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Germ plasm dynamics during oogenesis and early embryonic development in *Xenopus* and zebrafish

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Abstract

Specification of the germline and its segregation from the soma mark one of the most crucial events in the lifetime of an organism. In different organisms, this specification can occur through either inheritance or inductive mechanisms. In species such as *Xenopus* and zebrafish, the specification of primordial germ cells relies on the inheritance of maternal germline determinants that are synthesized and sequestered in the germ plasm during oogenesis. In this review, we discuss the formation of the germ plasm, how germline determinants are recruited into the germ plasm during oogenesis, and the dynamics of the germ plasm during oogenesis and early embryonic development.

KEYWORDS
Balbiani body, germ plasm, germline development, *Xenopus*, zebrafish

1 | INTRODUCTION

Germ cells have the responsibility of transferring genetic information from one generation to the next. Thus, they are considered the stem cells of sexually reproducing species (Wylie, 1999). Mutations in the germ cells can be inherited by subsequent generations and consequently, are more detrimental in the long run. Thus, it becomes imperative to specify and segregate the germline early on in development from the somatic cells, localizing them away from the body patterning signals (Dixon, 1994). This early specification and sorting attempts to better protect the germline. Once specified, there are mechanisms in place to ensure that these cells do not respond to the somatic inductive cues and that the germ cell fate is preserved. This is achieved by regulation of chromatin accessibility and global transcription for these specified cells (reviewed by Blackwell, 2004).

2 | GERMLINE SPECIFICATION

Germline specification happens through either an inductive or a cytoplasmic inheritance mechanism. The former relies on a series of inductive signals, which specify undifferentiated cells in early embryos as precursors of germ cells. Humans, mice, and axolotl are some organisms that employ induction via cell–cell signaling for germline specification. This mode is considered the more ancestral mode of specification (reviewed by Extavour & Akam, 2003). Members of the bone morphogenic protein (BMP) family, for instance, BMP4 in mice, serve as the key signal for germline specification (Lawson et al., 1999). In contrast, the cytoplasmic inheritance mechanism involves the acquisition of cytoplasm containing all germ cell determinants needed for the specification of primordial germ cells (PGCs). The cells which acquire this specialized cytoplasm, called the germ plasm, adopt the germ cell fate (Tada

et al., 2012). In organisms like *Xenopus*, zebrafish, *Drosophila*, and *C. elegans*, specification occurs via this route.

Although different organisms can specify their germline through these two mechanisms, the major molecular players for germline development are highly conserved. For instance, the DEAD-box RNA helicases, Nanos-related proteins, Sox family transcriptional factors, and DAZ family members are involved in germline specification in organisms that specify their germ cells through both mechanisms (reviewed by Hansen & Pelegri, 2021; Seydoux & Braun, 2006). A recent report (Colonnetta et al., 2021) suggests that BMP signaling, which is required for the induction of PGCs in mammals and axolotl (Chatfield et al., 2014; Johnson et al., 2003; Lawson et al., 1999), is required for the maintenance of germ cells once they have been specified in *Drosophila*. It has been reported that the majority of transcripts specifically expressed in *Xenopus* germ plasm and PGCs are enriched in human PGCs/PGC-like cells (PGCLCs) as well (Butler et al., 2017). Thus, understanding germ plasm regulation in lower vertebrates can also provide novel insights into human germline development. A comprehensive list of conserved germline components across different species is provided in Table 1. In this review, we will discuss the germline development in *Xenopus* and zebrafish, both of which rely on the acquisition of maternal determinants. We will focus on the germ plasm dynamics during oogenesis and early development.

3 | DISCOVERY OF THE Bb AND GERM PLASM

Xenopus and zebrafish are amongst the most well-studied vertebrate animal models that rely on the inheritance of maternal determinants for PGC specification. Ultrastructural studies conducted several decades ago identified the presence of germinal granules across a plethora of organisms. Elaborate reviews have been written describing these granules (Eddy, 1976; Guraya, 1979). Even though the exact description differed depending on the organism, most studies reported the presence of electron-dense nuage or granulofibrillar material (GFM) and abundant mitochondria in the germ plasm (Eddy, 1976; Guraya, 1979; Heasman et al., 1984; Kalt, 1973; Mahowald, 1962). In addition to that, the germ plasm contains RNA binding proteins (RBPs), RNAs, endoplasmic reticulum, ribosomes, and Golgi apparatus (Boke et al., 2016; Dhandapani et al., 2022; Heasman et al., 1984; Kalt, 1973; Kloc et al., 2001) It has been well known that the *Xenopus* germ plasm is necessary for germ cell specification and there have been studies to test if the germ plasm is itself sufficient (Buehr & Blackler, 1970; Ikenishi et al., 1986; Wakahara, 1978; Wylie et al., 1985). Tada et al. (2012) showed through transplantation experiments that germ plasm was indeed sufficient for PGC formation and that the gametes arising from germ plasm transplantation could be fertilized and further give rise to an embryo. Similar observations have been made in other species. For example, it has been shown that transplantation of pole plasm could rescue fertility in sterilized *Drosophila* eggs (Okada et al., 1974).

The accumulation of the germ plasm during development was first noted in early oogenesis in spiders, in a structure close to the nucleus, that was referred to as the Balbiani body (Bb). The *Xenopus* Bb was observed to contain electron-dense GFM, along with mitochondria (Heasman et al., 1984). For this reason, it is sometimes called the Mitochondrial Cloud. This GFM was reported to be persistently present later at the vegetal cortex of the oocyte and was speculated to be a precursor to the germ granules. Since the Bb and germ plasm is associated with abundant mitochondria, the germ plasm can be monitored all throughout development by tracking the enriched mitochondria.

TABLE 1 Conservation of components expressed in the germline across different species.

Components	Organisms expressing the components in the germline
Vasa or DDX family helicases	*Xenopus* (Komiya et al., 1994; MacArthur et al., 2000), zebrafish (Yoon et al., 1997), *Drosophila* (Hay et al., 1988), *C. elegans* (Roussell & Bennett, 1993), Mouse (Fujiwara et al., 1994), and Humans (Castrillon et al., 2000).
Nanos family	*Xenopus* (Mosquera et al., 1993), zebrafish (Köprunner et al., 2001), *Drosophila* (Asaoka et al., 1998; Lehmann & Nusslein-Volhard, 1991), *C. elegans* (Subramaniam & Seydoux, 1999), and Humans (Angeles Julaton & Reijo Pera, 2011; Jaruzelska et al., 2003).
DAZ-like (Dazl)	*Xenopus* (Houston et al., 1998), zebrafish (Maegawa et al., 1999), *Drosophila* (Cheng et al., 1998), Mouse (Cooke et al., 1996), and Humans (Lee et al., 1998).
Tudor-family	*Xenopus* (Ikema et al., 2002), zebrafish (Dai et al., 2017; Mo et al., 2005; Roovers et al., 2018), *Drosophila* (Golumbeski et al., 1991), *C. elegans* (Marnik et al., 2022), and Mice (Smith et al., 2004).
Sox-family	*Xenopus* (Butler et al., 2018; Koyano et al., 1997), zebrafish (Zhang et al., 2004), *Drosophila* (Mukherjee et al., 2006), Mice (Campolo et al., 2013), and Humans (De Jong et al., 2008).
Deadend (Dnd)	*Xenopus* (Aguero et al., 2017; Horvay et al., 2006; Koebernick et al., 2010; Taguchi et al., 2014), zebrafish (Gross-Thebing et al., 2017; Kedde et al., 2007; Weidinger et al., 2003), and Mice (Bhattacharya et al., 2007).
Kinesin-family	*Xenopus* (Oh & Houston, 2017; Robb et al., 1996; Tarbashevich et al., 2011), zebrafish (Campbell et al., 2015), and Mice (Czechanski et al., 2015; Kong et al., 2016; Lehti et al., 2015).
Sm proteins	*Xenopus* (Bilinski et al., 2004), *C. elegans* (Barbee et al., 2002), and Mice (Chuma et al., 2003).
Maelstrom	*Drosophila* (Findley et al., 2003) and Mice (Costa et al., 2006).

In *Drosophila*, germinal granules were observed by Mahowald (1962) in the pole plasm but the presence of a Bb wasn't reported until the early 2000s (Cox & Spradling, 2003). Since the first observation/sighting in the 1800s, Bb has been reported in the oocytes of almost all the organisms that have been tested for it. Currently, the consensus is that the Bb is a highly conserved organelle (reviewed by Jamieson-Lucy & Mullins, 2019). Interestingly, the presence of a Bb in mice remains debated (Dhandapani et al., 2022; Lei & Spradling, 2016; Pepling et al., 2007). The difference lies in the definition of what constitutes a Bb. Contrary to the definition of it being a mitochondrial aggregate, Pepling et al. (2007) identified a Golgi aggregate, surrounded by mitochondria and ER, which they defined as the mouse Bb. The work by Dhandapani et al. argues that this structure is in fact, not a Bb, as it (1) does not host RBPs, (2) is not connected to oocyte dormancy status, and (3) does not have mitochondria clustered within it. Further work is needed to better understand the structural details and function of this unique structure in mouse oocytes.

Conservation of the Bb across different species (Cox & Spradling, 2003; Eddy, 1974; Heasman et al., 1984; Hertig, 1968; Pepling et al., 2007), including the organisms that specify their germ cells through an inductive mechanism, suggests the possibility for alternative or secondary roles for the Bb. One such possibility that is widely accepted is the role of the Bb in the enrichment of "healthy" maternal mitochondria to be passed on to the embryo. Due to the central role of mitochondria in ATP synthesis and the generation of reactive oxidative species, mitochondrial DNA (mtDNA) is more prone to the accumulation of deleterious mutations than nuclear DNA (reviewed by Tworzydlo et al., 2020). Thus, the existence of a mitochondrial bottleneck effect to facilitate stringent selection into the Bb, rather than stochastic accumulation of mitochondria into the oocyte could be key to ensuring the inheritance of wild-type mtDNA (Bilinski et al., 2017; Hill et al., 2014; Marlow, 2017; Tworzydlo et al., 2016, 2020). Interestingly, in *Drosophila*, the majority of mitochondria passed onto PGCs are primarily derived from nurse cells, independent of the Bb (Hurd et al., 2016).

4 | GERM PLASM DYNAMICS DURING EARLY EMBRYONIC DEVELOPMENT

The embryo relies on the maternal products supplied by the egg till the zygotic genome activation. In *Xenopus*, the germ plasm in the oocyte is organized at the vegetal cortex into numerous fragmented islands. Upon fertilization, these "germ plasm islands" undergo fusion and aggregation along the furrow in cleavage stages, during which the germ plasm is acquired asymmetrically by daughter cells (Ressom & Dixon, 1988). The aggregation process depends on microtubules and is aided by the surface contraction waves (SCWs) at the vegetal pole (Ressom & Dixon, 1988; Robb et al., 1996; Savage & Danilchik, 1993). The kinesin-like protein Xklp1 is required for the aggregation and was seen to be essential for these SCWs (Quaas & Wylie, 2002).

Zebrafish differs from *Xenopus* in that upon egg activation, there is cytoplasmic streaming, transporting proteins and RNAs to the animal pole, where the blastodisc formation occurs and the embryo undergoes meroblastic cleavage. Like *Xenopus*, the zebrafish germ plasm also undergoes aggregation. As the germ plasm aggregates, the slow calcium wave (SCW) in the zebrafish embryos, with assistance from a furrow microtubule array (FMA) provides the directional cue for germ plasm enrichment along the furrow (Eno, Gomez, et al., 2018). It is believed that non-muscle myosin II functions to enrich the FMA at the cleavage furrows, allowing germ plasm aggregation (Urven et al., 2006). The role of cytoskeletal elements in assisting this aggregation has been studied elegantly by Pelegri et al. (reviewed by Moravec & Pelegri, 2020). They reported that a network of F-actin rings push germ plasm ribonucleoprotein (RNPs) toward the periphery of the blastoderm. Disruption of proper F-actin dynamics in the "aura" maternal effect mutants resulted in impaired RNP recruitment to the furrows (Eno & Pelegri, 2018; Eno et al., 2016). Unlike zebrafish, the germ plasm aggregation in *Xenopus* is fairly actin independent.

During the cleavage and blastula stages, the germ plasm remains tightly associated with the plasma membrane of the "future PGCs." In zebrafish, the germ plasm aggregate splits into four smaller aggregates during cleavage, acquired later by four pre-presumptive PGCs (reviewed by Hansen & Pelegri, 2021). After the zebrafish embryo undergoes several rounds of cleavage, in dome stage, the RNPs are released into the cytoplasm, specifying these cells as presumptive-PGCs. From here on, the germ plasm is passed on symmetrically to the daughter cells (Eno et al., 2019; Knaut et al., 2000; reviewed by Hansen & Pelegri, 2021). Before migration, there are around 30 PGCs, which further undergo proliferation upon reaching the gonad (Yoon et al., 1997). Similarly, during *Xenopus* gastrulation, germ plasm detaches from the plasma membrane and moves to the perinuclear region, specifying the PGCs, which can now divide symmetrically. These cells then undergo three rounds of division, resulting in a total of approximately 20–50 PGCs (reviewed by King, 2014).

Taguchi et al. (2012) generated "Dria" transgenic frogs (*Xenopus leavis*) with EGFP expression in the outer mitochondrial membrane. This EGFP signal can thus be used to monitor the germ plasm during oogenesis and early embryonic development. Figure 1 shows oocytes and embryos obtained from "Dria" frogs to observe the dynamic nature of the germ plasm organization during oogenesis and early development. In stage I oocyte, the germ plasm is packaged in the Bb (Figure 1a,b, white arrowheads). Over time, this spherical structure travels toward the vegetal pole and by stage II, the Bb is completely fragmented into numerous small germ plasm islands. Most of these germ plasm islands remain at the cortex in the fully grown stage VI oocyte, while others remain in the cytoplasm in the vegetal hemisphere. Upon fertilization, germ plasm islands aggregate along the cleavage furrows (Figure 1c,d). Before gastrulation, the germ plasm remains associated with the cell membrane in the pre-PGCs (Figure 1e,f). During gastrulation, it ultimately detaches from the cell membrane and moves to the perinuclear region of PGCs (Figure 1g,h). Numerous studies have also been conducted to look at the germ plasm localization in zebrafish, like the one by Yoon et al. (1997), utilizing the localization pattern of a germ plasm component, *vasa* RNA.

Through the years, there have been attempts to investigate the roles that different components of the germ plasm play during embryonic germline development in *Xenopus*. It has been reported that

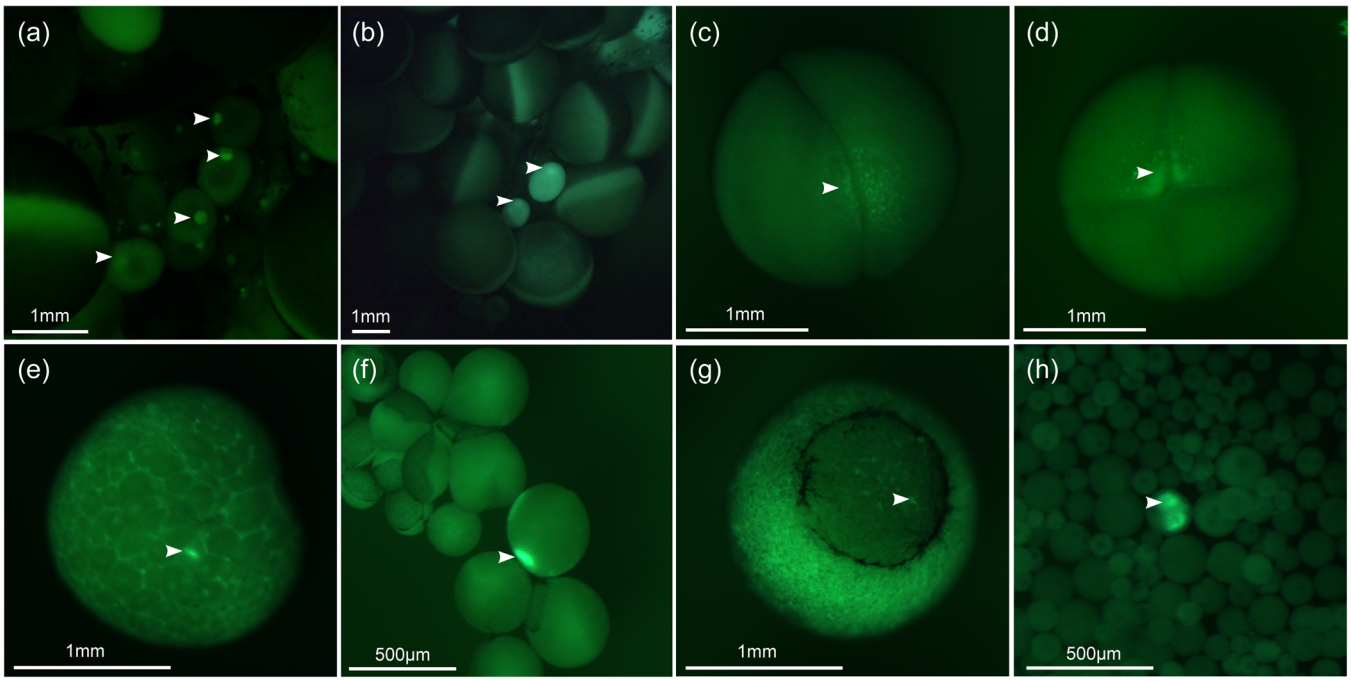

FIGURE 1 Dria frogs allow visualization of the dynamic nature of germ plasm during different stages of development. (a, b) Ovary tissue containing stage I and early-stage II oocytes. Arrowheads point to the Bb in these oocytes. (c) Two-cell stage embryo with germ plasm at the cleavage furrow. (d) Eight-cell stage embryo with germ plasm aggregated at the cleavage furrow. (e) Stage 8 embryo with germ plasm associated with the membrane of individual pre-PGCs. (f) Dissociated pre-PGCs obtained from a stage 8 embryo. (g) Stage 11 embryo with germ plasm in the perinuclear region of PGCs. (h) Dissociated PGCs from a stage 11 embryo. PGCs, primordial germ cells.

Nanos1 (Lai et al., 2011), whose translation is regulated by Dnd1 (Aguero et al., 2017, 2018) and Sox7 (Butler et al., 2017) preserves the totipotency of PGCs by suppressing premature zygotic transcription. Another crucial specification process, like the translocation of the germ plasm from the plasma membrane to the perinuclear region requires Sox7 and Syntabulin (Butler et al., 2017; Oh & Houston, 2017).

During the process of PGC migration in *Xenopus*, the PGCs form PIP3-enriched bleb-like protrusions to direct their movement. Kif13b is one of the proteins important for this polarized accumulation of PIP3, and in turn, for PGC migration (Tarbashevich et al., 2011). To migrate freely, the PGCs need to lose adhesin to the surrounding endodermal cells and to each other, which is achieved by lowered e-cadherin expression (Baronsky et al., 2016; Dzementsei et al., 2013). Dazl, Grip2, and Dnd1 are required for normal PGC migration as well (Horvay et al., 2006; Houston & King, 2000; Kirilenko et al., 2008). By the tailbud and tadpole stages, the PGCs migrate actively in the endoderm. Eventually, these cells make their way to the gonads and become differentiated into gametes.

It has been reported that zebrafish Dnd1, just like *Xenopus* Dnd1 preserves the germline fate of the PGCs by regulating Nanos (Draper et al., 2007; Gross-Thebing et al., 2017; Westerich et al., 2023). In zebrafish, once the PGCs are ready for migration, stromal-cell derived factor (SDF1) chemokines can direct their movement utilizing receptors like CXCR4, which are present in the PGCs (Aalto et al., 2021; Doitsidou et al., 2002; reviewed by Knaut et al., 2002 and Paksa & Raz, 2015). Like in *Xenopus*, E-cadherin also plays a role in restricting the regions in the PGCs where blebs form and thus, contribute to maintaining their direction (Grimaldi et al., 2020). Once migration is completed, PGCs proliferate and differentiate in the gonads, ultimately giving rise to the oocyte and the sperm. The germ cell life cycle thus continues and the assembly of the germ plasm in the oocytes begins again in the Bb.

5 | FORMATION OF THE Bb

Even after close to 170 years since the first visualization of the Bb, we do not fully understand molecular details regarding the formation and dispersal of this giant membrane-less aggregate. The first protein found to be important for Bb formation was the zebrafish protein Bucky Ball (Buc) (Dosch et al., 2004; Marlow & Mullins, 2008). Buc was identified from a maternal-effect mutant screen for genes involved in developmental processes before the mid-blastula transition. In the *bucky ball* mutant, Bb fails to form, and the localization of germ plasm components is disrupted. Additionally, the polarity of the mutant oocyte is severely affected. After fertilization, the formation of the blastodisc is impaired in embryos derived from the *bucky ball* mutant (Dosch et al., 2004). It turns out that the Buc protein specifically localizes to the Bb and germ plasm during oogenesis (Bontems et al., 2009). When Buc is overexpressed in early embryos, unlike any other zebrafish germ plasm component, it forms aggregates and protects germline RNAs from degradation in somatic cells, which ultimately converts somatic cells to PGCs (Bontems et al., 2009; Ye et al., 2019).

In comparison to the extensive studies conducted to understand the role of Buc, little work has been done on its *Xenopus* homolog, Velo1. Both Buc and Velo1 possess a C-terminal intrinsically disordered region (IDR) and a Prion-like domain (PLD) at their N-terminus, which can mediate the aggregation of these proteins. Some of the key residues at the PLD are conserved between these two and this stretch is referred to as the BUVE (Buc-Velo) motif (Bontems et al., 2009) (Figure 2a). In addition to being crucial for the self-aggregation of the protein, the PLD of Velo1 is important for its incorporation into the Bb. Fluorescence recovery after photobleaching studies established that Velo1 forms a stable condensate that recovers poorly after photobleaching (Boke et al., 2016). The Bb has been isolated from previtellogenic oocytes in both *Xenopus* and zebrafish (Butler et al., 2019; Jamieson-Lucy & Mullins, 2019). The physical nature of Bb in *Xenopus* and zebrafish is remarkably different from germ granules in some other species. Unlike P granules in *C. elegans*, which exhibit liquid-like behaviors (Brangwynne et al., 2009), the Bb in *Xenopus* can survive harsh conditions like high salt or temperature (Boke et al., 2016). This stable matrix and the ability to withstand seemingly difficult conditions can be seen as mechanisms evolved to protect germline determinants, especially germline-specific RNAs that are sequestered in the Bb or germ plasm. In agreement with this view, when trapped in the germ plasm, *Xenopus* and zebrafish germline RNAs are more resistant to RNA degradation machinery (Hwang et al., 2023). It is important to note that Velo1/Buc condensates exhibit amyloid-like properties. In contrast to pathological amyloids, these amyloids are reversible (Boke & Mitchison, 2017). Velo1/Buc first assembles into a spherical structure (the Bb) during oogenesis, and then is broken down into numerous small pieces. During oocyte maturation and early development, Velo1/Buc condensates disappear (Hwang et al., 2023). Thus, despite being amyloid-like structures, Velo1/Buc condensates are highly dynamic.

Surprisingly, overexpression of *Drosophila* Oskar (Osk) could induce ectopic PGCs in zebrafish, to the same extent as overexpression of Buc (Krishnakumar et al., 2018). Osk is a germ plasm component of *Drosophila* which is well known to induce ectopic germ cells in the fly (Ephrussi & Lehmann, 1992; Kistler et al., 2018; reviewed by Lehmann, 2016). The structure of Osk is very different from that of Buc and Velo1. In fact, Osk and Buc/Velo1 proteins do not even share the conserved BUVE motif (Figure 2). It was recently reported that the localization machinery for targeting Buc and Velo1 to the germ plasm in zebrafish recognizes a signal in the N-terminal BUVE motif. This machinery, however, is unable to recognize and localize injected Osk with the germ plasm (Rostam et al., 2022). The only common feature Osk, Buc and Velo1 possess is the IDR, which allows them to self-assemble and interact with germ plasm components. It has been proposed that Osk, Buc, and Velo1 have changed sequences through the course of evolution rapidly, as is seen in other IDR-containing proteins (Hultqvist et al., 2017;

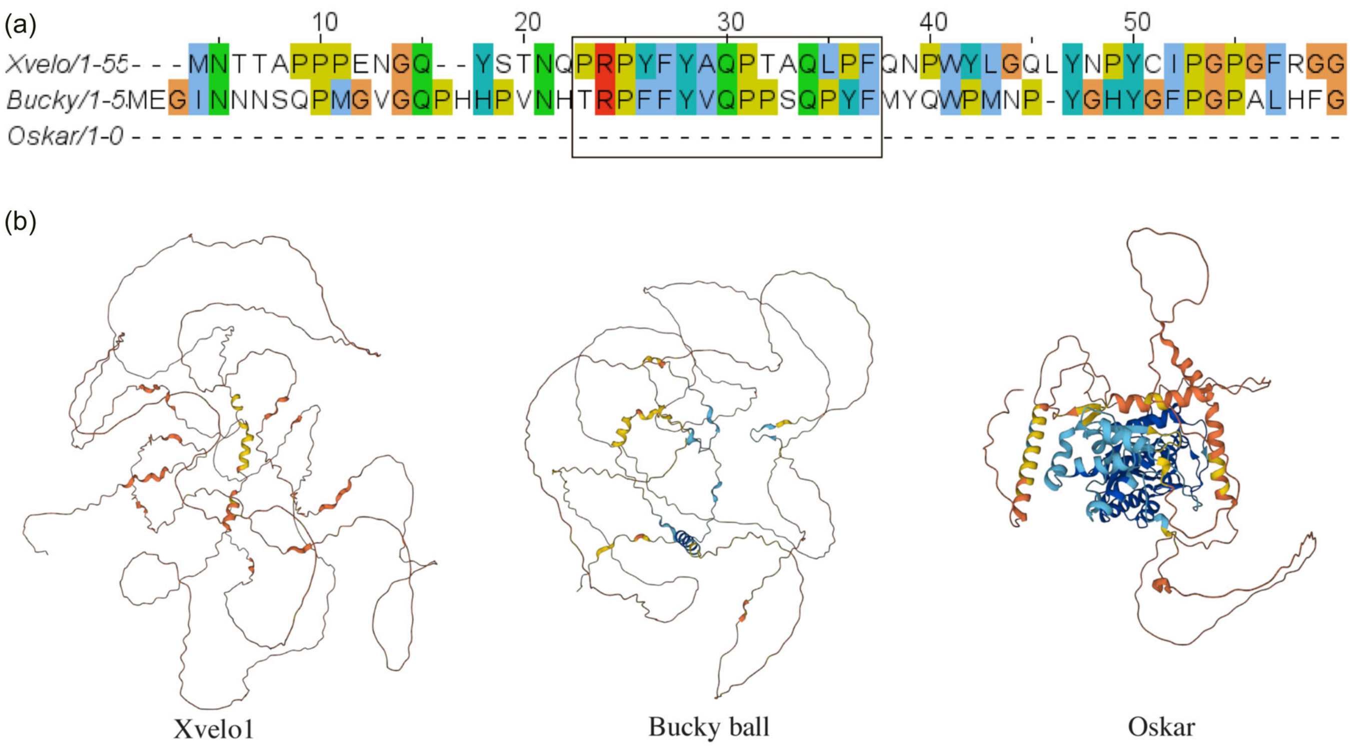

FIGURE 2 Comparison of Velo1, Bucky ball, and Oskar proteins. (a) Sequence alignment of the three proteins at the N-terminal BUVE motif (in box) obtained using Kalign (Lassmann & Sonnhammer, 2005) and Jalview (Waterhouse et al., 2009). (b) Predicted structures for the three proteins, obtained from alpha-fold (Jumper et al., 2021; Varadi et al., 2022).

discussed by Krishnakumar et al., 2018). The ability of these proteins to form very stable protein condensates appears to be the key to their function in organizing the germ plasm.

Some RBPs, for example, Rbpms2 and Tdrd6a, a tudor-domain containing protein, have been suggested to influence the Bb by affecting its architecture or growth of Buc granules respectively (Heim et al., 2014; Kaufman et al., 2018; Roovers et al., 2018). The zebrafish protein, Macf1 (microtubule actin cross-linking factor) was found to be important for the vegetal localization of RNAs and oocyte polarity (Gupta et al., 2010). Macf1 protein can interact with several cytoskeletal systems using different domains. The Bb is enriched in cytokeratins (CK) and it is speculated that Macf1 might be involved in Bb fragmentation by interacting with the CK in Bb and the F-actin at the vegetal cortex (Escobar-Aguirre et al., 2017). In zebrafish Magellan mutants, which carry mutations in the *macf1* gene, the Bb is enlarged and cannot be fragmented into small pieces (Gupta et al., 2010). *Drosophila* Milton is another protein that can interact with Kinesin and mediate the transport of mitochondria into the Bb, and thus, is one of the few proteins known to be important for the formation of Bb in this species (Cox & Spradling, 2006). A recent proteomic analysis has identified the RNA-binding proteins, Cirbpa and Cirbpb as novel components of the Bb in zebrafish oocytes (Jamieson-Lucy et al., 2022). This growing knowledge of the constituents of the Bb will hopefully bring us closer to understanding the detailed mechanisms governing the initial formation and subsequent dynamic regulation of Bb during early oogenesis.

6 | RECRUITMENT OF OTHER COMPONENTS TO THE Bb/GERM PLASM

The synthesis and accumulation of RNA and other components in the germ plasm start early in *Xenopus* oogenesis. Before stage I, there are multiple mitochondrial aggregates around the oocyte nucleus (Heasman et al., 1984). Over time, the aggregate around the centriole becomes larger and more spherical, and ultimately becomes the Bb (Heasman et al., 1984; Kloc et al., 1996). In *Xenopus*, while some components like *nanos1* and *dazl* are recruited into the germ plasm at this point, other RNA like *velo1* and *xdnd1* make their way to the germ plasm, which is essentially small pieces of fragmented Bb, during later stages of oogenesis. In addition to germline regulators, somatic cell fate determinants, especially those important for germ layers and axis specification, are colocalized with the vegetally localized germ plasm (reviewed by Heasman, 2006; King & Zhou, 2004). Thus, Bb and germ plasm are important for the vegetal localization of crucial determinants for both germline and somatic cell fates.

Vegetal localization of *Xenopus* germline RNAs can be regulated by three mechanisms, all of which require Bb/germ plasm. Based on the timing of the vegetal localization, transportation of RNAs to the vegetal hemisphere of the oocyte can occur through an early, late, or intermediate pathway (summarized in Figure 3). Localization via the early pathway occurs during stages I and II of oogenesis. Within the Bb, RNAs are associated with a dense ER network. The accumulation of germ plasm RNAs that localize through the early pathway occurs via a "diffusion entrapment" mechanism, involving the initial dispersal of diffused RNA, which eventually becomes immobilized into the Bb (Chang et al., 2004). This pathway is largely cytoskeleton independent. These RNAs accumulate in the "METRO region" (MEssenger TRansport Organizer region) of the Bb in stage I oocyte, on the side facing the vegetal pole of the oocyte (Kloc & Etkin, 1995; Kloc et al., 1996). The RNAs in the METRO region have distinct spatial arrangements. For instance, *nanos1* RNA is associated with the germ granules and forms a ring, *xwnt11* localizes to the center, while *xlsirts*, a long noncoding RNA, is present throughout the METRO region (Kloc et al., 2002). By stage II, the Bb transports its components to the vegetal cortex. A large proportion of RNAs important for the establishment of the germline, including *pgat*, *nanos1*, *dazl*, and *ddx25* are localized through the METRO pathway (Houston et al., 1998; MacArthur et al., 2000; Wilk et al., 2005). While many of these RNAs encode RNA-binding proteins, *pgat* encodes a protein important for the structure of the germ plasm islands. Ectopic expression of Pgat in the animal pole was shown to induce mitochondrial aggregation in the oocytes but these aggregates were unable to recruit the germ plasm RNA (Machado et al., 2005).

While the early pathway transports RNAs to the vegetal cortex, the late pathway delivers RNAs to a broader region of the vegetal hemisphere. The late pathway RNAs are initially present throughout the stage I oocyte but excluded from the Bb. The localization of these RNAs to the vegetal cortex utilizes microtubules and occurs much later (stage III–IV). Some of the best-studied late pathway transcripts include germline RNAs such as *xdnd1* and *velo1* (Claußen & Pieler, 2004; Horvay et al., 2006), and somatic cell fate determinants like *vegT* and *gdf1*, which are essential for meso-endoderm specification (Joseph & Melton, 1998; Zhang et al., 1998). Notably, although Velo1 protein forms the matrix of the Bb, *velo1* RNA is excluded from the Bb in a stage I oocyte. The localization elements (LEs) for these late pathway RNAs exist in their 3′UTR and generally direct their movement to the vegetal cortex. Interestingly, RNAs like *fatvg* and *grip2* can employ both the early and late pathways for their localization. These RNAs are categorized under the "intermediate pathway" (Chan et al., 1999, 2001; Tarbashevich et al., 2007). Thus, there is an overlap to some degree between the early and late localization machinery. Of note, RNAs that generally accumulate into the Bb and utilize the early pathway for vegetal localization can also employ the late pathway machinery to be transported to the vegetal cortex when injected into late-stage oocytes (Arthur et al., 2009; Claußen et al., 2004). Houston (2013) presents an extensive review on RNA localization in oocytes.

Several proteins important for the vegetal translocation of RNAs have been identified and characterized. For example, Vera/Vg1RBP (Kwon et al., 2002) and Staufen protein isoforms, Stau1 and Stau2 have also been shown to play a major role in this process (Allison et al., 2004; Yoon & Mowry, 2004). The Elr-type proteins, the *Xenopus* homologs of Hu/Elav family proteins, can bind to LEs in RNA and drive their vegetal localization (Arthur et al., 2009). Additionally,

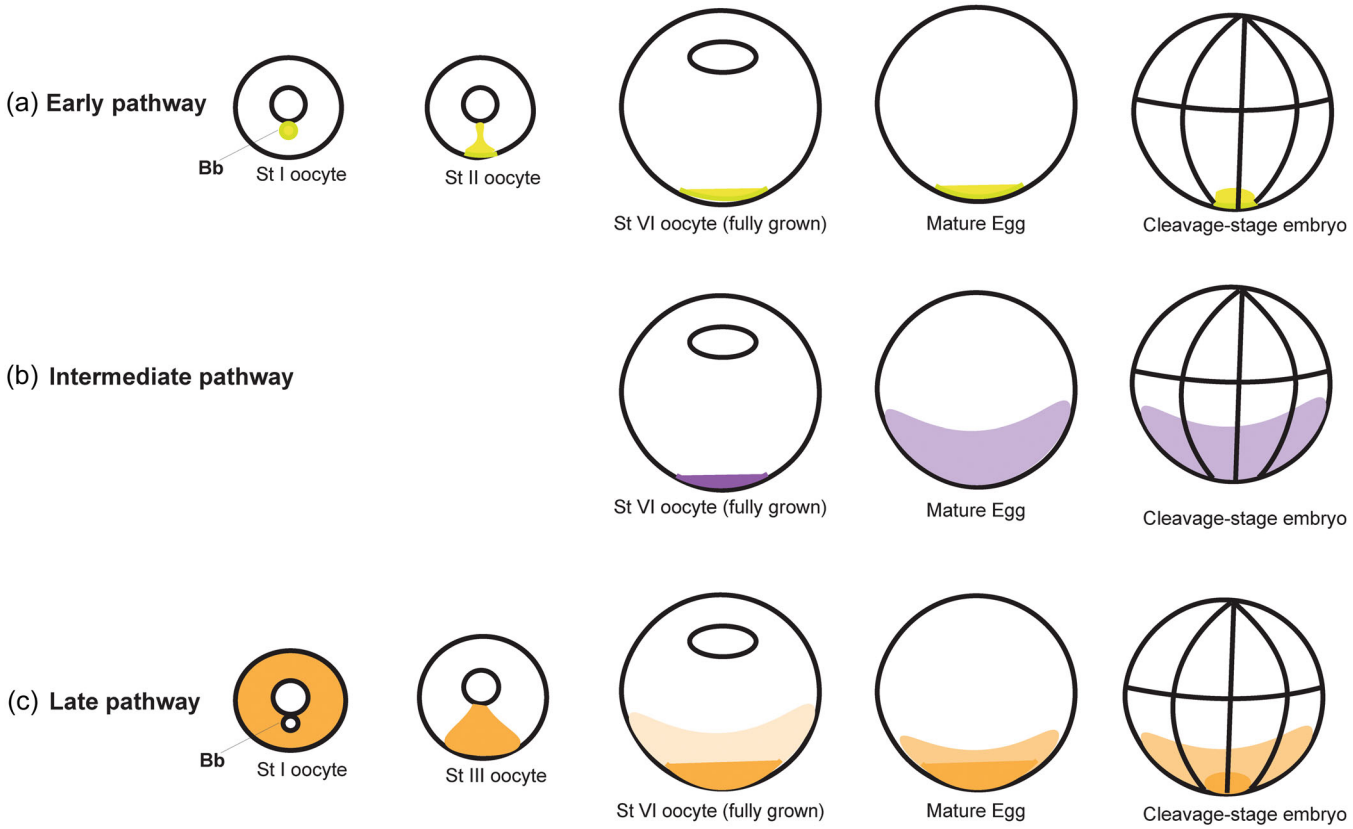

FIGURE 3 Schematic representation of the three pathways of RNA localization in *Xenopus* oocytes. (a) Early or METRO pathway utilizes the Bb to assemble and transport components like *nanos1* and *dazl* to the vegetal cortex (Houston et al., 1998; MacArthur et al., 2000; Wilk et al., 2023). (b) Intermediate pathway components like *fatvg* and *grip2* can be transported to the vegetal cortex utilizing both early and late pathway (Chan et al., 1999, 2001; Tarbashevich et al., 2007). (c) Late pathway components like *xdnd1*, *velo1*, *vegT*, and *gdf1* are transported to the vegetal cortex post-Bb disassembly (Clauβen & Pieler, 2004; Horvay et al., 2006; Joseph & Melton, 1998; Zhang et al., 1998).

42Sp50, an oocyte-specific isoform of EF1a can also interact with the RNA localization machinery involving Igf2bp3 (formerly known as Vg1RBP) and Staufen (Loeber et al., 2010). Celf1 is another component that colocalizes with other well-known localization components like Igf2bp3 and can interact with *dnd* LE (Bauermeister et al., 2015).

A recent study defined novel RNP granules, termed "localization bodies" (or L-bodies), which transport the late pathway RNAs, like *gdf1* to the vegetal cortex (Neil et al., 2021). The recruitment of RNAs into these condensates and their dynamics is mediated by multivalent interactions between the resident RNAs and the RBPs (Cabral et al., 2022). Another independent study suggests that the ATP provided by mitochondria from Bb remnants assists in the formation of such RNP granules (Yang et al., 2022). Once they reach the vegetal cortex, anchoring of RNA to the cortex ultimately requires F-actin (Kloc & Etkin, 1995; Yisraeli et al., 1990) and a network of CK (Alarcón & Elinson, 2001; Holowacz & Elinson, 1993). Strikingly, *vegt* and the long noncoding RNA *xlsirts*, were found to play a structural role in maintaining this CK cytoskeleton at the cortex (Heasman et al., 2001; Kloc et al., 2005). This arrangement is crucial for allowing proper localization of germ plasm and germline RNAs, and thus, for normal germline development.

In zebrafish, many germ plasm RNAs are localized to the Bb in stage I oocytes. However, after the fragmentation of the Bb, many germ plasm RNAs are dissociated from the germ plasm. In stage III oocytes, Buc protein aggregates are restricted to the vegetal cortex (Bontems et al., 2009; Riemer et al., 2015). Although *dazl* remains colocalized with these Buc aggregates in the vegetal cortex, germline RNAs such as *nanos*, *vasa*, and *buc* itself show different localization patterns (Bontems et al., 2009; Howley & Ho, 2000; Kosaka et al., 2007; Theusch et al., 2006). *Nanos* and *buc* localize toward the animal pole. *Vasa* localizes throughout the oocyte cortex initially but is enriched in the animal pole by the end of oocyte maturation. Depending on their location at the time of egg activation, zebrafish germ plasm RNAs can be divided into two categories (summarized in Figure 4). The RNAs like *buc*, *nanos*, *vasa*, *dnd*, and so forth which localize to the animal pole right after egg activation, are referred to as "animal germ plasm RNAs." The "vegetal germ plasm RNAs" are present at the vegetal pole upon activation. These RNAs need to be transported animally into the blastodisc through cytoplasmic streaming first and then be recruited into the germ plasm aggregates at the corners of cleavage furrow (Theusch et al., 2006). It is anticipated that the existence of distinct pathways ensures that the order in which RNAs enter the germ plasm aggregates can be maintained

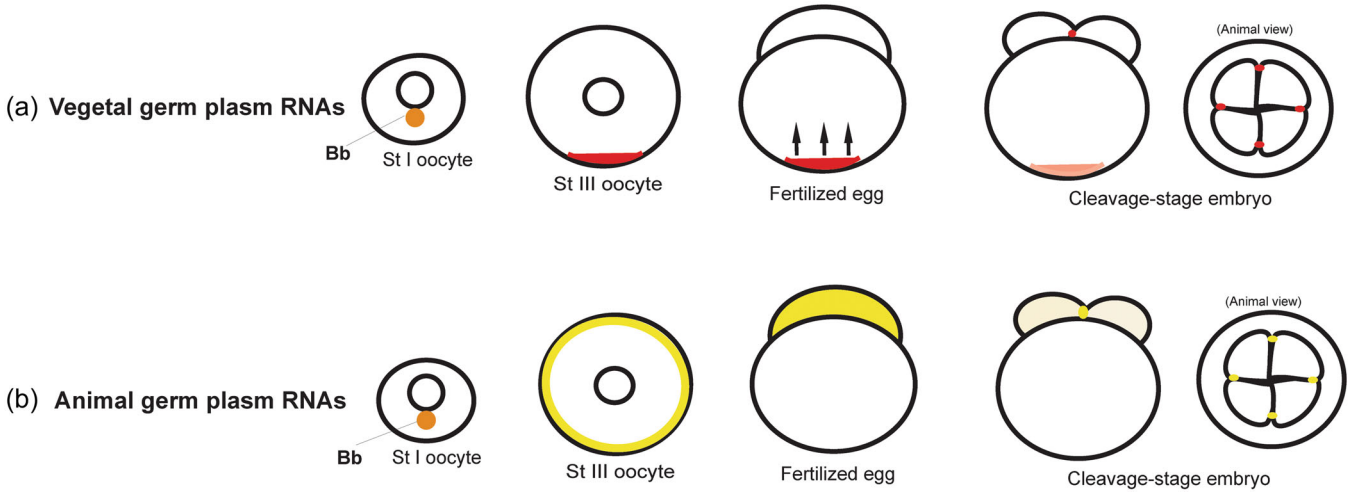

FIGURE 4 Schematic representation of the germ plasm RNA localization in zebrafish. (a) "Vegetal germ plasm RNAs" like *dazl* are localized at the vegetal pole at the time of egg activation and move animally (Kosaka et al., 2007; Theusch et al., 2006). (b) "Animal germ plasm RNAs" like *nanos1* and *vasa* are already localized at the animal hemisphere by the time of egg activation (Köprunner et al., 2001; Kosaka et al., 2007; Theusch et al., 2006; Yoon et al., 1997). Shown here is the localization pattern for *vasa* RNA.

(Moravec & Pelegri, 2020). Currently, the mechanisms by which some zebrafish germline RNAs detach transiently from Buc aggregates during mid-oogenesis remain unknown.

7 | REMODELING OF GERM PLASM DURING THE OOCYTE-TO-EMBRYO TRANSITION

It is well-known that excessive amounts of germline RNAs are synthesized during oogenesis. But only a fraction of them are needed for the specification of PGCs during early embryonic development. The remaining are degraded in somatic tissue to allow proper segregation of germline from the soma. Recent works have identified a germ plasm remodeling event during the oocyte-to-embryo transition, which facilitates the clearance of germline RNAs in soma during early embryonic development (Hwang et al., 2023).

In *Xenopus*, this germ plasm remodeling event occurs mainly during oocyte maturation. In fully-grown *Xenopus* oocytes, numerous germ plasm aggregates are distributed in a broad region in the vegetal cortex. After oocyte maturation, the majority of these aggregates are degraded. Only those localized at the tip of the vegetal pole remain. Due to the remodeling of the germ plasm, a large amount of germline RNAs are released into the cytoplasm. This remodeling event is mediated by the degradation of Velo1 protein (Hwang et al., 2023). In zebrafish, downregulation of Buc occurs during early embryonic development. While abundant Buc could be detected in the entire blastodisc right after fertilization, it declines rapidly during cleavage. By the 128-cell and dome stage, only four Buc protein aggregates remain in the embryo (Riemer et al., 2015). Consistently, zebrafish germline RNAs are released into the cytoplasm during early developmental stages.

When sequestered in the germ plasm, germline RNAs are protected from RNA degradation machinery (Hwang et al., 2023). Remodeling of the germ plasm during the oocyte-to-embryo transition thus increases the accessibility of germline RNAs to RNA degradation machinery, allowing effective turnover of germline RNAs in somatic tissues during early embryonic development. In zebrafish, overexpression of Buc, which forms ectopic germ plasm in somatic cells, prevents somatic clearance of germline RNAs, converting somatic cells into PGCs (Bontems et al., 2009; Ye et al., 2019). Currently, the molecular mechanisms responsible for the germ plasm remodeling during the oocyte-to-embryo transition remain unclear.

8 | CONCLUDING REMARKS

New findings through the years have continued to expand our understanding of germline development. Although maternal control of the germline specification has been the subject of investigation for decades, there are still major gaps in our current knowledge. The detailed mechanism behind the assembly and subsequent disassembly of the Bb remains unknown. Velo1/Buckyball is the only protein known to be indispensable for the formation of Bb. Similarly, besides Macf1, no other candidate proteins involved in aggregate disassembly have been identified. Through the course of development, the germ plasm undergoes massive reorganization. It is speculated that precisely regulated phase separation events are crucial for germ plasm dynamics (reviewed by So et al., 2021). The control of phase separation of germline P granules in *C. elegans* is certainly the most well-studied example in this context (Brangwynne et al., 2009; Putnam et al., 2019). In *Drosophila*, a recent study reported that the liquid-to-solid phase transition of the RNP granules of Oskar is crucial for germline development (Bose et al., 2022). The

Xenopus and zebrafish germ plasm matrix proteins, Velo1 and Buc also possess IDRs, which could contribute to the phase separation properties of the germ plasm in these species. The triggers for the change in phase separation behavior at different stages of the germline life cycle, and the components involved will be fascinating to uncover.

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DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no data sets were generated or analyzed during the current study.

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