believed to work by repressing translation initiation, rather than stimulating the decapping enzymes (Dcp1/Dcp2) (41). During mRNA deadenylation, Me31B interacts physically with a highly conserved deadenylation complex CCR4-NOT in yeast, fruit fly, and human cells (38, 117, 120-122). It is important to mention that in humans, DDX6 binds to the NOT1 protein which acts as the assembly scaffold of the CCR4-NOT complex. This binding stimulates DDX6's ATP hydrolysis activity (125) and is crucial for RNA repression and silencing (38, 111, 125). This suggests that DDX6's ATPase activity plays a significant role in its function as a repressor.

9.3 Interaction with ribosomes.

Research has shown that yeast Dhh1p interacts with ribosomes, potentially modulating translation. Recombinant Dhh1p at high concentrations can prevent the formation of translation preinitiation complexes (41)**.** Dhh1p also binds preferentially to mRNAs containing non-optimal codons (i.e., low translation efficiency mRNAs), leading to an accumulation of slow-moving ribosomes and the mRNA's degradation (126, 127). Additionally, Dhh1p can physically interact with eukaryotic ribosomes and certain ribosomal RNAs (126).

It should be noted that Me31B's mechanisms of action discussed in this section (Figure 4), such as RNA coating, interacting with RISC, decapping enzymes, deadenylation complexes, degradation enzymes, and modulating ribosomes, are interconnected and often converge as sequential processing steps involved in RNA

regulation.

10. Perspectives and Unanswered Questions

There are still a lot of questions when it comes to the molecular biology of the Me31B. We will delve into several of them, as we believe their answers will give us a better understanding of this conserved helicase family.

Box 1. Unanswered Questions

1. What is the role of Me31B in an RNP?
    a. If Me31B is a repressor, scaffold, and/or remodeler, how does Me31B transit between different functions to render different fates to mRNAs?
2. Does Me31B's function rely on the protein's localization in RNPs?
3. What's Me31B's molecular level working mechanism?
    a. How does Me31B interact with RNAs and protein partners?
    b. What are the biological implications of these interactions?
    c. How does Me31B adapt to changing cellular contexts to remodel the RNPs and output the appropriate functions?

What is the role of Me31B in an RNP? Based on the research reviewed, Me31B has been identified as a versatile player, serving as a general repressor, an RNP scaffold/hub, and an RNP remodeler. As a general repressor, Me31B exhibits low RNA-binding specificity and inhibits translation without modifying the RNA. As an RNP scaffold/hub, Me31B coordinates the assembly of a repressor RNP on a target RNA by recruiting and/or being recruited by other factors. Me31B's interaction with these factors will determine the RNA's fate. As an RNP remodeler, Me31B can dynamically alter an RNP's composition, morphology, and function in response to developmental changes

and cellular contexts. This allows Me31B-bound mRNAs to experience different outcomes, including degradation, repression for storage, or release for translational activation. This naturally raises the question of how Me31B can transit between these different functions. An earlier study proposed an intriguing model suggesting that Me31B alters RNP functions by component exchanges (89). This idea is supported by previous research indicating that Me31B's C-terminal RecA-like domain binds exclusively to different partners, including Edc3, Tral, ATX2, and NOT1 (33, 93, 128). The binding of Edc3 with DDX6 leads to the assembly of a decapping complex (containing decapping factors Dcp1, Edc4, and Dcp2), while the binding of Tral with DDX6 results in a repressing complex (containing eIF4E binding protein Cup, which can inhibit the decapping of bound mRNA). Such a transition in the fate of bound mRNA would be significant (129). Furthermore, Pat and Edc3's binding interferes and competes with the RNA binding of yeast Dhh1p (128), suggesting a flexible Dhh1p-RNA interaction and a possible mechanism for RNA release.

The role of Me31B and its dependence on the protein's localization into RNP granules has sparked much debate. However, a recent study shed light on this topic by introducing a *Drosophila* strain carrying mutations that prevent Me31B's localization to RNP granules (30). By disrupting Me31B's FDF-binding motif, the mutant Me31B is dispersed throughout the germline instead of aggregating onto RNP granules. Interestingly, this dispersed Me31B only slightly decreased fertility and did not appear to affect *Drosophila* oogenesis, embryogenesis, or the formation of germline RNPs (as indicated by other maker proteins Tral and Cup). These findings suggest that Me31B's localization to RNP granules may not be necessary for its function. One possible

explanation is that Me31B is abundant enough in the germline to interact with the RNPs and provide essential mRNA regulation functions. It would be valuable to investigate whether this concept applies to other species or cell systems.

Understanding the full working mechanism of Me31B is a complex yet crucial endeavor. How does Me31B interact with RNAs and various protein partners, and what are the implications of the interactions? Furthermore, how does Me31B adapt to changing cellular contexts to remodel the RNPs and output the appropriate functions? Although past research has provided some answers, a comprehensive understanding of Me31B's working mechanism remains elusive. One promising future approach may involve the systematic generation of weak, target-motif-mutation alleles of *me31B*. These tools could prove invaluable in uncovering the individual roles of different Me31B motifs, ultimately leading to a more complete understanding of the protein's overall mechanism and function.

Declaration of Competing Interest

There are no competing interests.

Data Availability

No research data was generated in the article.

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References

1. Trcek T, Lehmann R. Germ granules in Drosophila. Traffic. 2019;20(9):650-60. DOI: 10.1111/tra.12674.

2. St Johnston D. Polarity and axis formation in the Drosophila female germ line. Curr Top Dev Biol. 2023;154:73-97. DOI: 10.1016/bs.ctdb.2023.02.002.

3. Grmai L, Pozmanter C, Van Doren M. The Regulation of Germline Sex Determination in Drosophila by Sex lethal. Sex Dev. 2022;16(5-6):323-8. DOI: 10.1159/000521235.

4. Cassani M, Seydoux G. P-body-like condensates in the germline. Semin Cell Dev Biol. 2024;157:24-32. DOI: 10.1016/j.semcdb.2023.06.010.

5. Chiappetta A, Liao J, Tian S, Trcek T. Structural and functional organization of germ plasm condensates. Biochem J. 2022;479(24):2477-95. DOI: 10.1042/BCJ20210815.

6. Schisa JA, Elaswad MT. An Emerging Role for Post-translational Modifications in Regulating RNP Condensates in the Germ Line. Front Mol Biosci. 2021;8:658020. DOI: 10.3389/fmolb.2021.658020.

7. Mukherjee N, Mukherjee C. Germ cell ribonucleoprotein granules in different clades of life: From insects to mammals. Wiley Interdiscip Rev RNA. 2021;12(4):e1642. DOI: 10.1002/wrna.1642.

8. Schupbach T, Wieschaus E. Germline autonomy of maternal-effect mutations altering the embryonic body pattern of Drosophila. Dev Biol. 1986;113(2):443-8. DOI: 10.1016/0012-1606(86)90179-x.

9. Arkov AL, Ramos A. Building RNA-protein granules: insight from the germline. Trends Cell Biol. 2010;20(8):482-90. DOI: 10.1016/j.tcb.2010.05.004.

10. Wang JT, Seydoux G. Germ cell specification. Adv Exp Med Biol. 2013;757:17-39. DOI: 10.1007/978-1-4614-4015-4_2.

11. Gao M, Arkov AL. Next generation organelles: structure and role of germ granules in the germline. Mol Reprod Dev. 2013;80(8):610-23. DOI: 10.1002/mrd.22115.

12. Lehmann R. Germ Plasm Biogenesis--An Oskar-Centric Perspective. Curr Top Dev Biol. 2016;116:679-707. DOI: 10.1016/bs.ctdb.2015.11.024.

13. Swartz SZ, Wessel GM. Germ Line Versus Soma in the Transition from Egg to Embryo. Curr Top Dev Biol. 2015;113:149-90. DOI: 10.1016/bs.ctdb.2015.06.003.

14. Eichler CE, Hakes AC, Hull B, Gavis ER. Compartmentalized oskar degradation in the germ plasm safeguards germline development. Elife. 2020;9. DOI: 10.7554/eLife.49988.

15. Nakamura A, Amikura R, Hanyu K, Kobayashi S. Me31B silences translation of oocyte-localizing RNAs through the formation of cytoplasmic RNP complex during Drosophila oogenesis. Development. 2001;128(17):3233-42. DOI: 10.1242/dev.128.17.3233.

16. Balak C, Benard M, Schaefer E, Iqbal S, Ramsey K, Ernoult-Lange M, et al. Rare De Novo Missense Variants in RNA Helicase DDX6 Cause Intellectual Disability and

538 Dysmorphic Features and Lead to P-Body Defects and RNA Dysregulation. Am J Hum
539 Genet. 2019;105(3):509-25. DOI: 10.1016/j.ajhg.2019.07.010.
540 17. Alves-Rodrigues I, Mas A, Diez J. Xenopus Xp54 and human RCK/p54 helicases
541 functionally replace yeast Dhh1p in brome mosaic virus RNA replication. J Virol.
542 2007;81(8):4378-80. DOI: 10.1128/JVI.02246-06.
543 18. Weston A, Sommerville J. Xp54 and related (DDX6-like) RNA helicases: roles in
544 messenger RNP assembly, translation regulation and RNA degradation. Nucleic Acids
545 Res. 2006;34(10):3082-94. DOI: 10.1093/nar/gkl409.
546 19. de Valoir T, Tucker MA, Belikoff EJ, Camp LA, Bolduc C, Beckingham K. A
547 second maternally expressed Drosophila gene encodes a putative RNA helicase of the
548 "DEAD box" family. Proc Natl Acad Sci U S A. 1991;88(6):2113-7. DOI:
549 10.1073/pnas.88.6.2113.
550 20. Cargill M, Venkataraman R, Lee S. DEAD-Box RNA Helicases and Genome
551 Stability. Genes (Basel). 2021;12(10). DOI: 10.3390/genes12101471.
552 21. Li F, Fountzilas C, Puzanov I, Attwood KM, Morrison C, Ling X. Multiple functions
553 of the DEAD-box RNA helicase, DDX5 (p68), make DDX5 a superior oncogenic
554 biomarker and target for targeted cancer therapy. Am J Cancer Res. 2021;11(10):5190-
555 213. DOI: 10.1186/s13046-023-02787-x.
556 22. Xu C, Cao Y, Bao J. Building RNA-protein germ granules: insights from the
557 multifaceted functions of DEAD-box helicase Vasa/Ddx4 in germline development. Cell
558 Mol Life Sci. 2021;79(1):4. DOI: 10.1007/s00018-021-04069-1.

559 23. Weis K, Hondele M. The Role of DEAD-Box ATPases in Gene Expression and
560 the Regulation of RNA-Protein Condensates. Annu Rev Biochem. 2022;91:197-219.
561 DOI: 10.1146/annurev-biochem-032620-105429.
562 24. Ali MAM. DEAD-box RNA helicases: The driving forces behind RNA metabolism
563 at the crossroad of viral replication and antiviral innate immunity. Virus Res.
564 2021;296:198352. DOI: 10.1016/j.virusres.2021.198352.
565 25. Weis K. Dead or alive: DEAD-box ATPases as regulators of ribonucleoprotein
566 complex condensation. Biol Chem. 2021;402(5):653-61. DOI: 10.1515/hsz-2020-0381.
567 26. Overwijn D, Hondele M. DEAD-box ATPases as regulators of biomolecular
568 condensates and membrane-less organelles. Trends Biochem Sci. 2023;48(3):244-58.
569 DOI: 10.1016/j.tibs.2022.10.001.
570 27. Khemici V, Linder P. RNA helicases in RNA decay. Biochem Soc Trans.
571 2018;46(1):163-72. DOI: 10.1042/BST20170052.
572 28. Minshall N, Thom G, Standart N. A conserved role of a DEAD box helicase in
573 mRNA masking. RNA. 2001;7(12):1728-42. DOI: 10.1017/s135583820101158x.
574 29. Minshall N, Kress M, Weil D, Standart N. Role of p54 RNA helicase activity and
575 its C-terminal domain in translational repression, P-body localization and assembly. Mol
576 Biol Cell. 2009;20(9):2464-72. DOI: 10.1091/mbc.E09-01-0035.
577 30. Kara E, McCambridge A, Proffer M, Dilts C, Pumnea B, Eshak J, et al. Mutational
578 analysis of the functional motifs of the DEAD-box RNA helicase Me31B/DDX6 in
579 Drosophila germline development. FEBS Lett. 2023;597(14):1848-67. DOI:
580 10.1002/1873-3468.14668.

31. Tritschler F, Eulalio A, Helms S, Schmidt S, Coles M, Weichenrieder O, et al. Similar modes of interaction enable Trailer Hitch and EDC3 to associate with DCP1 and Me31B in distinct protein complexes. Mol Cell Biol. 2008;28(21):6695-708. DOI: 10.1128/MCB.00759-08.

32. Barbee SA, Estes PS, Cziko AM, Hillebrand J, Luedeman RA, Coller JM, et al. Staufen- and FMRP-containing neuronal RNPs are structurally and functionally related to somatic P bodies. Neuron. 2006;52(6):997-1009. DOI: 10.1016/j.neuron.2006.10.028.

33. Tritschler F, Braun JE, Eulalio A, Truffault V, Izaurralde E, Weichenrieder O. Structural basis for the mutually exclusive anchoring of P body components EDC3 and Tral to the DEAD box protein DDX6/Me31B. Mol Cell. 2009;33(5):661-8. DOI: 10.1016/j.molcel.2009.02.014.

34. Sankaranarayanan M, Emenecker RJ, Wilby EL, Jahnel M, Trussina I, Wayland M, et al. Adaptable P body physical states differentially regulate bicoid mRNA storage during early Drosophila development. Dev Cell. 2021;56(20):2886-901 e6. DOI: 10.1016/j.devcel.2021.09.021.

35. Huang JH, Ku WC, Chen YC, Chang YL, Chu CY. Dual mechanisms regulate the nucleocytoplasmic localization of human DDX6. Sci Rep. 2017;7:42853. DOI: 10.1038/srep42853.

36. Peter D, Ruscica V, Bawankar P, Weber R, Helms S, Valkov E, et al. Molecular basis for GIGYF-Me31B complex assembly in 4EHP-mediated translational repression. Genes Dev. 2019;33(19-20):1355-60. DOI: 10.1101/gad.329219.119.

37. Ozgur S, Basquin J, Kamenska A, Filipowicz W, Standart N, Conti E. Structure of a Human 4E-T/DDX6/CNOT1 Complex Reveals the Different Interplay of DDX6-Binding

Proteins with the CCR4-NOT Complex. Cell Rep. 2015;13(4):703-11. DOI: 10.1016/j.celrep.2015.09.033.

38. Chen Y, Boland A, Kuzuoglu-Ozturk D, Bawankar P, Loh B, Chang CT, et al. A DDX6-CNOT1 complex and W-binding pockets in CNOT9 reveal direct links between miRNA target recognition and silencing. Mol Cell. 2014;54(5):737-50. DOI: 10.1016/j.molcel.2014.03.034.

39. Layana C, Vilardo ES, Corujo G, Hernández G, Rivera-Pomar R. Distinct domains of Me31B interact with different eIF4E isoforms in the male germ line of Drosophila melanogaster. bioRxiv. 2021:2021.03.23.436655. DOI: 10.1101/2021.03.23.436655.

40. DeHaan H, McCambridge A, Armstrong B, Cruse C, Solanki D, Trinidad JC, et al. An in vivo Proteomic Analysis of the Me31B Interactome in Drosophila Germ Granules. FEBS Lett. 2017. DOI: 10.1002/1873-3468.12854.

41. Coller J, Parker R. General translational repression by activators of mRNA decapping. Cell. 2005;122(6):875-86. DOI: 10.1016/j.cell.2005.07.012.

42. Liao SE, Kandasamy SK, Zhu L, Fukunaga R. DEAD-box RNA helicase Belle posttranscriptionally promotes gene expression in an ATPase activity-dependent manner. RNA. 2019;25(7):825-39. DOI: 10.1261/rna.070268.118.

43. Thomson T, Liu N, Arkov A, Lehmann R, Lasko P. Isolation of new polar granule components in Drosophila reveals P body and ER associated proteins. Mech Dev. 2008;125(9-10):865-73. DOI: 10.1016/j.mod.2008.06.005.

44. Oulhen N, Wessel GM. Every which way--nanos gene regulation in echinoderms. Genesis. 2014;52(3):279-86. DOI: 10.1002/dvg.22737.

627 45. Kugler JM, Lasko P. Localization, anchoring and translational control of oskar,
628 gurken, bicoid and nanos mRNA during Drosophila oogenesis. Fly (Austin).
629 2009;3(1):15-28. DOI: 10.4161/fly.3.1.7751.
630 46. Warden MS, DeRose EF, Tamayo JV, Mueller GA, Gavis ER, Hall TMT. The
631 translational repressor Glorund uses interchangeable RNA recognition domains to
632 recognize Drosophila nanos. Nucleic Acids Res. 2023;51(16):8836-49. DOI:
633 10.1093/nar/gkad586.
634 47. McCambridge A, Solanki D, Olchawa N, Govani N, Trinidad JC, Gao M.
635 Comparative Proteomics Reveal Me31B's Interactome Dynamics, Expression
636 Regulation, and Assembly Mechanism into Germ Granules during Drosophila Germline
637 Development. Sci Rep. 2020;10(1):564. DOI: 10.1038/s41598-020-57492-y.
638 48. Bose M, Lampe M, Mahamid J, Ephrussi A. Liquid-to-solid phase transition of
639 oskar ribonucleoprotein granules is essential for their function in Drosophila embryonic
640 development. Cell. 2022;185(8):1308-24 e23. DOI: 10.1016/j.cell.2022.02.022.
641 49. Hakes AC, Gavis ER. Plasticity of Drosophila germ granules during germ cell
642 development. PLoS Biol. 2023;21(4):e3002069. DOI: 10.1371/journal.pbio.3002069.
643 50. Vo HDL, Wahiduzzaman, Tindell SJ, Zheng J, Gao M, Arkov AL. Protein
644 components of ribonucleoprotein granules from Drosophila germ cells oligomerize and
645 show distinct spatial organization during germline development. Sci Rep.
646 2019;9(1):19190. DOI: 10.1038/s41598-019-55747-x.
647 51. Valentino M, Ortega BM, Ulrich B, Doyle DA, Farnum ED, Joiner DA, et al.
648 Computational modeling offers new insight into Drosophila germ granule development.
649 Biophys J. 2022;121(8):1465-82. DOI: 10.1016/j.bpj.2022.03.014.

650 52. Voronina E, Seydoux G, Sassone-Corsi P, Nagamori I. RNA granules in germ
651 cells. Cold Spring Harb Perspect Biol. 2011;3(12). DOI: 10.1101/cshperspect.a002774.
652 53. Rangan P, DeGennaro M, Jaime-Bustamante K, Coux RX, Martinho RG,
653 Lehmann R. Temporal and spatial control of germ-plasm RNAs. Curr Biol.
654 2009;19(1):72-7. DOI: 10.1016/j.cub.2008.11.066.
655 54. Gotze M, Dufourt J, Ihling C, Rammelt C, Pierson S, Sambrani N, et al.
656 Translational repression of the Drosophila nanos mRNA involves the RNA helicase
657 Belle and RNA coating by Me31B and Trailer hitch. RNA. 2017. DOI:
658 10.1261/rna.062208.117.
659 55. Sankaranarayanan M, Emenecker RJ, Jahnel M, Trussina IREA, Wayland M,
660 Alberti S, et al. The arrested state of processing bodies supports mRNA regulation in
661 early development. bioRxiv. 2021:2021.03.16.435709. DOI:
662 10.1101/2021.03.16.435709.
663 56. Fare CM, Villani A, Drake LE, Shorter J. Higher-order organization of
664 biomolecular condensates. Open Biol. 2021;11(6):210137. DOI: 10.1098/rsob.210137.
665 57. Hubstenberger A, Noble SL, Cameron C, Evans TC. Translation repressors, an
666 RNA helicase, and developmental cues control RNP phase transitions during early
667 development. Dev Cell. 2013;27(2):161-73. DOI: 10.1016/j.devcel.2013.09.024.
668 58. Langerak S, Trombley A, Patterson JR, Leroux D, Couch A, Wood MP, Schisa
669 JA. Remodeling of the endoplasmic reticulum in Caenorhabditis elegans oocytes is
670 regulated by CGH-1. Genesis. 2019;57(2):e23267. DOI: 10.1002/dvg.23267.

59. Wang M, Ly M, Lugowski A, Laver JD, Lipshitz HD, Smibert CA, Rissland OS. ME31B globally represses maternal mRNAs by two distinct mechanisms during the Drosophila maternal-to-zygotic transition. Elife. 2017;6. DOI: 10.7554/eLife.27891.

60. Wang C, Lehmann R. Nanos is the localized posterior determinant in Drosophila. Cell. 1991;66(4):637-47. DOI: 10.1016/0092-8674(91)90110-k.

61. Gavis ER, Lehmann R. Translational regulation of nanos by RNA localization. Nature. 1994;369(6478):315-8. DOI: 10.1038/369315a0.

62. Bergsten SE, Gavis ER. Role for mRNA localization in translational activation but not spatial restriction of nanos RNA. Development. 1999;126(4):659-69. DOI: 10.1242/dev.126.4.659.

63. Wilhelm JE, Hilton M, Amos Q, Henzel WJ. Cup is an eIF4E binding protein required for both the translational repression of oskar and the recruitment of Barentsz. J Cell Biol. 2003;163(6):1197-204. DOI: 10.1083/jcb.200309088.

64. Nelson MR, Leidal AM, Smibert CA. Drosophila Cup is an eIF4E-binding protein that functions in Smaug-mediated translational repression. EMBO J. 2004;23(1):150-9. DOI: 10.1038/sj.emboj.7600026.

65. Nakamura A, Sato K, Hanyu-Nakamura K. Drosophila cup is an eIF4E binding protein that associates with Bruno and regulates oskar mRNA translation in oogenesis. Dev Cell. 2004;6(1):69-78. DOI: 10.1016/s1534-5807(03)00400-3.

66. Jeske M, Moritz B, Anders A, Wahle E. Smaug assembles an ATP-dependent stable complex repressing nanos mRNA translation at multiple levels. EMBO J. 2011;30(1):90-103. DOI: 10.1038/emboj.2010.283.

693 67. Forrest KM, Clark IE, Jain RA, Gavis ER. Temporal complexity within a
694 translational control element in the nanos mRNA. Development. 2004;131(23):5849-57.
695 DOI: 10.1242/dev.01460.
696 68. Peng Y, Gavis ER. The Drosophila hnRNP F/H homolog Glorund recruits dFMRP
697 to inhibit nanos translation elongation. Nucleic Acids Res. 2022;50(12):7067-83. DOI:
698 10.1093/nar/gkac500.
699 69. Andrews S, Snowflack DR, Clark IE, Gavis ER. Multiple mechanisms collaborate
700 to repress nanos translation in the Drosophila ovary and embryo. RNA. 2011;17(5):967-
701 77. DOI: 10.1261/rna.2478611.
702 70. Liu JL, Gall JG. U bodies are cytoplasmic structures that contain uridine-rich
703 small nuclear ribonucleoproteins and associate with P bodies. Proc Natl Acad Sci U S A.
704 2007;104(28):11655-9. DOI: 10.1073/pnas.0704977104.
705 71. Cauchi RJ, Sanchez-Pulido L, Liu JL. Drosophila SMN complex proteins Gemin2,
706 Gemin3, and Gemin5 are components of U bodies. Exp Cell Res. 2010;316(14):2354-
707 64. DOI: 10.1016/j.yexcr.2010.05.001.
708 72. Lee L, Davies SE, Liu JL. The spinal muscular atrophy protein SMN affects
709 Drosophila germline nuclear organization through the U body-P body pathway. Dev Biol.
710 2009;332(1):142-55. DOI: 10.1016/j.ydbio.2009.05.553.
711 73. Zabolotskaya MV, Grima DP, Lin MD, Chou TB, Newbury SF. The 5'-3'
712 exoribonuclease Pacman is required for normal male fertility and is dynamically
713 localized in cytoplasmic particles in Drosophila testis cells. Biochem J. 2008;416(3):327-
714 35. DOI: 10.1042/BJ20071720.

74. Lin MD, Jiao X, Grima D, Newbury SF, Kiledjian M, Chou TB. Drosophila processing bodies in oogenesis. Dev Biol. 2008;322(2):276-88. DOI: 10.1016/j.ydbio.2008.07.033.

75. Jensen L, Venkei ZG, Watase GJ, Bisai B, Pletcher S, Lee CY, Yamashita YM. me31B regulates stem cell homeostasis by preventing excess dedifferentiation in the Drosophila male germline. J Cell Sci. 2021;134(14). DOI: 10.1242/jcs.258757.

76. Layana C, Vilardo ES, Corujo G, Hernandez G, Rivera-Pomar R. Drosophila Me31B is a Dual eIF4E-Interacting Protein. J Mol Biol. 2023;435(5):167949. DOI: 10.1016/j.jmb.2023.167949.

77. Grzejda D, Mach J, Schweizer JA, Hummel B, Rezansoff AM, Eggenhofer F, et al. The long noncoding RNA mimi scaffolds neuronal granules to maintain nervous system maturity. Sci Adv. 2022;8(39):eabo5578. DOI: 10.1126/sciadv.abo5578.

78. Pushpalatha KV, Besse F. Local Translation in Axons: When Membraneless RNP Granules Meet Membrane-Bound Organelles. Front Mol Biosci. 2019;6:129. DOI: 10.3389/fmolb.2019.00129.

79. Bauer KE, de Queiroz BR, Kiebler MA, Besse F. RNA granules in neuronal plasticity and disease. Trends Neurosci. 2023;46(7):525-38. DOI: 10.1016/j.tins.2023.04.004.

80. Kipper K, Mansour A, Pulk A. Neuronal RNA granules are ribosome complexes stalled at the pre-translocation state. J Mol Biol. 2022;434(20):167801. DOI: 10.1016/j.jmb.2022.167801.

736 81. Arkov AL. Looking at the Pretty "Phase" of Membraneless Organelles: A View
737 From Drosophila Glia. Front Cell Dev Biol. 2022;10:801953. DOI:
738 10.3389/fcell.2022.801953.
739 82. Hillebrand J, Barbee SA, Ramaswami M. P-body components, microRNA
740 regulation, and synaptic plasticity. ScientificWorldJournal. 2007;7:178-90. DOI:
741 10.1100/tsw.2007.206.
742 83. Kiebler MA, Bassell GJ. Neuronal RNA granules: movers and makers. Neuron.
743 2006;51(6):685-90. DOI: 10.1016/j.neuron.2006.08.021.
744 84. Shiina N, Shinkura K, Tokunaga M. A novel RNA-binding protein in neuronal
745 RNA granules: regulatory machinery for local translation. J Neurosci. 2005;25(17):4420-
746 34. DOI: 10.1523/JNEUROSCI.0382-05.2005.
747 85. Krichevsky AM, Kosik KS. Neuronal RNA granules: a link between RNA
748 localization and stimulation-dependent translation. Neuron. 2001;32(4):683-96. DOI:
749 10.1016/s0896-6273(01)00508-6.
750 86. Fritzsche R, Karra D, Bennett KL, Ang FY, Heraud-Farlow JE, Tolino M, et al.
751 Interactome of two diverse RNA granules links mRNA localization to translational
752 repression in neurons. Cell Rep. 2013;5(6):1749-62. DOI: 10.1016/j.celrep.2013.11.023.
753 87. El Fatimy R, Davidovic L, Tremblay S, Jaglin X, Dury A, Robert C, et al. Tracking
754 the Fragile X Mental Retardation Protein in a Highly Ordered Neuronal
755 RiboNucleoParticles Population: A Link between Stalled Polyribosomes and RNA
756 Granules. PLoS Genet. 2016;12(7):e1006192. DOI: 10.1371/journal.pgen.1006192.
757 88. De Graeve F, Besse F. Neuronal RNP granules: from physiological to
758 pathological assemblies. Biol Chem. 2018;399(7):623-35. DOI: 10.1515/hsz-2018-0141.

759 89. Hillebrand J, Pan K, Kokaram A, Barbee S, Parker R, Ramaswami M. The
760 Me31B DEAD-Box Helicase Localizes to Postsynaptic Foci and Regulates Expression
761 of a CaMKII Reporter mRNA in Dendrites of Drosophila Olfactory Projection Neurons.
762 Front Neural Circuits. 2010;4:121. DOI: 10.3389/fncir.2010.00121.
763 90. Goss DJ, Kleiman FE. Poly(A) binding proteins: are they all created equal? Wiley
764 Interdiscip Rev RNA. 2013;4(2):167-79. DOI: 10.1002/wrna.1151.
765 91. Yokoshi M, Li Q, Yamamoto M, Okada H, Suzuki Y, Kawahara Y. Direct binding
766 of Ataxin-2 to distinct elements in 3' UTRs promotes mRNA stability and protein
767 expression. Mol Cell. 2014;55(2):186-98. DOI: 10.1016/j.molcel.2014.05.022.
768 92. Sudhakaran IP, Hillebrand J, Dervan A, Das S, Holohan EE, Hulsmeier J, et al.
769 FMRP and Ataxin-2 function together in long-term olfactory habituation and neuronal
770 translational control. Proc Natl Acad Sci U S A. 2014;111(1):E99-E108. DOI:
771 10.1073/pnas.1309543111.
772 93. Lee J, Yoo E, Lee H, Park K, Hur JH, Lim C. LSM12 and ME31B/DDX6 Define
773 Distinct Modes of Posttranscriptional Regulation by ATAXIN-2 Protein Complex in
774 Drosophila Circadian Pacemaker Neurons. Mol Cell. 2017;66(1):129-40 e7. DOI:
775 10.1016/j.molcel.2017.03.004.
776 94. Pushpalatha KV, Solyga M, Nakamura A, Besse F. RNP components condense
777 into repressive RNP granules in the aging brain. Nat Commun. 2022;13(1):2782. DOI:
778 10.1038/s41467-022-30066-4.
779 95. Formicola N, Heim M, Dufourt J, Lancelot AS, Nakamura A, Lagha M, Besse F.
780 Tyramine induces dynamic RNP granule remodeling and translation activation in the
781 Drosophila brain. Elife. 2021;10. DOI: 10.7554/eLife.65742.

96. Bauer KE, Bargenda N, Schieweck R, Illig C, Segura I, Harner M, Kiebler MA. RNA supply drives physiological granule assembly in neurons. Nat Commun. 2022;13(1):2781. DOI: 10.1038/s41467-022-30067-3.

97. Shih CY, Chen YC, Lin HY, Chu CY. RNA Helicase DDX6 Regulates A-to-I Editing and Neuronal Differentiation in Human Cells. Int J Mol Sci. 2023;24(4). DOI: 10.3390/ijms24043197.

98. Di Stefano B, Luo EC, Haggerty C, Aigner S, Charlton J, Brumbaugh J, et al. The RNA Helicase DDX6 Controls Cellular Plasticity by Modulating P-Body Homeostasis. Cell Stem Cell. 2019;25(5):622-38 e13. DOI: 10.1016/j.stem.2019.08.018.

99. Minshall N, Standart N. The active form of Xp54 RNA helicase in translational repression is an RNA-mediated oligomer. Nucleic Acids Res. 2004;32(4):1325-34. DOI: 10.1093/nar/gkh303.

100. Ernoult-Lange M, Baconnais S, Harper M, Minshall N, Souquere S, Boudier T, et al. Multiple binding of repressed mRNAs by the P-body protein Rck/p54. RNA. 2012;18(9):1702-15. DOI: 10.1261/rna.034314.112.

101. Iwakawa HO, Tomari Y. Life of RISC: Formation, action, and degradation of RNA-induced silencing complex. Mol Cell. 2022;82(1):30-43. DOI: 10.1016/j.molcel.2021.11.026.

102. Michlewski G, Caceres JF. Post-transcriptional control of miRNA biogenesis. RNA. 2019;25(1):1-16. DOI: 10.1261/rna.068692.118.

103. Gebert LFR, MacRae IJ. Regulation of microRNA function in animals. Nat Rev Mol Cell Biol. 2019;20(1):21-37. DOI: 10.1038/s41580-018-0045-7.

104. van den Berg A, Mols J, Han J. RISC-target interaction: cleavage and translational suppression. Biochim Biophys Acta. 2008;1779(11):668-77. DOI: 10.1016/j.bbagrm.2008.07.005.

105. Chu CY, Rana TM. Translation repression in human cells by microRNA-induced gene silencing requires RCK/p54. PLoS Biol. 2006;4(7):e210. DOI: 10.1371/journal.pbio.0040210.

106. Ozgur S, Stoecklin G. Role of Rck-Pat1b binding in assembly of processing-bodies. RNA Biol. 2013;10(4):528-39. DOI: 10.4161/rna.24086.

107. Ozgur S, Chekulaeva M, Stoecklin G. Human Pat1b connects deadenylation with mRNA decapping and controls the assembly of processing bodies. Mol Cell Biol. 2010;30(17):4308-23. DOI: 10.1128/MCB.00429-10.

108. Beilharz TH, Humphreys DT, Clancy JL, Thermann R, Martin DI, Hentze MW, Preiss T. microRNA-mediated messenger RNA deadenylation contributes to translational repression in mammalian cells. PLoS One. 2009;4(8):e6783. DOI: 10.1371/journal.pone.0006783.

109. Su H, Meng S, Lu Y, Trombly MI, Chen J, Lin C, et al. Mammalian hyperplastic discs homolog EDD regulates miRNA-mediated gene silencing. Mol Cell. 2011;43(1):97-109. DOI: 10.1016/j.molcel.2011.06.013.

110. Ayache J, Benard M, Ernoult-Lange M, Minshall N, Standart N, Kress M, Weil D. P-body assembly requires DDX6 repression complexes rather than decay or Ataxin2/2L complexes. Mol Biol Cell. 2015;26(14):2579-95. DOI: 10.1091/mbc.E15-03-0136.

111. Rouya C, Siddiqui N, Morita M, Duchaine TF, Fabian MR, Sonenberg N. Human DDX6 effects miRNA-mediated gene silencing via direct binding to CNOT1. RNA. 2014;20(9):1398-409. DOI: 10.1261/rna.045302.114.

112. Nicklas S, Okawa S, Hillje AL, Gonzalez-Cano L, Del Sol A, Schwamborn JC. The RNA helicase DDX6 regulates cell-fate specification in neural stem cells via miRNAs. Nucleic Acids Res. 2015;43(5):2638-54. DOI: 10.1093/nar/gkv138.

113. Teixeira D, Parker R. Analysis of P-body assembly in Saccharomyces cerevisiae. Mol Biol Cell. 2007;18(6):2274-87. DOI: 10.1091/mbc.e07-03-0199.

114. Sachdev R, Hondele M, Linsenmeier M, Vallotton P, Mugler CF, Arosio P, Weis K. Pat1 promotes processing body assembly by enhancing the phase separation of the DEAD-box ATPase Dhh1 and RNA. Elife. 2019;8. DOI: 10.7554/eLife.41415.

115. Kamenska A, Simpson C, Vindry C, Broomhead H, Benard M, Ernoult-Lange M, et al. The DDX6-4E-T interaction mediates translational repression and P-body assembly. Nucleic Acids Res. 2016;44(13):6318-34. DOI: 10.1093/nar/gkw565.

116. Nissan T, Rajyaguru P, She M, Song H, Parker R. Decapping activators in Saccharomyces cerevisiae act by multiple mechanisms. Mol Cell. 2010;39(5):773-83. DOI: 10.1016/j.molcel.2010.08.025.

117. Coller JM, Tucker M, Sheth U, Valencia-Sanchez MA, Parker R. The DEAD box helicase, Dhh1p, functions in mRNA decapping and interacts with both the decapping and deadenylase complexes. RNA. 2001;7(12):1717-27. DOI: 10.1017/s135583820101994x.

118. Eulalio A, Rehwinkel J, Stricker M, Huntzinger E, Yang SF, Doerks T, et al. Target-specific requirements for enhancers of decapping in miRNA-mediated gene silencing. Genes Dev. 2007;21(20):2558-70. DOI: 10.1101/gad.443107.

119. Nishihara T, Zekri L, Braun JE, Izaurralde E. miRISC recruits decapping factors to miRNA targets to enhance their degradation. Nucleic Acids Res. 2013;41(18):8692-705. DOI: 10.1093/nar/gkt619.

120. Hata H, Mitsui H, Liu H, Bai Y, Denis CL, Shimizu Y, Sakai A. Dhh1p, a putative RNA helicase, associates with the general transcription factors Pop2p and Ccr4p from Saccharomyces cerevisiae. Genetics. 1998;148(2):571-9. DOI: 10.1093/genetics/148.2.571.

121. Maillet L, Collart MA. Interaction between Not1p, a component of the Ccr4-not complex, a global regulator of transcription, and Dhh1p, a putative RNA helicase. J Biol Chem. 2002;277(4):2835-42. DOI: 10.1074/jbc.M107979200.

122. Temme C, Zhang L, Kremmer E, Ihling C, Chartier A, Sinz A, et al. Subunits of the Drosophila CCR4-NOT complex and their roles in mRNA deadenylation. RNA. 2010;16(7):1356-70. DOI: 10.1261/rna.2145110.

123. Nagarajan VK, Jones CI, Newbury SF, Green PJ. XRN 5'-->3' exoribonucleases: structure, mechanisms and functions. Biochim Biophys Acta. 2013;1829(6-7):590-603. DOI: 10.1016/j.bbagrm.2013.03.005.

124. Braun JE, Truffault V, Boland A, Huntzinger E, Chang CT, Haas G, et al. A direct interaction between DCP1 and XRN1 couples mRNA decapping to 5' exonucleolytic degradation. Nat Struct Mol Biol. 2012;19(12):1324-31. DOI: 10.1038/nsmb.2413.

125. Mathys H, Basquin J, Ozgur S, Czarnocki-Cieciura M, Bonneau F, Aartse A, et al. Structural and biochemical insights to the role of the CCR4-NOT complex and DDX6 ATPase in microRNA repression. Mol Cell. 2014;54(5):751-65. DOI: 10.1016/j.molcel.2014.03.036.

126. Radhakrishnan A, Chen YH, Martin S, Alhusaini N, Green R, Coller J. The DEAD-Box Protein Dhh1p Couples mRNA Decay and Translation by Monitoring Codon Optimality. Cell. 2016;167(1):122-32 e9. DOI: 10.1016/j.cell.2016.08.053.

127. Sweet T, Kovalak C, Coller J. The DEAD-box protein Dhh1 promotes decapping by slowing ribosome movement. PLoS Biol. 2012;10(6):e1001342. DOI: 10.1371/journal.pbio.1001342.

128. Sharif H, Ozgur S, Sharma K, Basquin C, Urlaub H, Conti E. Structural analysis of the yeast Dhh1-Pat1 complex reveals how Dhh1 engages Pat1, Edc3 and RNA in mutually exclusive interactions. Nucleic Acids Res. 2013;41(17):8377-90. DOI: 10.1093/nar/gkt600.

129. Jonas S, Izaurralde E. The role of disordered protein regions in the assembly of decapping complexes and RNP granules. Genes Dev. 2013;27(24):2628-41. DOI: 10.1101/gad.227843.113.

Figure Legends

Figure 1. *Drosophila* oogenesis and Me31B expression in the egg chambers. Me31B-GFP expression is visualized in an ovariole expressing GFP-tagged wildtype Me31B (30). The top image is a sketch outlining the egg chambers and nurse cells while the bottom image shows Me31B-GFP observed using confocal microscopy.

Figure 2. The Model of A Me31B Complex In Germline RNP. In this model of Me31B-containing RNP, the Me31B protein assumes a pivotal role as the central hub, orchestrating interactions with four groups of protein partners: RNA regulators, cytoskeleton/motor proteins, glycolytic enzymes, and core germ plasm proteins. Among these, the RNA regulators, including Me31B itself, provide post-transcriptional RNA regulations. Cytoskeleton/motor proteins facilitate the transportation of the RNP. Glycolytic enzymes contribute ATP resources to power the activities of other proteins within the assembly. Lastly, core germ plasm proteins play integral roles in the assembly of germ plasm/nuage and the formation of germ cells. The figure is adapted from DeHaan et al. (40)

Figure 3. The Model of A Me31B-containing Repressor Complex on *nos* mRNA. The model involves Smaug (Smg), functioning as an RNA repressor/degrader, binding to Smaug Recognition Elements (SREs) in the 3' UTR of *nos* during embryonic Maternal to Zygotic Transition (MTZ). Smg recruits Cup, which then interacts with eIF4E, Tral, and Me31B. Multiple Me31B-Tral complexes coat *nos* RNA, hindering translation. Additionally, Me31B or eIF4E may also engage Belle (Bel) to further suppress *nos* RNA.

910 Finally, Me31B and/or Smaug recruit the CCR4-NOT deadenylation complex, leading to
911 *nos* RNA degradation. The figure is adapted from Gotze et al. (54).
912

913 **Figure 4. A Schematic Illustration of The Mechanisms of How Me31B Affects**
914 **Target mRNAs.** In this model, Me31B shows various ways of influencing its target
915 mRNA. Firstly, it oligomerizes and coats the mRNA, functioning as a general repressor.
916 Secondly, it cooperates with RISC, a small RNA-equipped mRNA silencing machinery,
917 in mRNA degradation/repression. Thirdly, Me31B collaborates with decapping factors
918 (Decapper) to facilitate mRNA decapping. Additionally, it physically interacts with CCR4-
919 NOT complex, which induces mRNA deadenylation. Finally, Me31B may directly
920 interact with ribosomes, impeding mRNA translation. Notably, these interconnected
921 mechanisms of action may operate independently or synergistically in a unified RNA
922 regulation pathway.
923

Figure 1

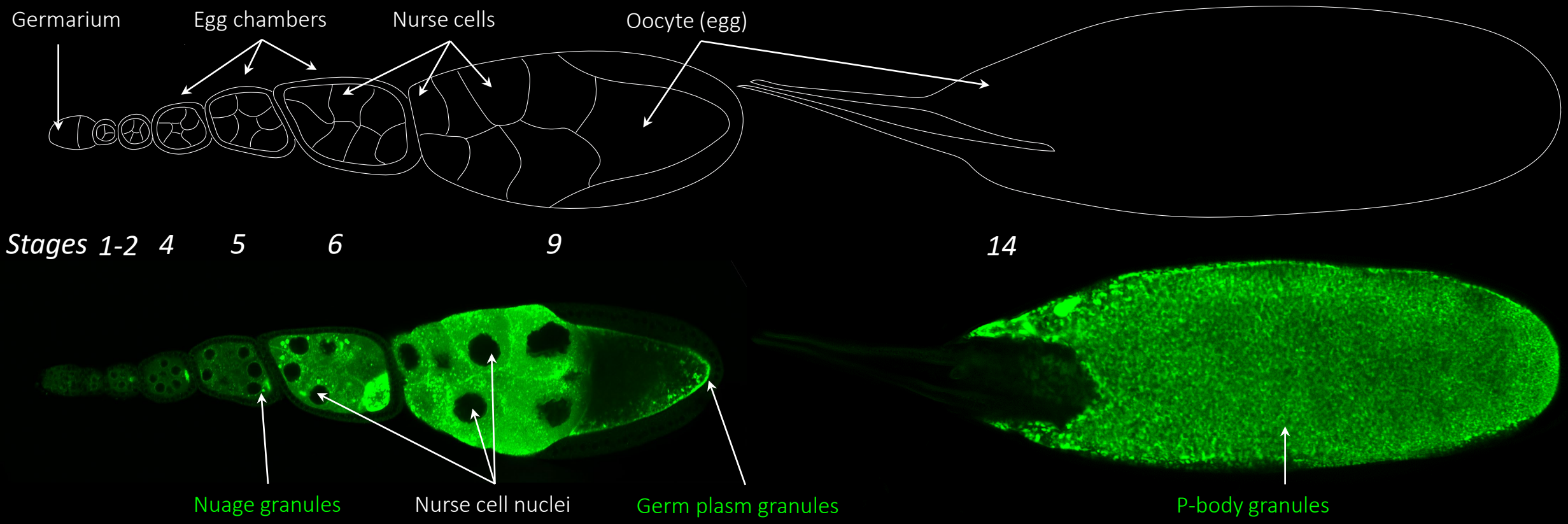
Germarium
Egg chambers
Nurse cells
Oocyte (egg)
Stages 1-2 4 5 6 9 14
Nuage granules
Nurse cell nuclei
Germ plasm granules
P-body granules

Figure 2

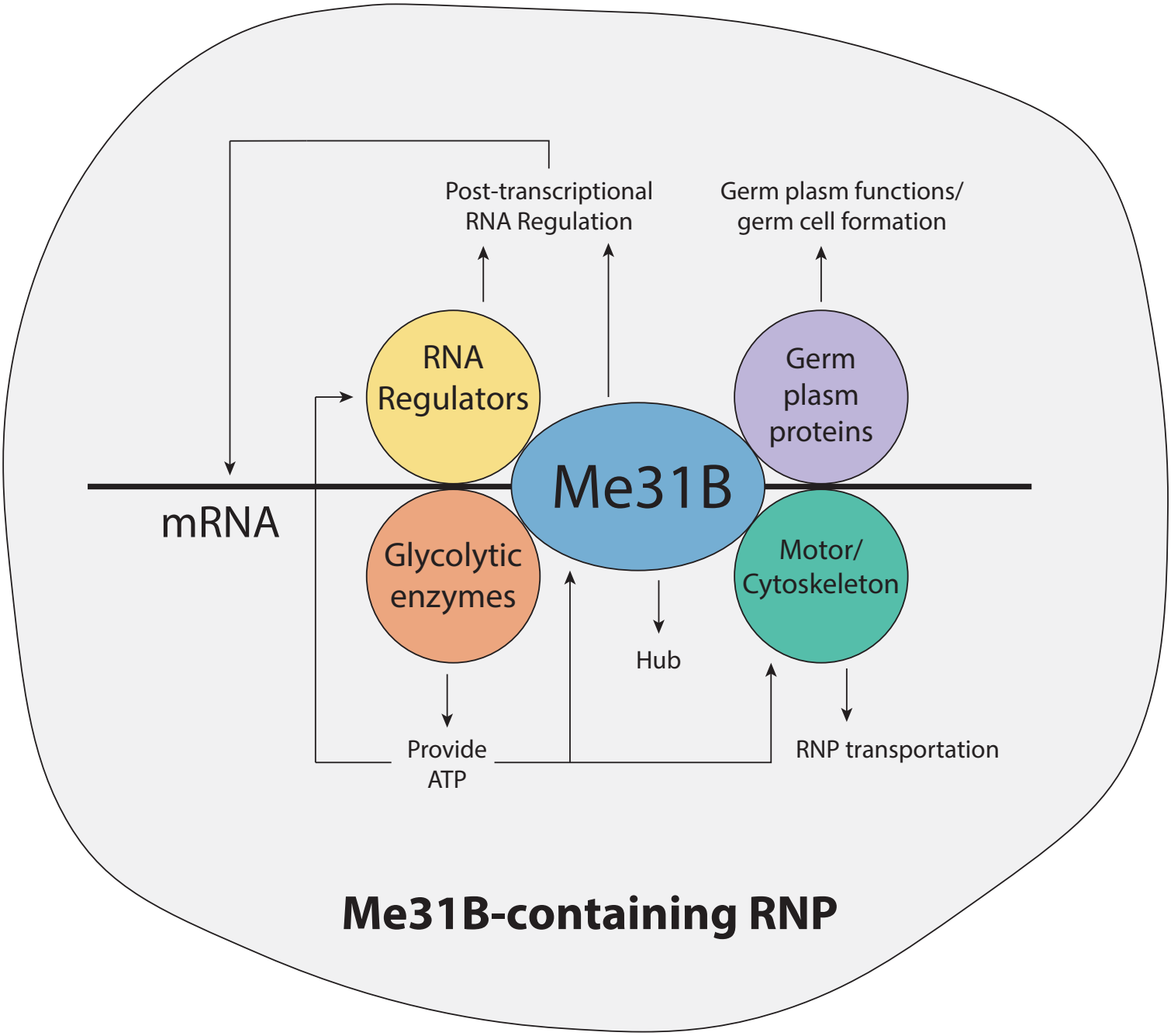

Figure 3

Figure 4