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Review

Decoding zebrafish oogenesis: From primordial germ cell development to fertilization

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ABSTRACT

Oogenesis – the formation and development of an oocyte – is fundamental to reproduction and embryonic development. Due to its accessibility to genetic manipulations and the ability to culture and experimentally manipulate oocytes *ex vivo,* zebrafish has emerged as a powerful vertebrate model system for studying oogenesis. In this review, we provide a comprehensive overview of zebrafish oogenesis, from early germ cell formation to oocyte maturation and fertilization. We discuss recent advances in uncovering the molecular and cellular mechanisms driving this complex process and highlight key knowledge gaps that remain to be addressed.

1. Introduction

The development of an oocyte into a fertilization-competent egg is a complex and highly regulated biological process. For successful fertilization and embryogenesis the oocyte has to duplicate its genome, establish polarity, form a sperm entry point, transcribe and store maternal RNAs and accumulate all resources required for early embryonic development. A deeper understanding of oocyte biology is essential for advancing our knowledge of fertility, reproduction, embryology and evolution.

Zebrafish (*Danio rerio*) has emerged as a powerful vertebrate model organism for studying oogenesis. Its large oocytes, the high number of oocytes per fish and well-established genetic manipulation techniques make zebrafish well-suited for investigating the mechanisms underlying oocyte development. Zebrafish oogenesis involves a series of regulated developmental events, including initiation of meiosis, oocyte growth, establishment of polarity, formation of cortical and yolk granules and final maturation of the oocyte. In zebrafish, oogenesis is conventionally divided into five distinct stages (see Fig. 1), which will be described in detail in this review. Additionally, this review highlights recent insights into the molecular and cellular mechanisms that underlie successful oocyte development and its transition into early embryogenesis, and identifies current gaps in our understanding of these complex processes.

2. From primordial germ cells to oogonia: cyst formation

Oocytes differentiate from primordial germ cells (PGCs), which are specified by maternal inheritance of germ plasm in the developing zebrafish embryo. Germ plasm is a specialized cytoplasmic structure that contains all germline factors, e.g. *nanos* (*nos*), *vasa* (*vas*), and *dazl* (*dzl*) [1–3]. In the early embryo, germ plasm aggregates at the cleavage furrows. Eventually, a subset of blastomeres inherit all germ plasm factors, priming them to develop into PGCs. Subsequently, these PGCs migrate to the developing gonad, where they differentiate into oogonia, the precursor cells of oocytes. Oogonia proliferate, but do not complete cytokinesis. Therefore, sister oogonia remain interconnected through cytoplasmic bridges and form a cyst [4,5]. It has been observed that the centrosome in oogonia consistently positions near the cytoplasmic bridge, suggesting that its position is set by the orientation of the last mitotic division plane [6]. Since centrosome positioning is important for oocyte polarity (discussed in the next section), cytoplasmic bridges may serve as an important polarity cue. As a last step before entering meiosis, oogonia undergo a final round of DNA replication without subsequent cell division, resulting in a duplicated genome required for the following two meiotic divisions [7].

3. Stage l: primary growth stage

During stage l, oogonia enter meiosis. The oogonia cyst breaks down and oocytes continue developing individually. A large condensate, the Balbiani body - which stores organelles, RNAs and proteins - forms and translocates to the future vegetal pole [6–8]. Oocytes start to grow substantially, increasing their diameter more than tenfold. Furthermore, precursors of granulosa and theca cells begin to engulf the oocyte [5–9].

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The zona pellucida, a precursor to the chorion that will later surround the embryo, forms between the oocyte and the surrounding pre-granulosa cells.

3.1. Start of meiosis

While still in the cyst, oogonia enter meiosis and differentiate into primary growth stage (stage l) oocytes [5]. First, the chromosomes start to condense (leptotene stage). This is followed by the zygotene stage, when homologous chromosomes pair and form a chromosomal bouquet at the side of the oocyte nucleus where the centrosome is located [6]. This process is tightly regulated by a recently discovered microtubule-based structure called the zygotene cilium, which anchors the centrosome (see Fig. 2). In absence of the zygotene cilium, the centrosome is mislocalized, which leads to failure of chromosomal bouquet formation, eventually resulting in oocyte apoptosis. This suggests that precise centrosome alignment with the chromosomal bouquet is essential for oogenesis progression [10]. Through a mechanism which is still unclear, the oocyte nucleus then forms a cleft on the side where the chromosomal bouquet has formed [6]. The centrosome becomes positioned within the cleft and Balbiani body starts to condense around the centrosome (see Fig. 2) (discussed in section "Balbiani body formation") [11].

3.2. Cyst breakdown

After zygotene stage the cyst breaks down and the individual oocytes transition into the pachytene stage. During cyst breakdown the cytoplasmic bridges disassemble, allowing oocytes to continue developing individually [5]. The mechanisms triggering cytoplasmic bridge disassembly remain unclear. Possibly, key bridge-associated proteins are degraded by proteolytic enzymes and/or signaling pathways destabilize the bridges. In mammals, such as mice, only a subset of oogonia survives this process, the remaining oogonia apoptose. This has been shown to be dependent on asymmetric organelle inheritance, whereby only a subset of oogonia receives the organelles essential for continued progression through oogenesis [12]. Whether oogonia apoptose during cyst breakdown in zebrafish remains to be addressed. Furthermore, the signals that stop oogonial proliferation and the factors that regulate the timing of cyst breakdown are still unknown.

After cyst breakdown oocytes develop further as individual cells. This is characteristic of zebrafish, as in contrast, mouse oocytes still remain in clusters after cyst-breakdown and only separate at a later stage [13]. Zebrafish oocytes at this stage are fully transparent and arrest during prophase in the meiosis I, until hormonal cues trigger resumption of meiosis once the oocyte is fully grown [5].

3.3. Early oocyte growth

During stage l, oocytes gradually enlarge, increasing in diameter from approximately 10 to 140 μm. This growth occurs through expansion of the cytoplasm, in which the oocyte actively synthesizes and accumulates organelles, including ribosomes, mitochondria, and Golgi [5]. Additionally, there is a significant increase in synthesized RNAs and proteins, which the oocyte stores for later use in development. Likely water uptake through osmotic regulation also contributes to oocyte growth during stage I, although direct evidence for such mechanism is still lacking.

3.4. Balbiani body formation

A key feature of stage l oocytes is the formation of the Balbiani body, a membrane-less phase separated condensate of organelles (mitochondria, ER, Golgi), RNAs and proteins conserved across species (e.g. *Xenopus*, flies and humans) [14–16].

The Balbiani body is important for storage and transport of its cargos within the oocyte cytoplasm. In zebrafish, the Balbiani body is crucial for establishing oocyte polarity and specifying germ cells in the embryo, as it localizes maternal determinants involved in embryonic axis and germ cell specification [4,17]. Bucky ball (Buc) is an essential protein for Balbiani body formation, as its absence in mutant oocytes prevents the assembly of the structure [4]. Additionally, the *Xenopus* Buc homolog Xvelo1 has been shown to contain a prion domain at its N-terminus, which drives Balbiani body condensation [18].

Condensation of the Balbiani body starts while the oocytes are still in the cyst by Buc condensating around the centrosome within the nuclear cleft (see Fig. 2) [6]. In zebrafish, a recent study revealed that Balbiani body assembly is microtubule-dependent, with dynein motor proteins transporting Buc-containing granules to the nuclear cleft. These granules condense in the cleft and undergo a liquid-to-solid transition, eventually forming the mature Balbiani body after the cyst has broken down. Microtubules scaffold the mature Balbiani body in order to prevent its overgrowth [11]. Despite these novel insights, several key aspects regarding the formation of the Balbiani body are still unclear. One crucial open question is whether factors beyond Buc contribute to Balbiani body formation. Proteomic analyses have identified several Balbiani body components and Buc interacting proteins, some of which may contribute to Balbiani body condensation [11,19]. Notably, this includes proteins like Cirbpa and Cirbpb, which contain prion-like domains that may function similarly to those of Buc in triggering Balbiany body condensation [20]. However, functional data confirming their role in Balbiani body formation is still lacking. Furthermore, it remains unclear how the specific constituents of the Balbiani body are recruited and whether their recruitment occurs simultaneously or step-wise. Directly connected to this, it still needs to be investigated whether the Balbiani body is a homogeneous structure or possesses intrinsic prepatterning,

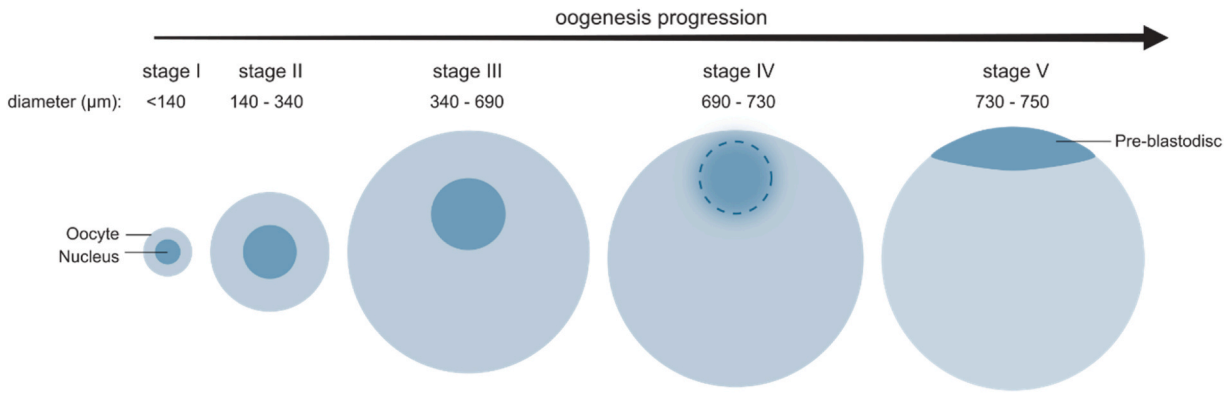

Fig. 1. Overview of zebrafish oogenesis. Zebrafish oogenesis is divided into five different stages.

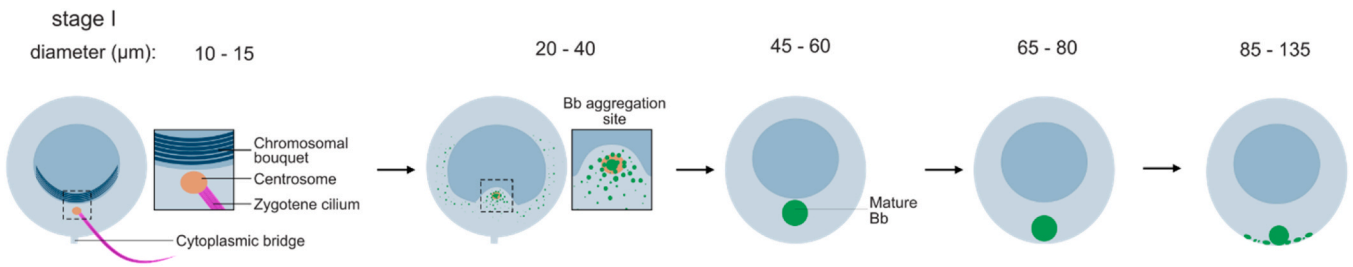

Fig. 2. Stage l. The chromosomal bouquet forms at the side of the nucleus where the centrosome is located. The centrosome is located close to the chromosomal bouquet and is anchored through the zygotene cilium. A nuclear cleft forms in which the Balbiani body (Bb) condensates. After the Balbiani body is fully formed, it translocates to the oocyte cortex.

which could be important for the later distribution of its constituents in the oocyte.

3.5. Balbiani body translocation to the vegetal pole

Once the Balbiani body is fully formed, it leaves its position at the nucleus and anchors to the oocyte cortex (see Fig. 2). One crucial factor for this translocation is *microtubule-actin crosslinking factor 1a* (*macf1a*). In *macf1a* mutant oocytes the Balbiani body fails to translocate to the cortex and instead stays attached to the nucleus [8]. Given that the Balbiani body is a large condensate, it likely requires precise active regulation to move to its correct position. It will be important to study in more detail the mechanisms by which *macf1a* and other regulators facilitate this transport. Once the Balbiani body reaches the cortex it starts to break down into fragments (see Fig. 2) (discussed in more detail in chapter "stage ll").

3.6. Start of follicle cell formation

Another essential aspect of stage I oogenesis is the formation of a pre-granulosa cell layer around the oocyte [5]. Pre-granulosa cells arise from somatic precursor cells. Around the pre-granulosa cells a basal lamina forms, shielding the oocyte from the outside. The mechanism for basal lamina formation remains unclear. Potentially, the proteins required for basal lamina formation are secreted by the pre-granulosa cells. The pre-granulosa cells will differentiate into granulosa cells, which communicate with the oocyte through gap junctions, enabling the exchange of nutrients and regulatory molecules that coordinate oocyte development [21,22]. Additionally, a zona pellucida forms between oocyte and pre-granulosa cells. The zona pellucida will later form the chorion that protects the developing embryo [5].

At the end of stage I, theca cell precursors differentiate from somatic cells and accumulate around the pre-granulosa cell and basal lamina layers, all of which together will give rise to the complete follicle surrounding the oocyte [9]. Granulosa and theca cells will be fully differentiated from their precursors at stage ll (see chapter "stage ll").

4. Stage ll: cortical alveolus stage

During stage ll, cortical alveoli, specialized vesicles containing glycoproteins, proteoglycans, and enzymes, are synthesized within the Golgi apparatus of the oocyte. Their main function is to prevent polyspermy during fertilization (discussed in more detail in the last chapter). Also, the zona pellucida, which will form the future chorion protecting the embryo, thickens. The follicle cells (granulosa and theca cells) become fully differentiated and surround the oocyte [5]. Balbiani body breaks down into fragments and spreads along the vegetal cortex [8]. At the animal pole, a micropyle precursor cell (MPC) is specified within the granulosa cell layer, which will later form the micropyle, a structure needed for sperm entry during fertilization [23]. Finally, while there is no yolk formation yet during stage II, Vitellogenin receptors start to get expressed at low levels at the oocyte plasma membrane, preparing the oocyte for yolk uptake and yolk granule (YG) formation during consecutive stages. During stage ll the oocyte grows further and reaches a diameter of around 340 µm [5].

4.1. Balbiani body break down and fragment spreading

After migrating to the oocyte cortex, the Balbiani body breaks down and spreads as fragments along the vegetal cortex (see Fig. 3). The breakdown of Balbiani body at the vegetal pole is essential for animal-vegetal (AV) axis establishment of the oocyte, as in *macf1a* mutant oocytes, where Balbiani body does not break down, mRNAs typically localized to the vegetal pole of the oocyte stay trapped within the intact Balbiani body [8]. The underlying mechanism of Balbiani body break down during stage ll is unknown. Changes in cytoskeletal dynamics, intrinsic phase separation mechanisms or mitochondrial activity might play a role, but direct supporting evidence for any such functions is still missing. Additionally, due to technical difficulties in long term culturing of oocytes, the temporal dynamics of Balbiani body break down are currently unknown. Interestingly, the Balbiani body has been shown to gradually fragment during a period where the oocyte nearly doubles in diameter [8]. It is thus conceivable that cortical actin filaments interacting with the Balbiani body pull it apart as the oocyte grows in size.

4.2. Completion of follicle cell formation

During stage ll the follicle cells (granulosa and theca cells) surrounding the oocytes become fully differentiated. Theca cells form the most outer layer around the oocyte (see Fig. 3). They are responsible for producing steroid hormones and provide structural integrity and mechanical support to the follicle [5,9]. How theca cells are recruited to the oocyte still needs to be investigated. It is possible that signals sent by granulosa cells are involved in theca cell attraction and differentiation. Furthermore, it remains unclear how theca cells are able to communicate with granulosa cells and thus with the oocyte. Potentially, they communicate via signals diffusing across the basal lamina separating theca and granulosa cells, yet evidence for such communication is still missing.

4.3. Micropyle precursor cell formation

At the end of stage ll, a group of granulosa cells at the animal pole of the oocyte becomes competent for forming the micropyle at late stages of oogenesis (see Fig. 3). The micropyle is a structure that allows sperm to pass through the chorion to fertilization the egg. It is crucial that only a single micropyle forms, as defects in oocyte polarity - as seen in mutants such as *buc* - can lead to the formation of multiple micropyles, resulting in polyspermy [4,24]. The establishment of a single micropyle is governed by a lateral inhibition mechanism, in which one granulosa cell at the animal pole, out of a group of several granulosa cells expressing early markers of micropyle precursor cells (MPCs), begins to

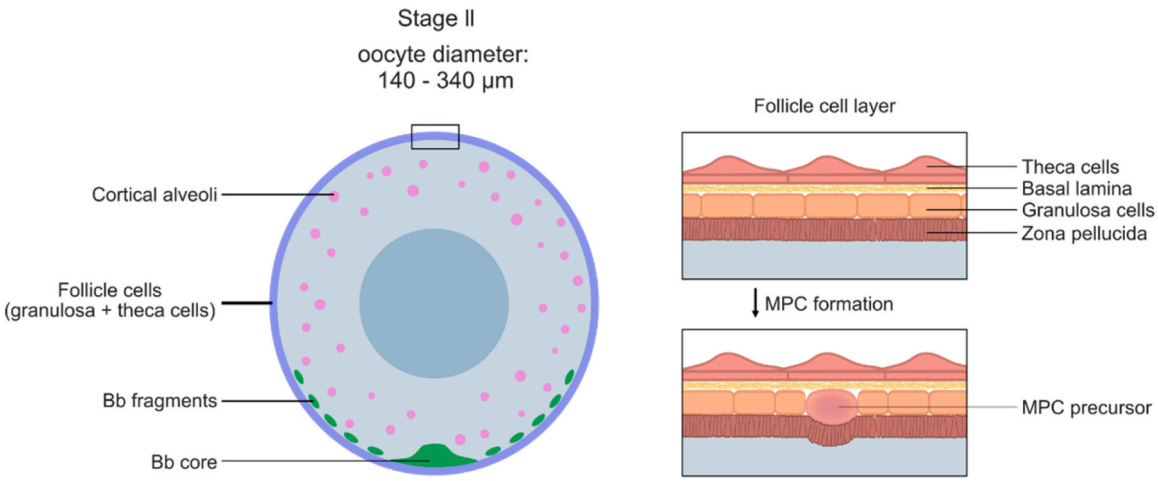

Fig. 3. Stage ll. Cortical alveoli form in the oocyte cytoplasm. Balbiani body breaks down into fragments at the vegetal pole. The micropyle precursor cell (MPC) forms in the granulosa cell layer at the animal pole.

grow more rapidly than its neighbors. This differential growth induces mechanical compression of the surrounding cells, preventing them from further differentiating into MPCs. This is achieved by the larger size of the faster growing micropyle precursor cell promoting the accumulation of the transcriptional co-activator TAZ in the cell nucleus, a key factor for micropyle precursor cell (MPC) differentiation [25], while the smaller size of the compressed neighbors leads reduces nuclear TAZ accumulation in these cells, preventing their MPC differentiation. This mechanical lateral inhibition process ensures that only one single MPC is formed at the animal pole, an essential process to prevent polyspermy during egg fertilization [23]. Why only cells at the animal pole become competent to adopt MPC fate is not yet clear. Likely, signals originating from the oocyte animal pole induce MPC precursor cell fates in the adjacent granulosa cell layer, but the nature of such signals still needs to be identified.

5. Stage lll: vitellogenesis stage

During the course of stage lll the oocyte grows further in size, reaching a diameter of approximately 690 µm at the end of stage III [5]. A hallmark of stage lll is the formation of yolk granules (YGs) in the oocyte cytoplasm [26]. Cortical alveoli differentiate into cortical granules (CGs), which preferentially locate closer to the oocyte cortex. Due to the accumulation of yolk and cortical granules the oocyte becomes opaque, losing its transparency from the earlier stages [5]. Balbiani body fragments remain located at the vegetal cortex, thereby retaining maternal RNAs, such as the germ plasm factor *dazl*, or dorsal axis specification factor, such as *wnt8a*, at the vegetal cortex. Additionally, the MPC initiates the formation of the micropyle, which channels sperm entry during fertilization [27].

5.1. Yolk granule formation

At stage lll, theca cells begin producing androgens, which diffuse into the granulosa cell layer [28,29]. Granulosa cells then convert these androgens into estrogens, which are critical for stimulating vitellogenin production in the liver [29]. Vitellogenin from the liver is transported via the blood stream and taken up by the oocyte through receptor-mediated endocytosis. Inside the oocyte, it is then enzymatically cleaved by cathepsins within endocytic vesicles, thereby producing yolk proteins such as lipovitellin and phosvitin. These vesicles then fuse with each other to form YGs, which eventually fill the majority of the oocyte's cytoplasm [30,31]. The YGs will later serve as an important energy and nutrient source for the developing embryo.

5.2. Micropyle formation

The MPC at the animal pole formed during stage ll, undergoes a drastic shape change, adopting a conical shape that faces the oocyte. It penetrates the zona pellucida, gradually forming a narrow canal into the oocyte. Upon completion of the canal, the MPC degenerates, resulting in the formation of a functional micropyle constituting the only entry point for sperm during fertilization (see Fig. 4) [27].

6. Stage IV - V: oocyte maturation

Stages IV and V mark the final stages of oogenesis. The oocyte exits prophase l arrest and resumes meiosis, completing its maturation into a fertilization-competent egg. The germinal vesicle (GV) migrates to the animal pole, where it undergoes germinal vesicle break-down (GVBD). The released nucleoplasm contributes to the formation of the pre-blastodisc at the animal pole, which serves as the precursor of the blastodisc, that will give rise to the embryo proper after fertilization. Yolk granules (YGs) fuse in the center of the oocyte, while CGs are transported to the oocyte cortex [32]. The fragments of the Balbiani body dissolve, releasing their contents into the ooplasm (unpublished observation). Additionally, yolk protein hydrolysis leads to accumulation of essential nutrients for embryonic development, and oocyte hydration increases egg buoyancy, an important adaptation for survival in aquatic environments [5,31]. During oocyte maturation meiosis I is completed, leading to extrusion of the first polar body [33].

6.1. Initiation of oocyte maturation

At the end of stage lll luteinizing hormone (LH) starts to bind to receptors on the granulosa cells, activating a signalling cascade leading to production of 17α,20β-dihydroxyprogesterone (DHP) from progesterone. DHP binds to receptors on the surface of the oocyte [34]. This hormonal signal triggers the translation of Cyclin B mRNA at the animal pole cortex [35,36], leading to activation of the maturation-promoting factor (MPF) - a complex of Cyclin B and CDK1, which shuttles into the GV [32], resulting into germinal vesicle break down (GVBD). Thereby, MPF initiates oocyte maturation and drives the progression to stage IV of oogenesis. As a result, the oocyte exits its prophase I arrest and resumes progression through meiosis [5].

6.2. Cytoplasmic reorganization in the oocyte

The first important morphological change during stage IV is the migration of the GV further to the animal pole [5]. Due to limitations in

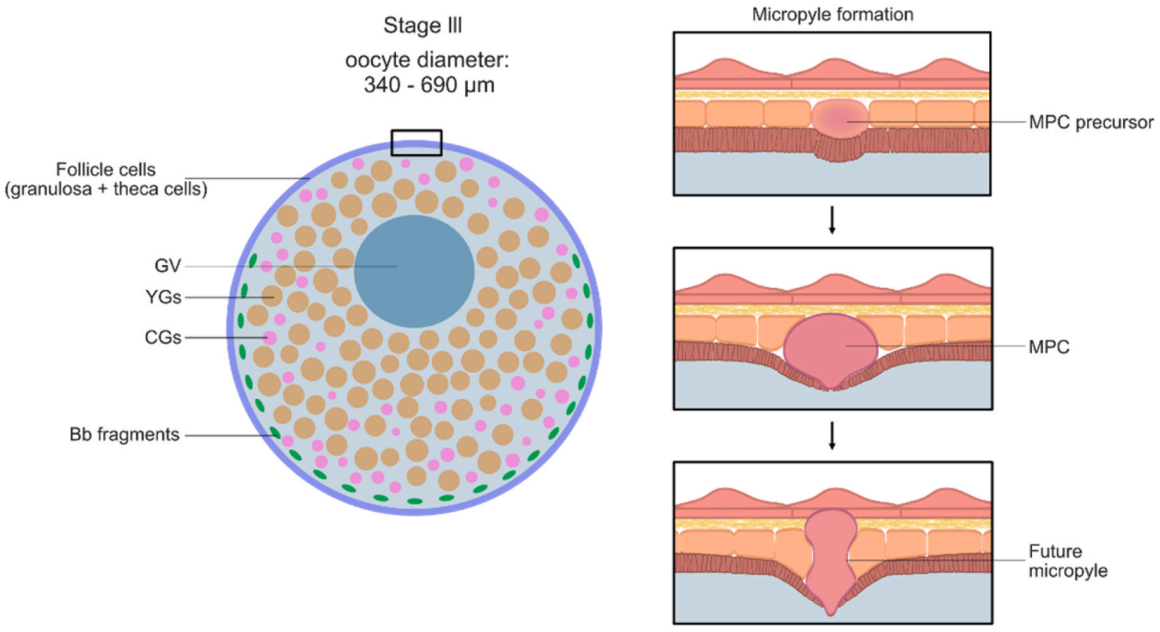

Fig. 4. Stage lll. Cytoplasm is filled with cortical granules (CGs) and yolk granules (YGs). Balbiani body is fully fragmented at the vegetal pole. The micropyle is formed by the MPC at the animal pole.

imaging deep into the oocyte, mechanistic details of GV migration are still lacking. At the animal pole the GV breaks down (GVBD) [5] and releases its nucleoplasm, containing the CDK1-Cyclin B complex, into the oocyte. The nucleoplasm accumulates at the animal pole, where it directly contributes to pre-blastodisc formation (see Fig. 5) [32]. Ooplasmic flows directed from the vegetal to the animal pole of the oocyte and generated by an bulk actin polymerization wave initiated at the animal pole, further drive the expansion of the pre-blastodisc. Simultaneously, YGs compact and fuse in the oocyte center, generating outwards directed cytoplasmic flows that transport CGs to the oocyte periphery. CycB-CDK1 released from the GV also triggers the reorganization of microtubules into asters, which transport Rab11 positive vesicles to the oocyte cortex. At the cortex, the Rab11 positive vesicles decorate CGs, thereby making them competent for undergoing exocytosis into the forming chorion once the egg is activated (egg activation discussed in more detail in the next chapter) (see Fig. 5) [32]. This mechanism of cortical granule exocytosis is distinct from that in mice, where CG exocytosis is mainly triggered by calcium waves and happens only after fertilization [37]. The reason for different mechanisms of CG exocytosis in mouse and zebrafish is unknown. Possibly, the missing YGs in mice led to the evolution of distinct developmental mechanisms. Additionally, the difference in timing of egg activation in zebrafish, which occurs when layed eggs get in contact with water before fertilization, and in mice, where egg activation is triggered by fertilization, might explain the difference in timing of CG exocytosis between these two organisms.

During stage lV, Balbiani body fragments dissolve, releasing Balbiani body constituents into the ooplasm (unpublished observation; note that this is a distinct event from Balbiani body breakdown described in section “stage ll”). Animal pole-directed ooplasmic flows (as described in the above paragraph) redistribute Balbiani body constituents in the oocyte, ensuring that e.g. germ plasm determinant Buc becomes relocalized towards the animal pole (unpublished observation). In contrast, dorsal axis specification factors within the Balbiani body, such as *wnt8a* or *syntabulin*, remain at the vegetal pole despite animally directed ooplasmic flows [28,29], suggesting that ooplasmic flows alone are not

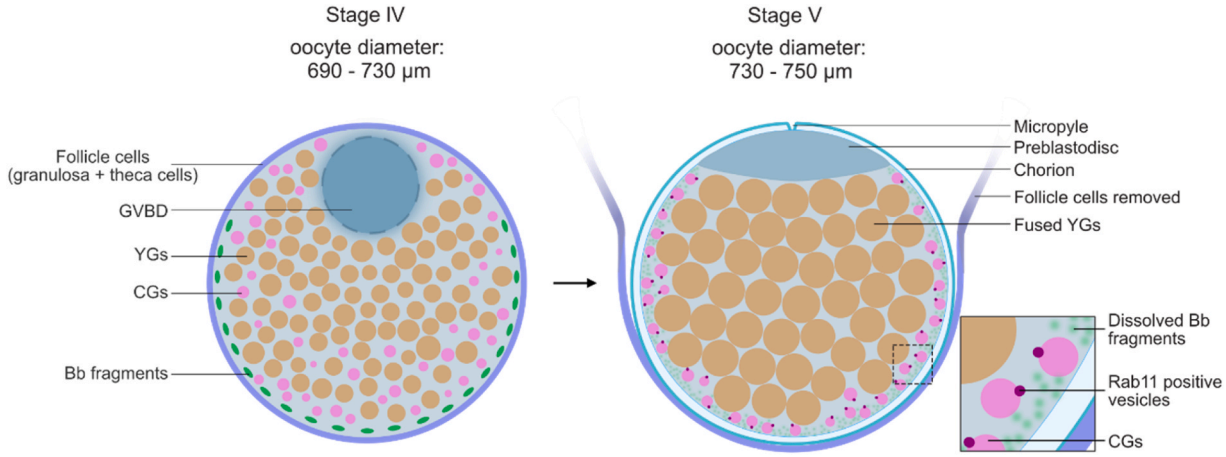

Fig. 5. Oocyte maturation. The germinal vesicle migrates to the animal pole and breaks down (GVBD). The released nucleoplasm contributes to pre-blastodisc formation. Yolk granules fuse in the center of the oocyte and push cortical granules to the periphery. To become competent for exocytosis cortical granules become decorated with Rab11 positive vesicles.

sufficient to explain the segregation pattern of all Balbiani body constituents at stage V. One potential mechanism by which some Balbiani body factors are retained at the vegetal pole is by specifically anchoring them to the cortical cytoskeleton at the vegetal pole, although direct evidence supporting this notion is still missing. Alternatively, ooplasmic flows might be too weak at the most vegetal region of the oocyte for efficiently transporting components to the animal pole [32]. Such a mechanism would require an unequal distribution of different factors within the Balbiani body fragments, something not yet clearly demonstrated.

6.3. Oocyte hydration and yolk protein hydrolysis

Further hallmarks of oocyte maturation during stage lV include oocyte hydration and yolk protein hydrolysis [5]. Vacuolar ATPase and chloride channels on the YG membrane facilitate acidification of the granule's interior and activation of cathepsin enzymes. These enzymes start to degrade yolk proteins, leading to a high concentration of free amino acids and polypeptides within the yolk granules, which get released into the cytoplasm [26,38]. Additionally, Na+ /K+ -ATPase pumps and pendrin transporters on the oocyte surface increase intracellular K+ and Cl- concentrations. The accumulation of these ions and free amino acids raises the osmotic pressure within the oocyte, which triggers water influx through aquaporin channels. This process, known as oocyte hydration, regulates egg buoyancy and supplies essential free amino acids and polypeptides for further embryonic development [39,40].

6.4. Follicle cell removal

After finishing maturation, the stage V oocyte arrests at Metaphase of meiosis II. Through a mechanism which is not fully understood, follicle cells surrounding the oocyte get removed and the mature oocyte (egg) is released into the ovarian lumen, where it becomes ready for fertilization [5].

7. Egg activation and fertilization

Once the mature oocyte (egg) reaches the ovarian lumen, the female can release it into the water through the genital pore during spawning. Contact of the egg with water triggers egg activation, leading to an increase in intracellular calcium, CG exocytosis, completion of meiosis, extrusion of the second polar body and formation of the haploid female pronucleus. In water, the egg is fertilized by sperm released by the male. Upon reaching the egg, sperm first encounters the chorion. Entry into the egg is only possible through the micropyle, as the chorion lacks any other openings (see Fig. 6), thereby preventing polyspermy. The sperm nucleus then de-condenses in the egg cytoplasm, giving rise to the haploid male pronucleus. The male and female pronuclei fuse to form the diploid zygote. After fertilization the chorion hardens and the micropyle becomes impermeable for sperm. A CDK1 wave triggers the formation of a second bulk actin polymerization wave travelling from the animal to the vegetal pole of the embryo [41]. This leads to an animal-to-vegetal actin gradient and flows of actin towards the animal pole. These actin flows preferentially drag ooplasm but not yolk granules towards the animal pole by exerting higher friction forces on the ooplasm than the yolk granules, thereby further expanding the blastodisc. Simultaneously, yolk granules get pushed down by actin comets [41]. Approximately 40 min after fertilization the embryo undergoes its first cell division. The cleavage furrow becomes the aggregation point of germplasm (see Fig. 6), which will be asymmetrically distributed to the future PGCs during subsequent cell cleavages.

8. Conclusion

Although significant advances have been made in understanding the transition of primordial germ cells (PGCs) into fertilization-competent eggs, several key questions remain unanswered. For example: What are the molecular mechanisms underlying cytoplasmic bridge formation and dissolution during the cyst stage? Do these cytoplasmic bridges play an instructive role in establishing early oocyte polarity along the animal–vegetal axis? How is the Balbiani body translocated to, and fragmented at, the vegetal cortex during stages I and II? What mechanisms drive the dissolution of Balbiani body fragments during oocyte maturation? How are granulosa and theca cells recruited to the oocyte? And what are the timeline and regulatory mechanisms governing oocyte growth?

Many of these open questions are challenging to address due to limitations in *ex vivo* oocyte culture systems. Current protocols allow *ex vivo* development only at early or late oocyte stages [42,43]. However, from late stage I through stage III, existing *ex vivo* culture conditions do not support continued oocyte development, precluding live imaging of these critical stages. Developing a robust system that enables real-time imaging of oocyte development across all stages will therefore be essential to elucidate the extensive cytoplasmic reorganization occurring during this period. One promising approach could be the implementation of *in vivo* imaging, which preserves the ovary in its natural physiological context.

Such an *in vivo* approach could also help determine the duration of individual steps and the overall timeline of oogenesis - currently unknown in zebrafish. Given that these processes likely span several days to weeks, the resources stored within the oocyte to support early embryonic development must be efficiently preserved over extended periods. While the Balbiani body offers a specialized means to store and

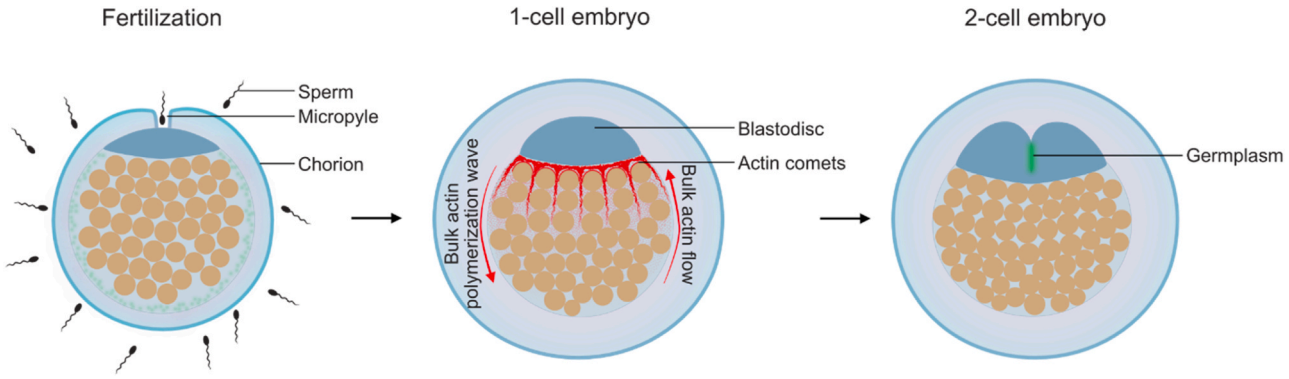

Fig. 6. Fertilization and onset of embryonic cleavages. Sperm enters the oocyte through the micropyle. After fertilization, the blastodisc expands through a combination of vegetally directed yolk granule pushing and animally directed cytoplasmic flows, which both originate from a bulk actin polymerization wave travelling from the animal to the vegetal pole of the egg. After the first cell division, the germ plasm aggregates and anchors to the cleavage furrow.

protect some of these components, others located outside of this structure must also be safeguarded, potentially through alternative preservation or storage compartments. For instance, in mammalian oocytes, many mRNAs are stored in a mitochondria-associated ribonucleoprotein domain (MARDO) located at the animal pole [44], although the existence of such a structure in zebrafish remains to be shown.

In addition to the oocyte itself, the role of the surrounding follicle cells in supporting and regulating oocyte development requires further investigation. A recent study in mice demonstrated that oocytes from aged females exhibited enhanced developmental potential when surrounded by follicle cells obtained from younger individuals, suggesting that follicle cell function declines with age [45]. Whether the quality of follicle cells decreases with age in zebrafish, and how follicle cells interact with each other and with the oocyte, remains to be explored.

In summary, oogenesis is a highly conserved process across metazoans, forming the foundation of reproduction and embryonic development. Unraveling the molecular principles that govern successful oocyte development is therefore of central interest to embryologists, as well as developmental, cell, and evolutionary biologists.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) used ChatGPT in order to improve language and readability of the text. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

Declaration of Competing Interest

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

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