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Abstract

The specification of germ cells in zebrafish mostly relies on an inherited mechanism by which localized maternal determinants, called germ plasm, confer germline fate in the early embryo. Extensive studies have partially allowed the identification of key regulators governing germ plasm formation and subsequent germ cell development. RNA-binding proteins, acting in concert with other germ plasm components, play essential roles in the organization of the germ plasm and the specification, migration, maintenance, and differentiation of primordial germ cells. The loss of their functions impairs germ cell formation and causes sterility or sexual conversion. Evidence is emerging that they instruct germline development through differential regulation of mRNA fates in somatic and germ cells. However, the challenge remains to decipher the complex interplay of maternal germ plasm components in germ plasm compartmentalization and germ cell specification. Since failure to control the developmental outcome of germ cells disrupts the formation of gametes, it is important to gain a complete picture of regulatory mechanisms operating in the germ cell lineage. This review sheds light on the contributions of RNA-binding proteins to germ cell development in zebrafish and highlights intriguing questions that remain open for future investigation.

Keywords: Germ plasm; Primordial germ cell; Germline stem cell; Gametogenesis; Zebrafish; RNA-binding protein; Posttranscriptional gene expression; Translational activation and repression

Introduction

Germline development in sexually reproducing organisms ensures the production of haploid gametes (sperm and oocytes) that fuse by the process of fertilization to create a new generation with a diploid genome. Primordial germ cells (PGCs) can be specified during embryonic development through an inherited or an inductive mechanism (Aguero et al., 2017a; Jamieson-Lucy and Mullins, 2019; Hansen and Pelegri, 2021). Among vertebrates, zebrafish, *Xenopus*, and Reptilia typically use an inherited mechanism to specify PGCs in the early embryos, whereas mammals (mice and humans) employ the strategy of inductive cell-cell signaling to confer germ cell identity at late developmental stages (Hansen and Pelegri, 2021). In the inherited mechanism or preformation, maternally derived germline determinants, organized as germ plasm, are already accumulated in specialized subcellular compartments within the oocyte and the early embryo; they are then asymmetrically encapsulated in the precursors of PGCs at the blastula period. It is well established that the germ plasm carries large amounts of mRNAs and proteins, which form membrane-free ribonucleoprotein biocondensates through liquid-liquid phase separation (Hansen and Pelegri, 2021). The dynamic molecular interactions between germ plasm components regulate germ plasm assembly and germline-specific gene expression program to establish the germ cell lineage and promote gametogenesis (Strome and Updike, 2015; Mercer et al., 2021; Chiappetta et al., 2022; Thomas et al., 2023).

Zebrafish has become an attractive model for studying gene regulation and function in male and female gamete development (Aharon and Marlow, 2021). It has also provided an important step forward in understanding the activity of maternal-effect genes during embryogenesis, although functional analyses of maternal germline determinants remain challenging (Dosch, 2015). The zebrafish model is particularly adapted for systematic genetic screens, genome editing, and transgenic gene expression, as well as omics and biochemical analyses. Extensive studies have demonstrated that molecular mechanisms underlying the specification and differentiation of germ cells are largely conserved between zebrafish and other

vertebrate models, despite notable differences in the localization and function of some germline components (Aguero et al., 2017a; Escobar-Aguirre et al., 2017).

RNA-binding proteins (RBPs) contribute to organizing the post-transcriptional gene expression networks. They are involved in all steps of RNA metabolism, ranging from pre-mRNA alternative splicing to mRNA localization, stability, and translation (Shi and Grifone, 2021). Importantly, RBPs in the form of mRNA or protein are major constituents of the germ plasm (Schisa and Elaswad, 2021; Albarqi and Ryder, 2023). At present, there is no evidence for the contribution of transcription factors to germ cell specification in preformation species such as zebrafish and *Xenopus*. However, several conserved maternal RBPs, such as DEAD-box polypeptide 4 (Ddx4), Nanos3, deleted in azoospermia-like (Dazl), dead end 1 (Dnd1), Piwil1/2, insulin-like growth factor 2 mRNA-binding protein 3 (Igf2bp3), and Tudor domain proteins, function in multiple aspects of post-transcriptional regulation to specify germ cell lineage (Wolke et al., 2002; Lehmann, 2012). Zygotic expression of these RBPs is essential for the migration, maintenance, and differentiation of germ cells (Houston and King, 2000; Wolke et al., 2002; Nguyen-Chi and Morello, 2011; Lehmann, 2012). Intriguingly, simultaneous overexpression of key germ plasm components shows the strong potential to induce the formation of PGCs from somatic cells of the early embryo (Wang et al., 2023a). Since RBPs are critically involved in regulating the germline-specific gene expression program, the loss of their function disrupts germ cell formation in the embryo or in the adult, leading to male or female sterility. Our knowledge of RBPs-regulated specification and differentiation of germ cells is rapidly evolving. This review summarizes the functions of known RBPs in establishing the zebrafish germline, including but not limited to germ plasm formation and PGC specification. By providing an in-depth analysis of the interplay between RBPs within the germ plasm, this review offers mechanistic insights into the intricacies of germ cell development and helps identify research gaps for future investigation.

Outline of primordial germ cell development

Whether preformation or induction, bipotential PGCs first appear in the posterior region of the embryo and then migrate into the gonads, where they are induced to form sperm or eggs. Therefore, PGCs are not initially specified in the gonads, which represents an important feature of germ cell development. In zebrafish, maternally derived germline-specific mRNAs and proteins are first stored in a specialized cytoplasmic compartment of primary oocytes, known as Balbiani body (Bb), which is a transient asymmetric structure conserved from insects to mammals (Dosch, 2015; Escobar-Aguirre et al., 2017; Jamieson-Lucy and Mullins, 2019). Germline determinants are then released to the cytoplasm at the vegetal pole region during oocyte maturation and are transported to the animal pole upon fertilization by cytoplasmic streaming. They undergo aggregation and compaction to become anchored at the distal end of each cleavage furrow in the 2- to 4-cell stage embryos (Fig. 1). This process is dependent on the interactions between germ plasm mRNAs and proteins as well as on cytoskeletal dynamics and specific tight junction proteins (Campbell et al., 2015; Eno et al., 2016; Eno and Pelegri, 2018; Roovers et al., 2018; Moravec and Pelegri, 2020; Rostam et al., 2022). At the blastula period, the germ plasm is asymmetrically segregated into one daughter cell, thus forming four future PGCs. During gastrulation, the PGCs begin to migrate and then align as two clusters on both sides of the notochord. They subsequently enter into the embryonic genital ridge located at the anterior end of the yolk extension by chemotactic guidance cues (Weidinger et al., 1999; Doitsidou et al., 2002; Aalto et al., 2021).

The mode of germline specification by localized maternal determinants is conserved across many species. In *Drosophila*, for example, germ cells are formed by incorporating the pole plasm that localizes to the posterior pole of the oocyte. It is well established that Oskar, Vasa, Nanos, Tudor, and Piwi families of RBPs critically contribute to the biogenesis of the germ plasm; they specify germ cell fate in the *Drosophila* embryo by regulating mRNA localization and/or translation (Lehmann, 2016). After precocious cellularization of polar nuclei at the syncytial blastoderm stage, the embryo will form 20-30 PGCs or pole cells (Williamson and Lehmann,

1996). Except for Oskar, all other RBPs that function in *Drosophila* germ cell development are important conserved components of the germ plasm. However, in other species, proteins seemingly unrelated to Oskar, such as Bucky ball (Buc) in zebrafish (Bontems et al., 2009), Xvelo in *Xenopus* (Boke et al., 2016), and Pgl family of constitutive granule components in *C. elegans* (Hanazaw et al., 2011), clearly play an essential role in the assembly of the germ plasm and the specification of PGCs.

The induction or epigenesis of PGC formation depends on extrinsic signals provided by zygotic gene products and emitted by neighboring cells. In mammals, Wnt and BMP (bone morphogenetic protein) signals are crucial for specifying the germ cell lineage (Ohinata et al., 2009; Kobayashi et al., 2017); they induce the expression of other conserved elements, including transcription factors Blimp1 or Prdm1 and Tfap2c, which then activate the transcription of germline-specific RBPs, such as Nanos3, Dnd1, Ddx4 and Dazl (Tang et al., 2015). However, there are also unique features in PGC specification between mice and humans. The analysis using human pluripotent stem cells suggests that Sox17 is expressed in the human germline and functions as an essential inducer of PGC fate. By contrast, Sox17 is unlikely a factor specifying PGCs in mice due to the absence of its expression in the germline (Kobayashi and Surani, 2018). Although the regulatory networks used to specify germ cell fate may vary among mammals, several closely associated processes coordinately separate the progenitors of PGCs from somatic cells, such as activation of germline-related gene expression program, repression of somatic fate and widespread epigenetic reprogramming (Magnúsdóttir and Surani, 2014). As a result, approximately 30-40 PGCs are specified in the extraembryonic mesoderm of the gastrula stage mouse embryo; they are committed to germline fate after migration into the genital ridge at the tail-bud stage (Ohinata et al., 2009). It is of note that the above-mentioned RBPs are important in activating germline-specific programs. Some RBPs, such as Vasa, Nanos, and Dazl, are crucial for PGC and/or germ cell development across the animal kingdom, regardless of preformation or epigenesis (Hansen and Pelgri, 2021).

Overview of RBPs in germ cell development

As important components of the germ plasm and key regulators of the germline gene expression program, RBPs contribute to multiple processes of germline development both outside and inside the gonads. Functional studies using a variety of models suggest that they regulate germ plasm assembly, PGC specification, transition of PGCs to germline stem cells (GSCs), germ cell maintenance, progression of mitotic to meiotic identity, differentiation of gametes, and regeneration of ovary. Therefore, the loss of germline-associated RBPs impairs germ cell development and causes infertility of both sexes. In zebrafish, it can produce all-male or sterile adults. This is because zebrafish sex determination operates largely in a chromosome-independent manner. The absence of germline or reduced numbers of PGCs makes the bipotential gonad to adopt testis fate (Slanchev et al., 2005; Siegfried and Nüsslein-Volhard, 2008). The implication of RBPs in the biological processes of germline development will be reviewed below, focusing on zebrafish and comparing RBPs with those in other models. Importantly, RBPs are required for germline development in a species- and sex-dependent manner, often displaying sexually dimorphic functions. Generally, these RBPs act in concert or interact to regulate the germline-specific post-transcriptional gene expression profiles from PGC specification to gamete differentiation. For example, Ddx4 interacts with many other RBPs to build germ granules and regulate various aspects of germ cell development (Xu et al., 2021); Nanos proteins form a post-transcriptional repressor complex with Pumilio to regulate germ cell survival and pluripotency, with Nanos3 involved in preventing apoptosis of PGCs during their migration (Suzuki et al., 2008). It is of note that RBPs are assembled into germ granules before and after the formation of PGCs. This represents an important cytoplasmic mechanism of gene expression control, mostly through translational activation and repression of germ cell-specific transcripts. It functions to protect germ cell fate, maintain the survival of PGCs, and regulate gamete differentiation during germline development.

Functions of germline-associated RBPs in zebrafish

In the past two to three decades, a large number of evolutionarily conserved RBPs have been identified and functionally characterized in the zebrafish germline (Table 1). Many of them, either in the form of mRNA or protein, also show specific localization in the germ plasm at early cleavage stages and thus represent important maternal determinants for germ cell formation (Fig. 2). At least 12 RNAs (including 6 RBP-coding mRNAs and 1 non-coding RNA) and 4 proteins (including 3 RBPs) show specific accumulation in the germ plasm, which is recruited to the cleavage furrows in a kinesin-dependent manner (Campbell et al., 2015). It is intriguing that the mRNA and its corresponding protein, or vice versa, may not be jointly localized to the germ plasm. Thus, it remains to be determined whether some RBP-coding mRNAs could function as mRNA components in the formation of the germ plasm. In addition, as discussed below, several RBPs that do not show characteristic localization in the germ plasm also contribute to its formation, such as Igf2bp3, raising the possibility that they can also function as maternal determinants of germ cells. The following sections will present a more detailed analysis of RBPs in preformation germline development, from germ plasm formation to PGC differentiation and gametogenesis, by briefly mentioning their functions in the induction models.

Ddx4 in meiosis, differentiation and maintenance of germline stem cells

Ddx4, best known as Vasa, is a member of the DEAD (Asp-Glu-Ala-Asp) box family RBPs and positively regulates the translation of multiple mRNAs through its ATP-dependent RNA helicase activity (Carrera et al., 2000). It is clearly associated with germ cell development from *Drosophila* to humans, although its requirement for germ plasm formation and PGC specification in vertebrate early embryos remains elusive (Gustafson et al., 2010; Xu et al., 2021). In zebrafish, *ddx4* mRNA represents the first described germ plasm component and germline-specific marker. During oogenesis, *ddx4* mRNA accumulates in the Bb of stage I oocytes and then concentrates in the cytoplasm or vegetal cortex, whereas the protein is localized around the germinal vesicle (Braat et al., 1999; Knaut et al., 2000; Kosaka et al., 2007). After fertilization, *ddx4* mRNA is translocated to the animal pole region and subsequently localized to the distal ends of cleavage

furrows in 2- to 4-cell stage embryos (Olsen et al., 1997; Yoon et al., 1997). Zygotic expression of *ddx4* is exclusively activated in PGCs throughout early development (Yoon et al., 1997).

Intriguingly, Ddx4 protein is ubiquitously expressed in cleavage-stage embryos (Knaut et al., 2000), and is only specifically detected in PGCs from late blastula stage onward (Braat et al., 2000; Knaut et al., 2000). This suggests that Ddx4 protein may not be a germ plasm component, but there is a possibility that it becomes active only in the germ plasm or early PGCs as a result of specific post-translational modifications (Raz, 2000). Supporting the activity of Ddx4 protein in germ cell specification, overexpression of a protein interaction motif in Ddx4, which likely prevents the formation of an active Ddx4-containing complex and may inhibit maternal Ddx4 function, leads to a reduced number of PGCs (Perera et al., 2021). Morpholino-mediated inhibition of *ddx4* mRNA translation does not seem to affect germ cell formation (Braat et al., 2001); zygotic *ddx4* mutant embryos have normal specification and migration of PGCs, but they show defective meiosis, differentiation, and maintenance of GSCs, developing into infertile males (Hartung et al., 2014). Thus, at least to some extent, maternal Ddx4 participates in germline establishment, whereas zygotic Ddx4 functions to promote GSC development. Future studies that eliminate maternally derived *ddx4* gene products are also necessary to definitely clarify the maternal implication of Ddx4 in germ plasm formation and PGC specification. Interestingly, the loss of Ddx4 causes sex-specific germline defects in other species. It impairs oocyte differentiation in *Drosophila* (Styhler et al., 1998) and leads to male infertility in mice (Tanaka et al., 2000). Ddx4 most likely functions as a translational activator that interacts with translation initiation factors to regulate germ cell-specific gene expression (Adashev et al., 2023).

Nanos proteins regulate migration of primordial germ cells and maintenance of germline stem cells

RBPs of the Nanos family contain a highly conserved carboxyl-terminal zinc finger motif $(CCHC)_2$ that mediates binding with target mRNAs and interaction with protein partners (De Keuckelaere et al., 2018). Zebrafish genome harbors three *nanos* paralogs, *nanos1*, *nanos2*, and *nanos3*, but *nanos1* does not seem to play a role in germ cell development. In situ hybridization screening has identified *nanos3* (previously called *nanos1*) mRNA as another germ plasm-

specific marker with localization at the cleavage furrows of 4-cell stage embryos and in PGCs from blastula stage until before 5 days after fertilization (Köprunner et al., 2001). The specific accumulation of *nanos3* mRNA in the germ plasm is tightly controlled by a post-transcriptional regulatory mechanism. It is well established that the 3'-UTR (3'-untranslated region) of *nanos3* mRNA stabilizes the transcripts in the germline but directs their degradation in somatic cells (Köprunner et al., 2001). This is executed by another germ cell-specific RBP, Dnd1, which binds to the 3'-UTR of *nanos3* mRNA to counteract the activity of miR-430 in maternal mRNA clearance and translational repression. Knockdown of *nanos3* in the zebrafish embryo does not affect the specification of PGCs but leads to abnormal migration and a reduced number of germ cells (Köprunner et al., 2001). In the gonads of larval and adult zebrafish, *nanos3* is enriched in the Bb of stage Ib oocytes but is undetectable in the testis (Draper et al., 2007; Kosaka et al., 2007). Adult female zebrafish carrying a *nanos3* mutation that results in the expression of a truncated protein lacking the zinc finger motif fail to maintain the production of oocytes, leading to the early onset of sterility (Draper et al., 2007). Together, these observations indicate that Nanos3 is required for the migration and survival of PGCs during development as well as for the maintenance of oocyte production in the adult. Studies in different species suggest that Nanos proteins regulate germ cell fate by forming a complex with the Pumilio family of RBPs and functioning as translational repressors to control the activity of germ cell-specific genes (Lai and King, 2013). However, germ cell-specific target transcripts of this complex at specific steps of germline development remain elusive. In addition, it is still unclear whether maternal *nanos3* mRNA or its coded protein contributes to germ plasm formation. In mice, Nanos2 is predominantly expressed in male germ cells and functions in spermatogenesis, while Nanos3 is expressed in migrating PGCs and is required for germ cell development in both sexes (Tsuda et al., 2003).

Within the ovary and testis, *nanos*2 mRNA is expressed in mitotic and early meiotic germ cells, which likely represent GSCs transitioned from PGCs, but it is not present in the germ plasm of cleavage embryos (Beer and Draper, 2013). Nanos2 is required for the maintenance but not for the specification of GSCs, because the loss of its function leads to a reduced number of these cells and causes the development of sterile males (Cao et al., 2019). Defective maintenance of

GSCs has also been observed in *nanos3* mutants, suggesting that Nanos2 and Nanos3 likely display partial redundant functions in the regulation of GSCs (Beer and Draper, 2013). In addition, the ovary in *nanos2* mutants shows defective regeneration after amputation due to the absence of GSCs, suggesting a key role of GSCs in this process (Cao et al., 2019).

Dnd1 promotes primordial germ cell migration and protects germline fate

Germ cell-specific expression of *dnd1* mRNA was initially discovered in zebrafish and then reported in other vertebrates, while invertebrate *dnd1* homologs have not been identified to date (Gross-Thebing and Raz, 2020). Maternal *dnd1* mRNA accumulates at the cleavage furrows of 4-cell stage embryos, and Dnd protein localizes to perinuclear granules in PGCs similarly to Ddx4 and Nanos3 proteins (Weidinger et al., 2003). Knockdown of *dnd1* leads to abnormal migration and progressive loss of PGCs in the early embryo (Weidinger et al., 2003; Gross-Thebing et al., 2017). A more detailed analysis of Dnd1-deficient PGCs reveals their transdifferentiation into somatic cells without apoptosis, suggesting that the absence of Dnd1 causes germline-to-soma reprogramming, thus inducing PGCs to adopt somatic cell fates and integrate into different germ layers (Gross-Thebing et al., 2017). Mechanistically, Dnd1 functions to maintain the stability of germline-specific mRNAs and repress somatic gene expression profiles in PGCs (Gross-Thebing et al., 2017). Moreover, Dnd1 prevents the inhibitory effects of miR-430 to relieve the translational repression of those mRNAs encoding proteins regulating PGC motility, such as Zeb1 (zinc finger E-box binding homeobox 1) and myosin light chain kinase (Goudarzi et al., 2012).

Dnd1 can also regulate translational activation of germ cell-specific mRNAs through other mechanisms, such as preventing eIF3 repressive preinitiation complex (Aguero et al., 2017b) and remodeling the translational control element through its ATPase-dependent helicase activity (Liu and Collodi, 2010; Aguero et al., 2018). Thus, it may act as a molecular scaffold to orchestrate post-transcriptional gene expression in PGCs (Gross-Thebing and Raz, 2020). Indeed, a more recent study suggests that Dnd1 regulates the spatial organization and translation of mRNAs within germ cell-specific phase-separated organelles (Westerich et al., 2023). Ribosomes and *nanos3* mRNA are enriched at the periphery of germ granules in a manner that is dependent on Dnd1 activity; the loss of Dnd1 disrupts the association of *nanos3* mRNA and likely other germline

mRNAs with ribosomes. As a consequence, this prevents translation and causes mis-localization of germline mRNAs, resulting in the loss of germ cell fate (Westerich et al., 2023). These observations reveal a Dnd1-mediated sub-granule post-transcriptional regulation of germline mRNA localization and function. Nevertheless, in the absence of functional analysis by depleting the maternal gene products, there is still no evidence demonstrating its contribution to germ plasm formation and early PGC specification. In mice, Dnd1 can also destabilize mRNAs encoding positive regulators of apoptosis and modulators of stem cell pluripotency, by binding to a UU(A/U) trinucleotide motif predominantly present in the 3'-UTR; this contributes to the survival of mouse PGCs and spermatogonial stem cells (Yamaji et al., 2017).

Dazl regulates germline stem cell specification

Dazl is a conserved RBP with essential roles in male and female fertility (Fu et al., 2015). Maternally derived zebrafish *dazl* mRNA is present in the Bb of early oocytes and translocated to the vegetal cortex during oogenesis; it is then localized to the cleavage furrows of 4-cell stage embryos (Hashimoto et al., 2004; Kosaka et al., 2007). Therefore, there is a colocalization of *ddx4*, *nanos3, dnd1*, and *dazl* mRNAs in the Bb of early oocytes and in the germ plasm of cleavage embryos. However, Dazl protein is not present in Ddx4-expressing PGCs, but it can be detected in Ddx4-positive germ cells prior to cyst formation in the gonads. Consistently, *dazl* mutants show defective cyst formation and GSC specification (Bertho et al., 2021). The molecular mechanism underlying zebrafish Dazl function in germ cell development remains elusive, but there is evidence that it promotes mRNA stability and translational efficiency of germline mRNAs, such as *tdrd7a* (Maegawa et al., 2002; Takeda et al., 2009). Supporting this observation, it has been shown that Dazl activates translationally silent mRNAs through interaction with poly(A)-binding proteins in the germ cells of other vertebrates (Collier et al., 2005). In mammals, Dazl is required for both male and female fertility. It regulates the activation and repression of a large mRNA translation program during oocyte maturation and spermatogenesis (Mikedis et al., 2020; Yang et al., 2020).

Piwi-like proteins function in germ cell maintenance and differentiation

Piwi proteins, alongside their interacting small non-coding RNAs (piRNAs), are abundantly expressed in the germline, and they play an important role in the post-transcriptional regulation of germline specification and stem cell maintenance (Juliano et al., 2011). The first known and best-described function of the Piwi-piRNA pathway is its activity in repressing transposable elements in germ cells, but recent studies suggest that this pathway also regulates a large variety of coding and non-coding germline-specific genes (Ramat and Simonelig, 2021; Wang and Lin, 2021; Wang et al., 2023b). Genetic studies in mice have unveiled an essential role of the Piwi pathway in spermatogenesis and male fertility (Juliano et al., 2011; Chuma and Nakano, 2013). Zebrafish genome encodes Piwil1 (also known as Ziwi) and Piwil2 (also known as Zili), which are specifically expressed in the testis and ovary (Houwing et al., 2007, 2008). The interacting piRNAs of Piwi1 and Piwi2 are also detected in the gonads. However, Piwil1 shows both overlapping and distinct localization with these piRNAs. Thus, it may have piRNA-dependent and independent functions (Houwing et al., 2007). Piwil2 is critically required for oocyte meiosis, but this may also be independent of its function in transposon defense (Houwing et al., 2008).

During early development, maternal Piwil1 is localized to germ plasm at the cleavage furrows of 4-cell stage embryos (Houwing et al., 2007), whereas Piwil2 can be detected in the nucleus and cytoplasm of PGCs around 3 days of development (Houwing et al., 2008). In homozygous *piwil1* mutants, there is a progressive loss of germ cells due to increased apoptosis, suggesting that Piwil1 is required for germ cell maintenance (Houwing et al., 2007). It is still unclear whether Piwil1 functions in germ cell survival through transposon silencing. However, homozygous *piwil2* mutants show defective germ cell differentiation and loss of germ cells in juvenile zebrafish, which is associated with an increase in transposon transcripts (Houwing et al., 2008). This suggests a possible piRNA-dependent function of Piwil2 for germ cell maintenance at more late stages. Similar to the loss of many other germline-specific genes, *piwil1* or *piwil2* mutants develop into sterile adult males.

Tudor domain family proteins in germ plasm and germ cell formation

Tudor domain-related (Tdrd) proteins are characterized by the presence of Tudor domains initially predicated as RNA-binding motifs but more likely implicated in the interaction

with methylated proteins (Thomson and Lasko, 2005; Arkov and Ramos, 2010). In mammals, there are approximately 30 Tudor domain-containing proteins; many of them have been shown to interact with Piwi proteins to regulate spermatogenesis (Chuma and Nakano, 2013). Several members of the Tdrd family (Tdrd1, Tdrd6, Tdrd7a, and Tdrd12) have been characterized as regulators of germ cell development in zebrafish.

Tdrd1 protein is detected in the testis and immature oocytes; it also shows granular distribution in the cytoplasm of PGCs around 4 days after fertilization. The loss of Tdrd1 disrupts Piwil2 localization in the oocyte, causes defects in transposon silencing, and weakly reduces the number of germ cells, similar to the *piwil1* and *piwil2* mutant phenotypes (Huang et al., 2011). It has been shown that the carboxyl-terminal Tudor domains of Tdrd1 interact with Piwil2 containing symmetrically dimethylated arginine residues and with piRNAs (Huang et al., 2011). Thus, it may promote germ cell formation by functioning as a molecular scaffold to enhance the piRNA pathway.

Zebrafish Tdrd6 (previously named Tdrd6a), a putative ortholog of *Drosophila* Tudor protein, is localized in the Bb of oocytes, germ plasm of 4-cell stage embryos, and PGCs at 1 day of development. Maternal-zygotic *tdrd6* mutants show reduced numbers of PGCs, with biased development into males (Roovers et al., 2018). Mechanistically, maternal Tdrd6 interacts with a demethylated tri-RG (arginine-glycine) motif within the C-terminus of Buc protein, which shows no homology to known proteins but functions as an organizer of the germ plasm (Bontems et al., 2009), to promote germ plasm aggregation. The absence of Tdrd6 causes interrupted or scattered germ plasm (Roovers et al., 2018). Thus, Tdrd6 contributes to the growth of germ plasm in cleavage stage embryos by inducing the merge of particles containing Buc and associated mRNAs. In addition, Tdrd6 binds to known germ plasm mRNAs and regulates their stoichiometry in PGCs, whereas its loss of function leads to reduced PGC numbers due to insufficient loading of germ plasm mRNAs into individual PGCs after the blastula period (Roovers et al., 2018).

Maternal *tdrd7a* (previously known as *tdrd7*) transcripts aggregate at the cleavage furrows of 4-cell stage embryos, and zygotic *tdrd7a* is specifically expressed in PGCs when these cells reach the embryonic genital ridge at 1 day after fertilization (Thisse and Thisse, 2004; Mishima et

al., 2006; Strasser et al., 2008). As with *nanos3*, the differential stabilization of *tdrd7a* mRNA in soma and germ cells is regulated by miR-430 (Mishima et al., 2006). Morpholino-mediated knockdown of *tdrd7a* disrupts the size and integrity of germ granules but nevertheless without effects on germ cell development (Strasser et al., 2008). There is a possibility that maternal Tdrd7a protein, which is not affected by the morpholino, contributes to germ plasm formation and germ cell specification. More recent studies suggest that Tdrd7a defines PGC characters by regulating chromatin accessibility and transcriptional profile, which is associated with the relocalization of germ granules during PGC migration. Therefore, the knockdown of *tdrd7a* leads to mildly reduced numbers of PGCs due to their tendency to adopt somatic fate (D'Orazio et al., 2021).

Tdrd12 is involved in germ cell maintenance at juvenile stages. Its transcripts are present during early development and in adult gonadal tissues, but it is unclear how they are localized in the germ plasm or in the PGCs (Dai et al., 2017). The loss of zygotic Tdrd12 leads to defective meiosis of germ cells during the gonad-transitioning period, and the mutants develop into infertile males, suggesting that Tdrd12 is required for gametogenesis (Dai et al., 2017). However, the maternal function of Tdrd12 in germ plasm and germ cell formation during early development awaits further investigation.

Igf2bp3 regulates assembly of the germ plasm and migration of primordial germ cells

Maternal *igf2bp3*, previously called *vg1RBP*, is localized to the Bb of early oocytes and then accumulates in the animal pole region (Zhang et al., 1999; Bontems et al., 2009). Although *igf2bp3* transcripts and Igf2bp3 protein do not display characteristic accumulation in the germ plasm, the loss of maternal Igf2bp3 affects the localization of germ plasm-specific mRNAs and disrupts germ plasm formation, resulting in reduced numbers of PGCs (Ren et al., 2021; Vong et al., 2021). Maternal-zygotic *igf2bp3* mutants show decreased expression of germline mRNAs and impaired collective migration of PGCs, with biased development into male adults (Vong et al., 2021). Mechanistically, Igf2bp3 can bind to m6A (N6-methyladenosine)-modified germ plasm mRNAs to prevent their degradation in early cleavage stage embryos (Ren et al., 2021); it may also function in the translational control by forming a complex that binds the 3'-UTR of maternally

or zygotically expressed mRNAs (Vong et al., 2021). Thus, maternal Igf2bp3 may represent a molecular determinant of germ cell fate.

Another member of the Igf2bp family, Igf2bp2a (or Igf2bp2-A isoform), also plays a role in PGC development. Maternal *igf2bp2a* transcripts are present exclusively in the oocytes but become undetectable soon after fertilization, while zygotic transcription of the gene is activated in PGCs and other germ layers around 1 day of development; knockdown of *Igf2bp2a* progressively reduces PGC numbers from gastrula stages onward (Li et al., 2021). However, the post-transcriptional mechanism by which Igf2bp2a regulates germ cell fate and PGC formation remains unclear.

Rbpms2a and Rbpms2b maintain oocyte fate and regulate female gonad differentiation

There are two zebrafish *rbpms2* (RNA-binding protein with multiple splicing variants 2) paralogs, *rbpms2a* and *rbpms2b* (also known as *rbpms2* or *hermes*), which are likely homologous to the *Xenopus hermes* gene (Kaufman et al., 2018). Maternal Rbpms2a and Rbpms2b proteins are localized to the Bb of early-stage oocytes (Kosaka et al., 2007; Kaufman et al., 2018). It is unclear whether they are present in the germ plasm of cleavage-stage embryos, but exogenous Rbpms2b can be integrated into germ granules in PGCs (Kaufman et al., 2018). Rbpms2a and Rbpms2b are redundantly required for the localization of germ plasm mRNAs in the Bb at least by interacting with Buc protein and *buc* mRNA through their RNA-binding domains (Heim et al., 2014; Kaufman et al., 2018). However, the loss of *rbpms2a* and *rbpms2b* function perturbs the distribution of Buc or the morphology of Bb but does not affect germline development until sexual maturation. After the initiation of sexual differentiation, *rbpms2a* and *rbpms2b* double mutants show the loss of oocytes and develop into fertile males, suggesting an important role in female gonad development (Kaufman et al., 2018). The molecular mechanisms underlying their function in female fate development are not clear. There is a possibility that they act epistatically to Dmrt1, a critical regulator of male development, to prevent the germline from adopting a male fate by promoting the activity of female genes and/or repressing the expression of male factors (Romano et al., 2020). A recent study shows that zebrafish Rbpms2 promotes nucleolar assembly and

female fate via the mTorc1 signaling pathway by functioning as a translational regulator of missing oocyte (*mios*) mRNA (Wilson et al., 2024).

Other RNA-binding proteins in germ cells

Maternal mRNAs coding for Staufen double-stranded RNA binding proteins (Stau1 and Stau2) are expressed in the oocytes and early embryos but without apparent localization in the germ plasm (Bateman et al., 2004; Ramasamy et al., 2006). There is evidence that Stau1 and Stau2 bind germline-specific mRNAs, such as *nanos3*, through the RBD4 sequence to regulate germ cell development (Ramasamy et al., 2006). Disrupting the function of Stau1 and Stau2 does not affect germ plasm aggregation at cleavage stages, but this leads to defective expression of germline markers associated with abnormal migration and loss of PGCs (Ramasamy et al., 2006).

Pumilio (Pum) proteins generally regulate stem cell fate through translational repression by complexing with Nanos proteins (Lai and King, 2013). Zebrafish Pum1 associates with *cyclin B1* mRNA to form granules in the cytoplasm of oocytes and controls the timing of its translational activation (Kotani et al., 2013). Prior to oocyte maturation, phosphorylation of Pum1 promotes the disassembly of *cyclin B1* mRNA granules and polyadenylation of the mRNA, leading to the activation of maturation/M-phase-promoting factor (Saitoh et al., 2018). Pum3 (also known as Puf-A) plays a role in early stages of germ cell development. Its loss of function has been shown to affect the formation and migration of PGCs, but the precise molecular mechanism remains unclear (Kuo et al., 2009).

In addition to the aforementioned Igf2bp3, other m^6A reader proteins have also been shown to function as regulators of germ cell development. Ythdf1, Ythdf2, and Ythdf3 display redundant functions in female development, because only double homozygous mutations disrupting two of these genes cause defective formation of the ovary, whereas single mutation has no effect (Kontur et al., 2020). However, the post-transcriptional mechanisms by which Ythdf proteins function in oogenesis are likely more complex because they are differentially involved in regulating RNA metabolism. Ythdf1 cooperates with Ythdf3 to promote mRNA translation, whereas Ythdf2 interacts with Ythdf3 to reduce mRNA stability (Shi et al., 2017). Thus, how these m6A readers cooperatively control mRNA stability and/or translation to promote oogenesis

requires further investigation. In contrast to Ythdf, the loss of Ythdc2 causes an absence of meiotic germ cells in the testis and leads to the development of infertile males, which is independent of m6A modifications in target mRNAs but requires its RNA helicase activity. These defects are likely due to a failure in the progression of the germline transcriptome further into meiotic identity, as reported for mouse Ythdc2, which forms a protein complex with XRN1 (5′–3′ exoribonuclease), MEIOC (meiosis specific with coiled-coil domain), and Rbm46 to bind U-rich motifs in target transcripts (Li et al., 2022).

Maternal *rbm46* is specifically expressed in germ cells of gonadal tissues and is required for both female and male development. The deficiency of *rbm46* impairs the progression of gametes through meiosis similarly as the loss of Ythdc2, resulting in female-to-male conversion and male sterility (Dai et al., 2021). There is evidence that Rbm46 associates with Ythdc2 to separate mitotic and meiotic transcriptome in the germ cells (Li et al., 2022).

The double-stranded RBP Adad1 (adenosine deaminase domain containing 1) is necessary for the maintenance of male and female germ cells, which are totally absent in *adad1* mutant adult fish (Islam et al., 2023). The loss of *adad1* leads to a failure of spermatocytes to progress beyond the zygotene stage and prevents the formation of ovarian follicles at the bipotential gonad stage. It is possible that Adad1 may function upstream of many other RBPs that are involved in germ cell development (Islam et al., 2023).

Analysis of zebrafish Bb proteome has allowed the identification of novel resident RBPs, such as Cirbp (cold-inducible RBP), Zar1 (zygote arrest 1), and Ybx1 (Y-box binding protein 1), which may play a role in the organization of Bb or in germline development (Jamieson-Lucy et al., 2022). Cirbpa and Cirbpb could contribute to the assembly of Bb components by interacting with maternal mRNAs through their RNA-recognition motifs and with other proteins through their disordered C-terminal regions (Jamieson-Lucy et al., 2022). Two other RBPs, Zar1 and Ybx1, function as translational repressors to regulate oogenesis (Miao et al., 2017; Sun et al., 2018). Zar1 binds to zona pellucida mRNAs and represses their translation, likely by recruiting components of the cytoplasmic polyadenylation complex, including poly(A) binding protein cytoplasmic 1-like and cytoplasmic polyadenylation element-binding protein 1. The loss of Zar1

causes increased endoplasmic reticulum stress and unfolded protein response, leading to apoptosis of early oocytes and female to male conversion in a p53-dependent manner (Miao et al., 2017). Similarly, Ybx1 is required for oocyte maturation, but it functions to repress the global translation of maternal mRNAs in a manner that is dependent on eukaryotic translation initiation factor 4E. The loss of maternal Ybx1 triggers translational derepression and induces egg activation defects (Sun et al., 2018).

Functional interactions among germ plasm components

It is fascinating that the interplay between germ plasm components promotes the growth and localization of phase-separated aggregates. In the oocyte, Rbpms2 cooperates with Buc to organize Bb architecture at least by promoting the localization of germ plasm mRNAs (Kaufman et al., 2018). Maternal Tdrd6 protein also interacts with germ plasm mRNAs, including *ddx4*, *nanos3*, *dazl*, and *hook2*, to promote their accumulation in the germ plasm and regulate their stoichiometry in single PGCs (Roovers et al., 2018). Buc is an important germ plasm organizer that can induce the formation of germ cells (Bontems et al., 2009). It contains intrinsically disordered regions possibly involved in protein aggregation and recapitulates germ plasm functions similarly to the *Drosophila* Oskar protein (Krishnakumar et al., 2018). Tdrd6 interacts with the C-terminal region of Buc protein containing symmetrically dimethylated arginine residues to regulate its aggregation in the germ plasm. An absence of Tdrd6 leads to the formation of incomplete germ plasm structures due to the limited merge of ribonucleoprotein particles (Roovers et al., 2018). As a scaffold, Buc can interact with tight junction proteins through its N-terminal region to anchor its associated germ plasm mRNAs and proteins at the early cleavage furrows (Rostam et al., 2022). Therefore, complex interactions between germ plasm proteins and mRNAs contribute to the proper localization and formation of germ plasm aggregates, which are important for inducing appropriate numbers of PGCs.

Molecular interactions among germ plasm components are also important during the entire process of germline development, which contribute to specify the germ cell lineage,

safeguard germline identity, and promotes gametogenesis (Fig. 3). Post-transcriptional regulation is critically involved in germline-specific gene expression. Dnd1 can bind to the 3'-UTRs of several germ cell-specific mRNAs, such as *nanos3*, *tdrd7a*, and *elavl2*, to protect them in the germline against degradation mediated by miR-430, thereby promoting their translation (Mishima et al., 2006; Kedde et al., 2007; Mickoleit et al., 2011). Although it is still unclear how this protection contributes to mRNA translation, there is evidence that in *Xenopus*, Dnd1 can promote *nanos* translation by directly interacting with and relieving the inhibitory function of the eukaryotic initiation factor 3f (eIF3) repressive preinitiation complex (Aguero et al., 2017b). In zebrafish, as discussed above, Dnd1 regulates the spatial distribution and translation of germline mRNAs within germ granules to specify germ cell fate (Westerich et al., 2023). Dazl can bind at least to the 3'-UTR of exogenously expressed *tdrd7a* mRNA as well as its own mRNA to relieve miRNA-430 translational repression and promote polyadenylation (Takeda et al., 2009). The activity of germ plasm proteins is also dependent on their interaction. An important example is the evolutionarily conserved Nanos/Pumilio complex, which represses the translation of many mRNAs essential for somatic differentiation to protect and set aside the germline (Lai and King, 2013). It is also of note that a conserved C-terminal region of Buc, called Vasa-binding motif, is involved in binding with and promoting the ATPase activity of Ddx4. Inhibition of this interaction leads to reduced germ cell numbers (Perera et al., 2021). Several Tudor-related proteins generally function as molecular adaptors that mediate protein-protein interactions by binding to methylated arginine or lysine residues of target molecules (Pek et al., 2012). They are clearly involved in different aspects of germ cell development by interacting with Piwi proteins and regulating the Piwi-piRNA pathway, which plays an important role in gametogenesis. Overall, the interplay among RBPs as well as between RBPs and other germline components establishes the germ cell lineage and regulates the differentiation of gametes.

Conclusions and perspectives

The inherited mode of germ cell establishment in zebrafish largely depends on maternal germline determinants that assemble into the germ plasm and specify PGCs in a cell-autonomous

manner. Dynamic molecular interactions between germ plasm components coordinate the stage-specific expression of germline transcriptome and the progression of germ cell development. RBPs are enriched in germ cells throughout development and function as essential regulators of germline fate. Although several conserved RBPs are critically involved in different aspects of germ cell development, there are still many interconnected questions that remain largely unanswered.

First, the inventory of germline-specific RBPs, as well as other regulators, including proteins and mRNAs, is far from complete. Thus, data available at present are insufficient for a full understanding of molecular interactions underlying germ plasm assembly and localization, PGC fate specification, and germ cell differentiation. Indeed, systematic identification of germ plasm resident proteins has the potential to uncover new RBPs and provide insights into their roles in these processes (Jamieson-Lucy et al., 2022). Second, for several RBP-coding mRNAs, as well as for other germ plasm-specific mRNAs, whether, when, where, and how they are translated during PGC development remains enigmatic. This could be elucidated, at least partially, by investigating the functional interactions among germ plasm components and monitoring the dynamic features of endogenous RBP localization during germ plasm formation using high-resolution living imaging. Third, the maternal functions of many known germline-specific RBPs are not clear, often due to technical barriers to inhibit the function of maternal gene products or to generate maternal mutants because of zygotic lethality or sterility. With the advent of genome-editing technology, several approaches can be used to overcome these obstacles in studying maternal gene functions (Zhang et al., 2021; Shi, 2022). This will undoubtedly provide further insights into the preformation mechanism by which RBPs-mediated post-transcriptional events contribute to setting aside germ cells during early development. Fourth, for some RBPs, an intriguing question is to determine the respective functions of their mRNAs and proteins in germ plasm formation. This is particularly true for Ddx4 because its mRNA but not the protein is specifically accumulated in the germ plasm (Knaut et al., 2000). Extending this question, it is clearly important to decode the regulated translational activation or repression of germ plasm mRNAs as well as the specific post-translational modifications of germ plasm proteins. Last but not least, emerging evidence indicates that the interplay of RBPs contributes to setting up the

germline transcriptome in a stage-specific manner. However, mechanistic insights into the complex interactions of RBPs in the dynamic regulation of germline gene expression programs are quite elusive.

Deciphering the combinatorial actions of RBPs in coordinating the formation of germ plasm and the post-transcriptional gene expression program in the germ cells will shed light on the molecular control of germ cell fate and gametogenesis. Since a failure to control the developmental outcome of germ cells can lead to sterility or infertility and the occurrence of germ cell tumors (Sanchez and Amatruda, 2016; Oosterhuis et al., 2019), functional analyses of RBPs-mediated post-transcriptional regulatory networks underlying germline development may contribute to a better understanding of diseases related to germ cells.

Conflict of interest

The author declares no competing interests.

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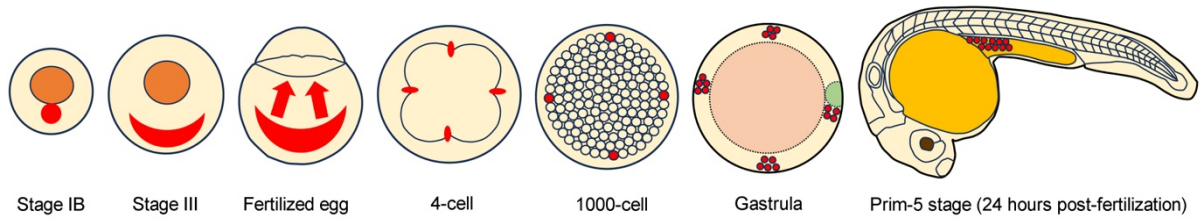

Fig. 1. Formation of PGCs in zebrafish. Maternal germline determinants are accumulated in the Bb (red dot) below the nucleus of early oocytes (stage IB). After Bb breakdown during oocyte maturation, germline determinants are released to the vegetal cortex (stage III). After fertilization, germ plasm components are transported to the animal pole region and anchored at the distal ends of cleavage furrows as large aggregates (oval red dots). At the blastula period (beginning at the 1000-cell stage), the four germ plasm aggregates are asymmetrically segregated into individual blastomeres located at the margin, which are the precursors of PGCs (red circled dots). In gastrula-stage embryos, PGC precursors give rise to four clusters of PGCs, which migrate toward the future gonadal ridge. At 24 hours post-fertilization, PGCs enter into the gonadal ridge located at the anterior region of the yolk extension and will further differentiate to form male or female gametes. Lateral views for oocytes, the fertilized egg, and the prim-5 stage embryo; other embryos are animal pole views. The green area in the gastrula represents the embryonic shield.

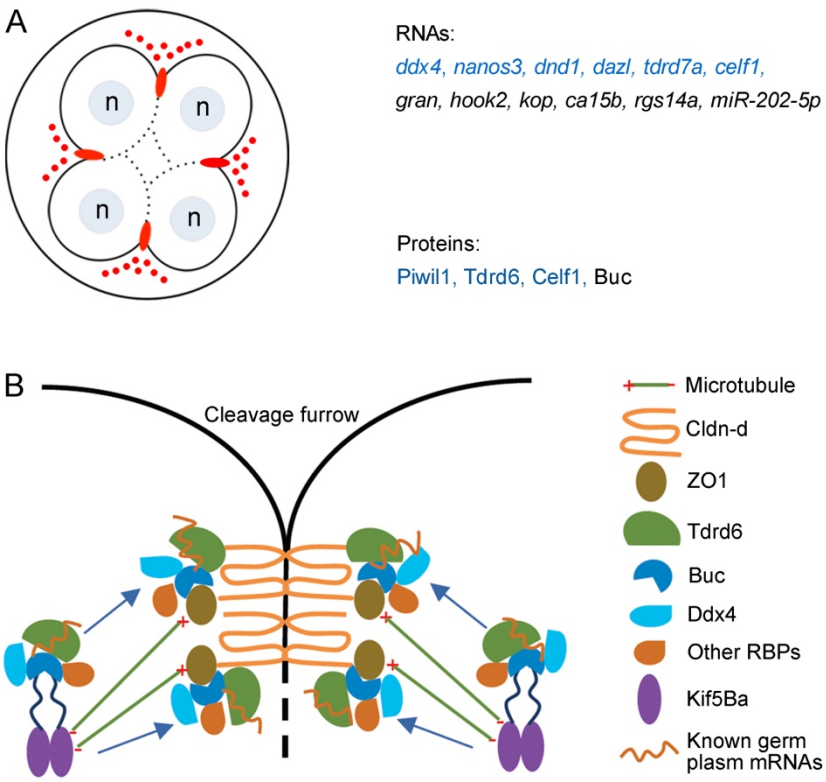

Fig. 2. Schematic of germ plasm aggregation at the cleavage furrows in the zebrafish early embryo. **A**: Formation and components of the germ plasm. On the left, animal pole view of a 4-cell stage embryo (n, nucleus). Beginning at the first division, germ plasm ribonucleoparticles (round red dots) are transported to the animal pole region and anchored at the distal ends of cleavage furrows to form germ plasm aggregates (oval red dots). On the right, localized mRNA and protein components of the germ plasm, with RBP-coding mRNAs and RBPs indicated in blue. **B**: Kinesin-dependent recruitment of germ plasm component to the cleavage furrows. Tdrd6 interacts with Buc and binds to germ plasm mRNAs to promote the growth of germ plasm ribonucleoparticles (Roovers et al., 2018). Buc can also interact with Ddx4 and other germ plasm RBPs, and the complex is recruited to the cleavage furrows by Kif5Ba, likely in a microtubule-dependent manner (Campell et al., 2015; Perera et al., 2021). The interaction between Buc and the tight junction protein ZO1 further anchors the germ plasm at the cleavage furrows (Rostam et al., 2022).

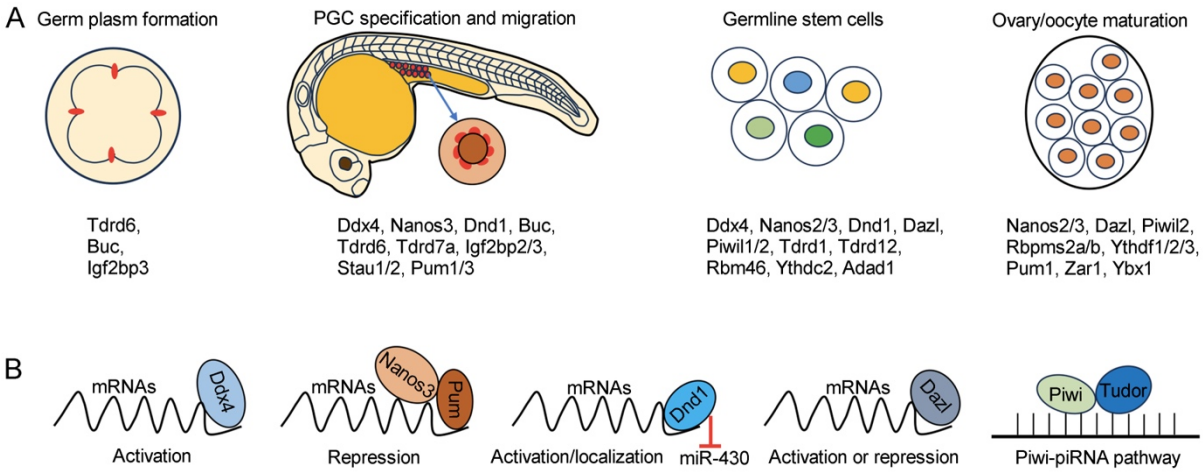

Fig. 3. Function and interaction of RBPs in the germ cells. **A**: Summary of RBPs involved in major steps of germ cell development (see Table 1 for more details). Buc may be considered a noncanonical RBP. In PGCs, RBPs are localized in perinuclear germ granules. **B**: Functional interactions among germ plasm components. RBPs regulate the translational activation or repression and localization of germ cell-specific mRNAs to promote germ cell specification and differentiation. The interaction between Piwi and Tudor domain proteins contributes to germ cell maintenance and gametogenesis in a manner that is both dependent and independent of transposon silencing.

Table 1. Functions of RBPs during germ cell development in zebrafish.

RBPs	Expression and localization	Molecular interactions	Biological functions	References
Ddx4 (aka Vasa)	The mRNA localizes to Bb, cleavage furrows, and PGCs; the protein accumulates in PGCs from late blastula stage onward	Interaction with Buc via a Buc-binding motif to modulate germ cell specification	Meiosis, differentiation and maintenance of GSCs	Olsen et al., 1997; Yoon et al., 1997; Braat et al., 2000; Knaut et al., 2000; Kosaka et al., 2007; Hartung et al., 2014; Perera et al., 2021;
Nanos3 (aka Nanos1)	The mRNA localizes to Bb and cleavage furrows	Interaction with Pum1 in translational repression	PGC migration and survival; maintenance of GSC and oocyte production	Köprunner et al., 2001; Draper et al., 2007; Kedde et al., 2007
Nanos2	The mRNA is expressed in pre-meiotic spermatogonia and oogonia	Unknown	Maintenance of GSCs; ovary regeneration	Beer and Draper, 2013; Cao et al., 2019
Dnd1	The mRNA localizes to cleavage furrows; the protein accumulates in perinuclear granules of PGCs	Interaction with 3'-UTRs of *nanos3* and *Tdrd7a* mRNAs to inhibit degradation by miR-430	Organization and translation of *nanos3* mRNA within germ granules; PGC localization and migration; protection of germline fate	Weidinger et al., 2003; Kedde et al., 2007; Gross-Thebing et al., 2017; Westerich et al., 2023
Dazl	The mRNA is localized to Bb and cleavage furrows; the protein is first detected in Ddx4-positive germ cells in the gonads	Interaction with 3'-UTRs of *tdrd7a* and its own mRNAs to promote poly(A) tail elongation and translation by antagonizing miR-430	Germline cyst formation and GSC specification	Maegawa et al., 2002; Hashimoto et al., 2004; Kosaka et al., 2007; Takeda et al., 2009; Bertho et al., 2021
Piwil1 (aka Ziwi)/Piwil2 (aka Zili)	Piwil1 localizes to cleavage furrows and perinuclear granules in early PGCs; Piwil2 accumulates in the nucleus and cytoplasm of late PGCs	Transposon silencing; interaction with coding and non-coding germline mRNAs to regulate stability and translation	Germ cell maintenance and differentiation; oocyte meiosis	Houwing et al., 2007, 2008
Tdrd1	It shows granular distribution in late PGCs and is expressed in the testis and early oocytes	Interaction with Piwil2 and association with piRNAs to enhance Piwi-piRNA pathway	Germ cell formation	Huang et al., 2011
Tdrd6 (aka Tdrd6a)	It localizes to Bb, cleavage furrows, and early PGCs	Interaction with Buc; binding to all known germ plasm mRNAs	Aggregation of germ plasm; loading of germ plasm mRNAs into PGCs	Roovers et al., 2018

Tdrd7a	The mRNA localizes to cleavage furrows; Tdrd7a-GFP fusion protein is present in germ granules	The 3'-UTR of its mRNA is bound by Dnd1 and Dazl	Maintenance of germ granule integrity; epigenetic reprogramming in PGCs	Strasser et al., 2008; D'Orazio et al., 2021
Tdrd12	The mRNA is expressed in testis and ovary	Unknown	Maintenance of germ cells; meiosis of germ cells	Dai et al., 2017
Igf2bp3 (aka Vg1RBP)	The mRNA localizes to Bb of early oocytes	Interaction with m6A-modified germ plasm mRNAs to prevent their degradation	Germ plasm assembly and germline progenitor cell migration	Bontems et al., 2009; Ren et al., 2021; Vong et al., 2021
Igf2bp2a	The mRNA is expressed in PGCs and oocytes	Unknown	PGC development	Li et al., 2021
Rbpms2a/2b (aka Hermes or Rbpms2)	The proteins localize to Bb of early oocytes	Interaction with Buc protein and *buc* mRNA; binding to the 3'-UTR of *mios* mRNA	Maintenance of Bb architecture and oocyte fate; female gonad differentiation	Kosaka et al., 2007; Heim et al., 2014; Kaufman et al., 2018; Romano et al., 2020; Wilson et al., 2024
Stau1/2	Their mRNAs localize to cortical patches of oocytes	Binding to *nanos3* mRNA	PGC migration and survival	Ramasamy et al., 2006
Pum1/3	Unknown	Interaction of Pum1 with *cyclin B1* mRNA to repress translation	Oocyte maturation; PGC formation and migration	Kuo et al., 2009; Kotani et al., 2013; Seitoh et al., 2018
Ythdf1/2/3	Unknown	Interaction with m6A-modified mRNAs	Female development	Kontur et al., 2022
Ythdc2	Unknown	Regulation of transcriptome to confer meiotic identify	Formation of male germ cells	Li et al., 2022
Rbm46	The mRNA is expressed in undifferentiated and transitioning gonadal germ cells	Regulation of *nanos3* and *dazl* expression	Maintenance of female germ cells; meiosis of male gametes	Dai et al., 2021
Adad1	It is detected in male and female germ cells	Involved in DNA damage and repair; regulating the expression of pluripotency genes	Germ cell maintenance and meiosis	Islam et al., 2023
Cirbpa/b	They are Bb resident proteins	Possible interaction with Bb mRNAs	Unknown	Jamieson-Lucy et al., 2022

Zar1	It is a Bb resident protein	Interaction with zona pellucida mRNAs to repress translation	Early oogenesis	Miao et al., 2017; Jamieson-Lucy et al., 2022
Ybx1	It is a Bb resident protein	Interaction with processing body components to repress translation	Oocyte maturation	Sun et al., 2018; Jamieson-Lucy et al., 2022