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REVIEW

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Cellular dynamics of double fertilization and early embryogenesis in flowering plants

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Abstract

Flowering plants (angiosperms) perform a unique double fertilization in which two sperm cells fuse with two female gamete cells in the embryo sac to develop a seed. Furthermore, during land plant evolution, the mode of sexual reproduction has been modified dramatically from motile sperm in the early-diverging land plants, such as mosses and ferns as well as some gymnosperms (*Ginkgo* and cycads) to nonmotile sperm that are delivered to female gametes by the pollen tube in flowering plants. Recent studies have revealed the cellular dynamics and molecular mechanisms for the complex series of double fertilization processes and elucidated differences and similarities between animals and plants. Here, together with a brief comparison with animals, we review the current understanding of flowering plant zygote dynamics, covering from gamete nuclear migration, karyogamy, and polyspermy block, to zygotic genome activation as well as asymmetrical division of the zygote. Further analyses of the detailed molecular and cellular mechanisms of flowering plant fertilization should shed light on the evolution of the unique sexual reproduction of flowering plants.

KEYWORDS

asymmetric zygotic division, cytoskeleton, double fertilization, flowering plants, gamete nuclear migration, karyogamy, polyspermy block, zygotic genome activation

1 | INTRODUCTION

Sexual reproduction is accomplished by the mixture of genomes from parents resulting from fertilization, a fusion of female and male gametes that forms a zygote. In flowering plants, unlike animals, two nonmotile sperm cells are encapsulated in a pollen grain. Sperm cells are delivered through a tube extended from the pollen grain into an embryo sac (female gametophyte), which contains two female gamete cells, the egg cell, and central cell. The egg cell is haploid while the ploidy of the central cell is diverse (Baroux, Spillane, & Grossniklaus, 2002). In most flowering plants, including *Arabidopsis thaliana*, the embryo sac has dimorphic female gametes, a haploid egg cell (*n*), and a homodiploid central cell (2*n*; Figure 1a). Besides the female gamete cells, the embryo sac contains synergid cells that secrete chemical attractants that guide pollen tube growth toward the unfertilized female gametes (Figure 1b). The sperm cells fuse with the egg cell and central cell to develop an embryo (2*n*) and endosperm (3*n*), respectively, in a typical developing seed (Figure 1d). This distinctive process of flowering plants is called double fertilization (Kawashima & Berger, 2011). The endosperm nourishes the developing embryo at the early stage and either keeps nutrients for germination in monocots, such as rice and maize, or is absorbed before seed maturation in eudicots, such as soybean and *Arabidopsis* (Hands, Rabiger, & Koltunow, 2016).

The advances in the understanding of the double fertilization process and the regulatory mechanisms of early zygotic events in flowering plants have been achieved by recent progress in microscopy techniques with in vivo and in vitro fertilization systems.

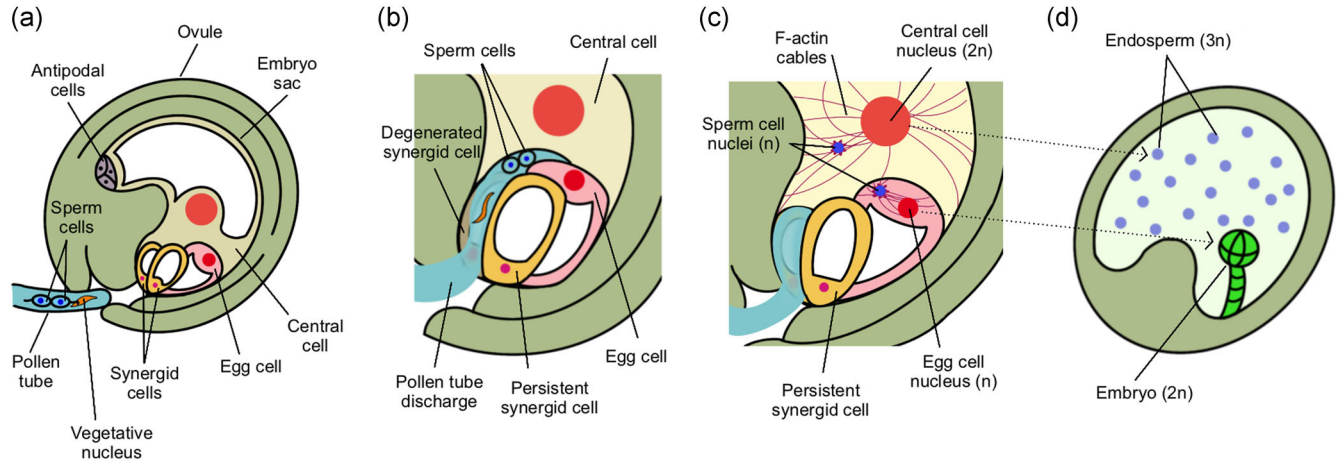

FIGURE 1 Schematic representation of double fertilization in *Arabidopsis*. (a) Two sperm cells are delivered into an embryo sac via a pollen tube, which is attracted by chemical cues secreted from synergid cells. (b) The pollen tube bursts and releases two sperm cells, which are subsequently located between the plasma membranes of the egg cell (*n*) and central cell (2*n*), leading to plasmogamy. One of the synergid cells receives the pollen tube contents and degenerates. (c) After plasmogamy, one sperm nucleus each migrates toward the egg cell nucleus and central cell nucleus by the constant F-actin inward movement, followed by karyogamy. Assembly of an F-actin aster around the migrating sperm nucleus is apparent in the *Arabidopsis* central cell, and it remains to be determined in the egg cell. Successful double fertilization triggers degeneration of the persistent synergid cell, which can terminate pollen tube attraction. (d) The fertilized egg cell and central cell generate the embryo (2*n*) and endosperm (3*n*), respectively, in a developing seed [Color figure can be viewed at wileyonlinelibrary.com]

For successful fertilization in flowering plants, the sperm cells adhere to the egg cell and central cell, and the plasma membrane fusion of female and male gametes (plasmogamy) is followed by gamete nuclear migration and gamete nuclear fusion (karyogamy). In animals, maternal gene transcripts already deposited in the egg cell are utilized to support early embryogenesis, followed by de novo transcription from the zygotic genome through minor and major zygotic genome activation (ZGA; Schulz & Harrison, 2019). Unlike in animals, fertilization in flowering plants triggers an immediate maternal-to-zygotic transition (MZT), promoting the zygote elongation necessary for asymmetric cell division with distinct cell fates. Recent understanding of flowering plant fertilization mechanisms from pollen tube guidance to plasmogamy has been well summarized (Sprunck, 2020); here, we provide an update with recent advances in knowledge of flowering plant fertilization mechanisms after plasmogamy, from gamete nuclear migration to first division of the zygote, and we compare those processes in flowering plants and animals.

2 | GAMETE NUCLEAR MIGRATION

After gamete fusion in the fertilized egg, pronuclei/nuclei of the female and male gametes migrate toward each other, before the fusion of female and male gamete nuclei (Fatema, Ali, Hu, Clark, & Kawashima, 2019). Animal cells contain centrioles constituting the centrosome that serves as the microtubule-organizing center. In many mammalian species, the spermatozoal centrosomes (pericentriolar materials dispersed in ooplasm in rodents) form the microtubule sperm asters around the sperm pronucleus in the fertilized egg. Microtubule bundles extending from the sperm asters interact with the egg pronucleus, drawing it toward the sperm pronucleus for the completion of fertilization (Hochi, 2016). In contrast to the essential role of microtubules in pronuclear migration, treatment with inhibitors for filamentous actin (F-actin), another cytoskeleton component, does not disturb pronuclear migration in most animals with few exceptions (Fatema et al., 2019).

Unlike animals, flowering plants lack centrosomes (Carvalho-Santos, Azimzadeh, Pereira-Leal, & Bettencourt-Dias, 2011) and evolved F-actin-based sperm nuclear migration (Kawashima, Maruyama, et al., 2014; Ohnishi, Hoshino, & Okamoto, 2014; Peng, Yan, & Sun, 2017). Both the egg cell and central cell generate a constant F-actin active inward movement from the plasma membrane periphery to the center of the cell where the nucleus resides (Figures 1c and 2a). This F-actin inward movement is already taking place in the female gamete even before sperm cell delivery by the pollen tube, in preparation for rapid sperm nuclear migration right after plasmogamy. In *Arabidopsis*, a sperm nucleus is released into the central cell, becomes surrounded by F-actin meshwork and is transferred to the central cell nucleus by an inward moving F-actin (Figure 1c; Kawashima & Berger, 2015; Kawashima, Maruyama, et al., 2014). How F-actin is assembled and its movement is facilitated for sperm nuclear migration during double fertilization remain largely unknown. Whether de novo actin polymerization is initiated around the sperm nucleus or pre-existing F-actin in the female cytoplasm adheres to the sperm nucleus needs to be determined.

3 | KARYOGAMY

Like in yeast (*Saccharomyces cerevisiae*) karyogamy, gametes of sea urchins and sea stars undergo fusion of pronuclear envelopes,

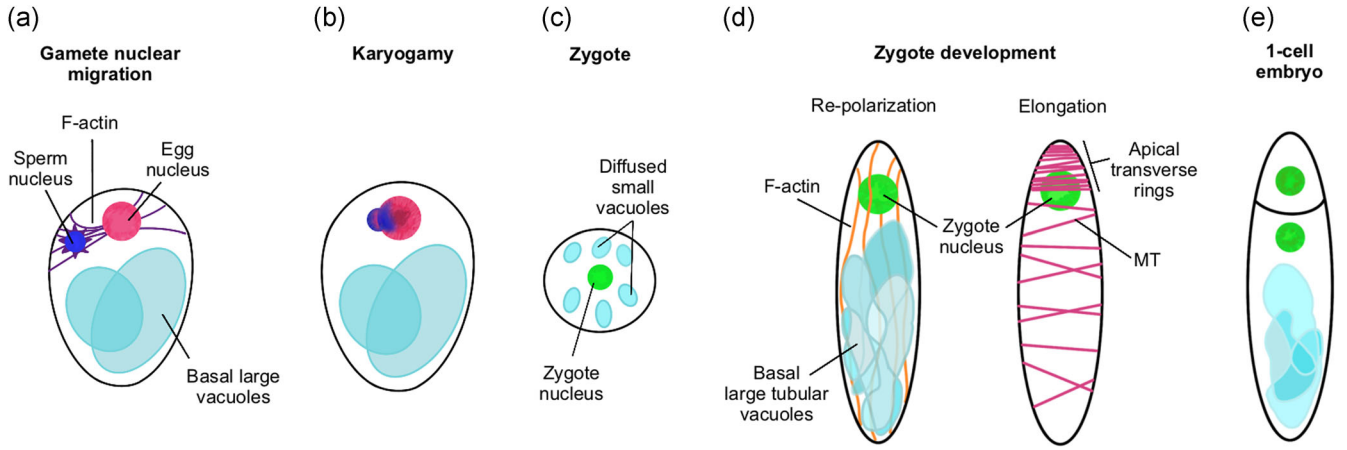

FIGURE 2 Schematic diagrams showing dynamics of the *Arabidopsis* zygote. (a) The mature egg cell has a polarity with the nucleus (shown in pink) in the apical position and large vacuoles in the basal position. After plasmogamy, Ca^{2+} is transiently increased in the fertilized egg cytoplasm, and the sperm nucleus (shown in blue) moves toward the egg nucleus by F-actin active inward movement. In the in vitro-fertilized egg cell in rice and maize, the cell wall is formed immediately after fertilization. (b) While nuclear membranes of the egg and sperm nuclei are fusing, sperm chromatin decondensation is rapidly occurring, and maternal and paternal genomes blend. (c) After karyogamy, maternal transcripts inherited from the egg cell are rapidly degraded, and then transcripts are synthesized de novo from the zygotic genome (zygotic genome activation; ZGA). The maternal factor clearance, maternal-to-zygotic transition, and ZGA occur in the zygote nucleus (shown in green). Fertilization triggers zygote cell shrinkage and the zygote loses its polarity with disassembly of the large vacuoles and the zygote nucleus returning to the center of the cell. (d) The zygote is repolarized and elongates; the nucleus moves to an apical location and large vacuoles reorganize at the basal end. Longitudinal F-actin bundles are arranged along the apical–basal axis. Large vacuoles accumulate at the basal region and form tubular strands along the F-actin. F-actin cables promote the formation of tubular vacuoles in the perinuclear region so the F-actin-dependent polar vacuole distribution results in migration of the zygote nucleus toward the apical region (left). At the same time as the events in (d, left), microtubules (MT) form subapical transverse rings, promoting zygote elongation (right). (e) The mature zygote asymmetrically divides into two daughter cells with distinct cell fates, the one-cell embryo proper and basal cell, which develops into the suspensor [Color figure can be viewed at wileyonlinelibrary.com]

composed of two lipid bilayers, the outer and inner nuclear membranes. *Ascaris*, mammals, and most arthropods including insects, on the contrary, exhibit different modes of parental genome fusion (Combelles & Rawe, 2013; Gibeaux & Knop, 2013; Loppin, Dubruille, & Horard, 2015; Poccia & Collas, 1996). In *Ascaris* and most mammals, pronuclei remain separate until the initiation of the first zygotic mitosis. The parental chromosomes are blended at the metaphase plate. Pronuclei of most arthropods attach to each other but the pronuclear envelopes do not fuse and the parental chromosomes do not blend until the end of the first zygotic mitosis (Kawamura, 2001; Longo, 1973; Loppin et al., 2015; Poccia & Collas, 1996).

In flowering plants, the fusion of both nuclear envelope membranes between male and female gamete nuclei occurs in the zygote right after fertilization (Figure 2b; Dresselhaus, Sprunck, & Wessel, 2016; Mori, Igawa, Tamiya, Miyagishima, & Berger, 2014). The first mitosis of the endosperm (fertilized central cell) in *Arabidopsis* occurs within a few hours after plasmogamy (Boisnard-Lorig et al., 2001), and therefore rapid decondensation of sperm chromatin must happen in the fertilized central cell. The constant F-actin inward movement for sperm nuclear migration, even before plasmogamy, in the female gametes likely prepares for the rapid completion of karyogamy including sperm decondensation (Kawashima, 2020).

Using in vitro-fertilized rice zygotes, Ohnishi et al. (2014) discovered that female-derived histones, labeled by a fluorescent marker, start to accumulate in the condensed sperm chromatin before the completion of karyogamy when the inner nuclear membrane of the sperm nuclear envelope appears to remain intact. In *Arabidopsis*, inner membrane fusion defective mutants show successful decondensation of the sperm chromatin in the central cell (Maruyama, Higashiyama, Endo, & Nishikawa, 2019). In contrast, outer membrane fusion defective mutants cannot decondense the sperm chromatin, causing a seed development failure (Maruyama et al., 2019; Portereiko et al., 2006). These results suggest that histone exchange in the sperm chromatin can occur before the completion of karyogamy and that sperm decondensation is the key for the successful onset of mitosis and the subsequent seed development. It still remains unclear whether histones in the egg nucleus directly move to the sperm nucleus for histone exchange or histones in the zygote cytoplasm are transported to the sperm nucleus.

An *Arabidopsis* mutant of mitochondrial ribosomal protein exhibits a defective fusion of the outer nuclear membranes of polar nuclei forming the central cell nucleus as well as failure of karyogamy in both the egg cell and the central cell, suggesting that ATP synthesis is important to nuclear membrane fusion (Portereiko et al., 2006). GAMETE EXPRESSED 1 (GEX1) in the unicellular green alga, *Chlamydomonas reinhardtii*, and yeast is localized in the nuclear envelope during sexual reproduction and plays an important role in karyogamy (Ning et al., 2013). In *Chlamydomonas*, the *gex1* mutant gametes adhere

to each other successfully but their nuclear fusion is strongly inhibited. In *Arabidopsis*, *GEX1* is expressed in both the embryo sac and pollen grain and is involved in female and male gametophyte development (Alandete-Saez, Ron, Leiboff, & McCormick, 2011). However, the GEX1 function for karyogamy in *Arabidopsis* is still not clear, and further experiments are awaited.

4 | POLYSPERMY BLOCK

In animals and fucoid algae, polyspermy (fertilization of the egg by multiple sperm) causes zygote (embryo) lethality by multipolar or supernumerary mitotic spindles in the zygote due to transmission of extra centrioles from multiple sperm, resulting in aberrant nuclear and cell division (Nagasato, Motomura, & Ichimura, 1999; Navara, First, & Schatten, 1994; Santelices, 2002; Schuel, 1984). To restrict the number of sperm simultaneously approaching the egg plasma membrane, animals have the egg's extracellular coats such as the jelly layers and vitelline envelope in amphibians, mollusks, and crustaceans; zona pellucida in mammals; and chorion in teleosts (Iwao & Izaki, 2018; Wong & Wessel, 2006). The number of sperm reaching the egg membrane is dramatically reduced by the physical barriers of these extracellular coats; however, many sperm still possibly arrive at the egg simultaneously (Gardner & Evans, 2006; Iwao & Izaki, 2018; Wong & Wessel, 2006). Therefore, fast blocking of additional sperm entry (polyspermy block) is achieved in many animals by an increase in Ca^{2+} in the egg cytoplasm. The intracellular Ca^{2+} increase acts as a signal, resulting, for instance, in dephosphorylation of mitogen-activated protein kinase (MAPK). This inhibits extra sperm–egg fusions and sperm attraction in jellyfish and, in monospermic frogs, induces a reversal of electrical properties between the interior and exterior of the egg membrane, blocking additional sperm entry by activating an efflux of Cl^- (Arakawa, Takeda, Tachibana, & Deguchi, 2014; Iwao & Izaki, 2018; Watabe et al., 2019).

In contrast, polyspermy at fertilization does occur in some animals such as birds, newts, and salamanders, and, especially in birds, polyspermy is necessary for normal embryo development (Hemmings & Birkhead, 2015; Iwao, Kimoto, Fujimoto, Suda, & Hara, 2019). In urodele amphibians including newts and salamanders, a principle sperm pronucleus forms prominent sperm asters, enabling organized pronuclear migration to produce a zygote nucleus (Iwao et al., 2019). Other accessory sperm nuclei form smaller asters, which do not act as functional sperm asters for pronuclear migration, and they are removed by chromatin pyknosis and centrosome degradation.

In contrast to animals, flowering plants have lost the centrioles (Carvalho-Santos et al., 2011), and polyploidization is a common phenomenon mainly caused by cell cycle defects which can result in somatic doubling or unreduced gametes (Blanc & Wolfe, 2004; Tekleyohans & Groß-Hardt, 2019; Wendel, 2000). Does polyspermy occur in flowering plants and can it contribute to polyploidization? In vitro fertilization experiments in maize and rice eggs can mimic polyspermy events and the triploid embryos form viable plants (Kranz & Lörz, 1993; Toda, Ohnishi, & Okamoto, 2016). In planta, two sperm cells delivered by the pollen tube are simultaneously released into the ovule, yet one sperm cell fuses with the egg cell and the other with the central cell for double fertilization (Huang, Ju, Wang, Zhang, & Sodmergen, 2015; Igawa, Yanagawa, Miyagishima, & Mori, 2013). This suggests that flowering plants possess a mechanism to prevent two sperm cells from fusing to one female gamete cell; however, flowering plant polyspermy does occur in nature and is accomplished by multiple pollen tubes leading into the embryo sac (polytubey; Beale, Leydon, & Johnson, 2012; Kasahara et al., 2012). Grossniklaus (2017) carried out a polyspermy/polytubey experiment in maize using a mixture of pollens from two genetically distinct male parents which convey different pigmented phenotypic patterns to their endosperm offspring. A mixture of two pigmented patterns in the endosperm indicates polyspermy in the central cell. The polyspermy frequency of the central cell is much higher than that of the egg cell in maize (Grossniklaus, 2017) and the results are consistent with a study in *Arabidopsis* (Scott, Armstrong, Doughty, & Spielman, 2008). These results indicate that the polyspermy block is likely weaker in the central cell compared with the egg cell. It is possible that the difference in the level of polyspermy block between the egg cell and central cell may contribute to one sperm cell with one female gamete cell fusion event in simultaneous double fertilization, and further work should clarify the biological significance of the difference of the polyspermy block levels. Nevertheless, polyspermy events in both female gamete cells are extremely rare in nature (Grossniklaus, 2017; Nakel et al., 2017), raising the question of whether flowering plants indeed possess a highly stringent polyspermy block in the egg cell and/or a functional polytubey block mechanism to minimize such events.

In *Arabidopsis*, a first transient Ca^{2+} rise in the egg cell occurs at pollen tube rupture for sperm cell release. A second transient Ca^{2+} rise in the fertilized egg cell at plasmogamy has also been observed (Denninger et al., 2014; Hamamura et al., 2014). It is still not clear, however, whether these Ca^{2+} influxes play a role in signaling, leading to polyspermy block and/or activation of other reproductive processes such as polytubey block in the fertilized egg cell. The cell wall in flowering plants can also be a physical barrier for polyspermy block. The egg cell in flowering plants does not generate an obvious cell wall, and the release of cell wall material to initiate cell wall formation starts 30 s after plasmogamy in maize, followed by the deposition of cell wall around the whole surface 20 min after plasmogamy (Kranz, Wiegen, & Lörz, 1995). In vitro polyspermic rice zygotes are efficiently obtained when the second in vitro fertilization process is carried out within 10 min of the first egg–sperm fusion, but are hardly observed 20 min after the first fusion (Toda et al., 2016), suggesting that cell wall formation may contribute to polyspermy block.

While there are possible polyspermy block mechanisms in flowering plants, these blocks are not as vigorous as those in animals. However, polyspermy remains very rare in flowering plants, and this is likely due to polytubey block. In flowering plant double fertilization, two synergid cells, which lie adjacent to the egg cell and central cell, secrete small peptide chemical attractants to guide pollen tube

growth and assist the delivery of two sperm cells into the embryo sac (Figure 1a–c; Higashiyama, 2002; Márton, Cordts, Broadhvest, & Dresselhaus, 2005; Okuda et al., 2009). With unknown mechanisms of pollen tube–pollen tube repulsion, preventing additional pollen tubes from invading (Shimizu & Okada, 2000), flowering plants achieve the lowest mating ratio of male to female gametes (1:1 sperm to egg and central cells) at fertilization, lower than those of animals (Spielman & Scott, 2008). Furthermore, successful fertilization triggers the degeneration of synergid cells, resulting in the termination of pollen tube attraction (Maruyama et al., 2015; Völz, Heydlauff, Ripper, von Lyncker, & Groß-Hardt, 2013). Although it seems that a polyspermy barrier is not strictly required in flowering plants, how exactly Ca^{2+} influx in the fertilized egg cell, cell wall formation right after plasmogamy, and low pollen tube to embryo sac ratio affect the rate of polyspermy is currently unclear. Further molecular and cellular dissections of the polyspermy block system in flowering plants, including the investigation of the consequence of polyspermy in triparental plant lines, might reveal the evolutionary reason for the polyspermy rate being kept low even though polyspermy-derived plants are viable.

5 | ZYGOTIC GENOME ACTIVATION

After completion of the fertilization process, animal zygotes undergo rapid cell divisions supported by maternal factors stored in the egg cell, followed by minor ZGA with clearance of the maternal transcripts in the developing embryo. Major ZGA then occurs to complete the transition from maternal control to de novo transcripts expressed from the zygotic genome (MZT; Kawashima & Berger, 2014; Lee, Bonneau, & Giraldez, 2014). In land plants, fertilization itself gives rise to transition from the gametophytic haploid life phase to the sporophytic diploid life phase, a clear shift of developmental control from haploid-to-diploid genomes (Gilbert, 2000). However, until recently, it was unclear how flowering plants undergo MZT and ZGA after fertilization. Zhao et al. (2019) used genetically distinct geographic varieties of *Arabidopsis*, known as ecotypes, as maternal or paternal lines to distinguish which of the zygotic transcripts are from the maternal or paternal genome by identifying ecotype-specific single nucleotide polymorphisms. Transcriptome analyses of the egg cells, spherical zygotes, elongated zygotes, one-cell embryos, and 32-cell embryos discovered a significant reduction of maternally inherited transcripts in the zygote after fertilization, showing that plant MZT starts with rapid clearance of maternal transcripts in the zygote shortly after fertilization. Furthermore, ZGA takes place in the zygote before the first cell division (Zhao et al., 2019). ZGA shortly after fertilization is also evident by the rapid accumulation of RNAPII Ser2P (phosphorylated serine 2 of the carboxy-terminal domain of RNA polymerase II) in the zygote nucleus, which marks active transcription, compared with the unfertilized egg cell in *Arabidopsis* (Kao & Nodine, 2019). Transcriptome analysis in maize and rice also showed that ZGA takes place shortly after fertilization, revealing that the timing of ZGA in the zygote is similar among flowering plants (Anderson et al., 2017; Chen et al., 2017; Zhao et al., 2019).

In the *Arabidopsis* zygote after karyogamy, egg-derived histone H3 variants are actively removed and rapidly replaced with de novo synthesized H3 (Ingouff et al., 2010). The analysis of three-dimensional genome structures of rice egg cells, sperm cells, and zygotes by chromatin conformation capture (3C) and high-throughput 3C (Hi-C) also provides evidence of active chromatin reorganization by fertilization (Zhou, Jiang, Zhao, & Zhou, 2019). Interestingly, the ectopic expression of sperm-specific gene, BABY BOOM 1 (BBM1), a member of the plant-specific APETALA2 transcription factor family, in the egg cell can initiate rice embryo development without fertilization (Khanday, Skinner, Yang, Mercier, & Sundaresan, 2019). This result is consistent with paternal gene activation being essential for the initiation of embryo development in flowering plants and BBM1 is one of the paternal factors that are expressed immediately after fertilization. How exactly sperm chromatin decondensation and chromatin reorganization play their roles in rapid ZGA in the flowering plant zygote will be the next questions to be addressed.

Reduced length of the reproductive phase, such as decreased time between flower maturation and fertilization, has evolved in the flowering plants, increasing seed production under seasonally deteriorating environments (Hackenberg & Twell, 2019; Snell & Aarssen, 2005). Rapid ZGA might also positively contribute to the adaption to short lifecycles by assigning embryo proper and suspensor cell fates immediately after the first division of the zygote (ten Hove, Lu, & Weijers, 2015). However, the biological significance of the immediate ZGA in flowering plants compared with the "delayed" ZGA like in animals is still largely unknown. It would be interesting to know when immediate maternal factor clearance and ZGA in the zygote were acquired during land plant evolution.

6 | ASYMMETRIC DIVISION OF THE ZYGOTE

The formation of the body axis is one of the first developmental events in offspring resulting from successful fertilization in multicellular eukaryotes. Oocytes and unfertilized eggs in most animals show a clear cell polarity, but the body axis is changed by the site of sperm entry (Houston, 2017). In flowering plants, the mature egg cell also has polarity; however, different species have different sperm cell adhesion site positions relative to the axis of the embryo sac, and whether the zygotic polarization is inherited from the egg cell or is determined after fertilization remains unknown (Hamamura et al., 2011; Mansfield & Briarty, 1991; Mansfield, Briarty, & Erni, 1991; Olson & Cass, 1981). In the *Arabidopsis* mature egg cell, the nucleus is at the apical position and large vacuoles occupy the basal region (Figures 1a–c and 2a,b). After fertilization, the zygote volume is remarkably reduced, the vacuoles are evenly distributed, and the position of the zygote nucleus is in the center of the cell (Figure 2c). Zygote elongation from the apical side then follows together with repolarization, which is marked by the migration of the

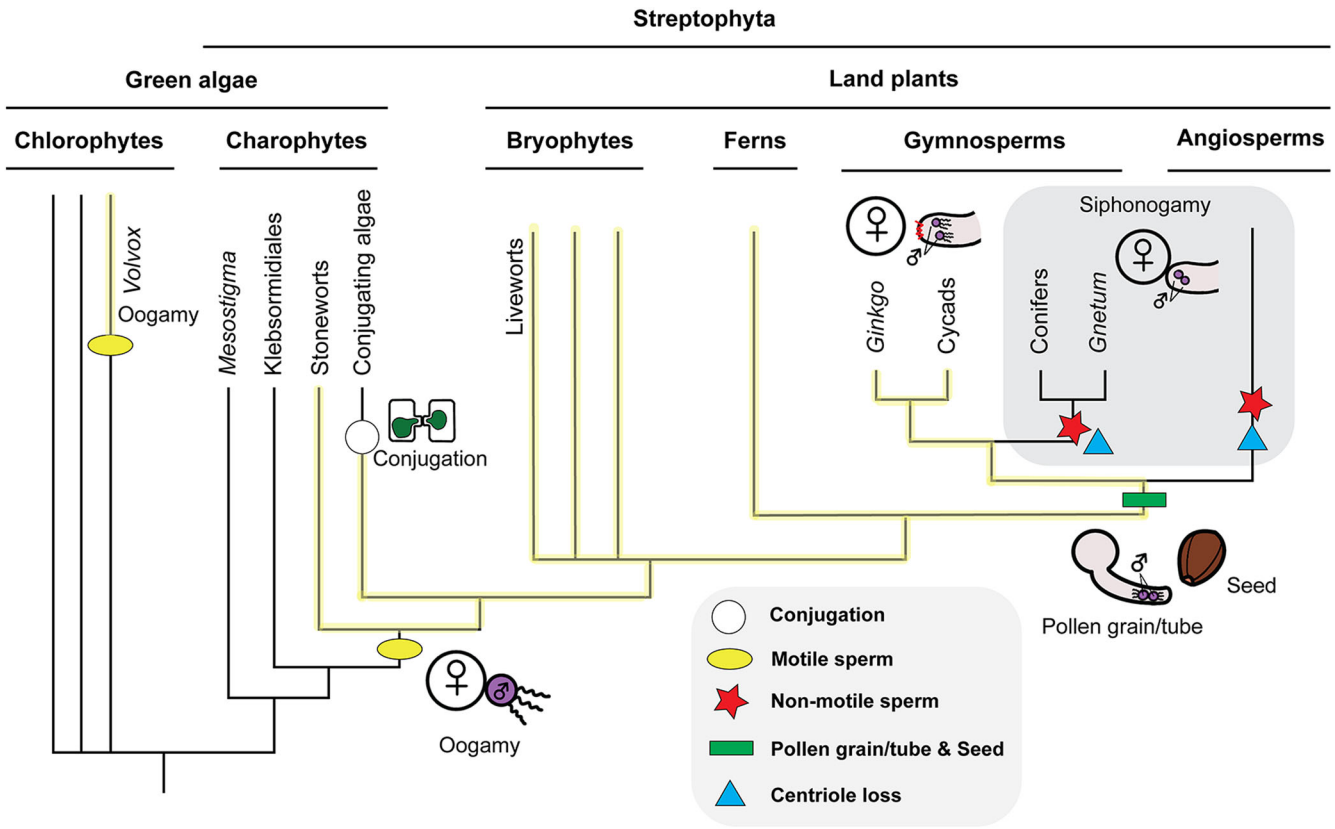

FIGURE 3 A phylogeny of green plants. Sperm motility evolved first in an ancestor of the freshwater green algae Charophyceae (stoneworts). Conjugating algae, a sister group of the land plants, have lost sperm motility and reproduce via conjugation. The pollen grain/tube was acquired by an ancestor of the gymnosperms and angiosperms (flowering plants). Centrioles and sperm motility have been lost in the angiosperms and a part of the gymnosperms (i.e., conifers and *Gnetum**). Other gymnosperms (i.e., *Ginkgo* and cycads) retain sperm motility. The white circle (conjugation), yellow ellipse (motile sperm), red star (nonmotile sperm), green rectangle (pollen grain/tube and seed), and blue triangle (centriole loss) on the phylogenetic tree branch indicate the appearance of each characteristic during evolutionary divergence. *Confirmation of centriole loss in *Gnetum* is awaited [Color figure can be viewed at wileyonlinelibrary.com]

nucleus toward the apical part and reformation of large and tubular vacuoles at the basal region (Figure 2d). Subsequently, the zygote divides asymmetrically into a smaller apical cell and a larger vacuolated basal cell with distinct cell fates, leading to the embryo proper and suspensor, respectively (Figure 2e). Live-imaging analysis revealed that both cytoskeleton and vacuole dynamics lead directional zygote elongation and polar nuclear migration coordinately and determine the plane of the first asymmetric division in the zygote (Figure 2d; Kimata et al., 2016, 2019). Like vacuoles, both microtubules and F-actin become disorganized right after fertilization and are subsequently rearranged differently in the elongating zygote to support directional elongation and nuclear migration toward the apical tip, respectively (Kimata et al., 2016). The activation of WUSCHEL HOMEOBOX 8 (WOX8), a homeodomain transcription factor, in the *Arabidopsis* zygote is essential for asymmetric zygotic division (Breuninger, Rikirsch, Hermann, Ueda, & Laux, 2008; Ueda et al. 2017; Ueda, Zhang, & Laux, 2011). *WOX8* is directly upregulated by maternally inherited transcription factors HOMEODOMAIN GLABROUS 11/12 (HDG11/12) and biparentally derived plant-specific transcription factor WRKY2. Antecedently, the function of WRKY2 as a transcription factor is activated via phosphorylation by the YODA (YDA) MAPK signaling cascade (Lukowitz, Roeder, Parmenter, & Somerville, 2004). YDA is a MAPKK kinase and is activated in the zygote by the Pelle/interleukin-1 receptor (IL-1R)-associated kinase (IRAK)-like kinase SHORT SUSPENSOR (SSP). The *SSP* gene transcripts are delivered to the zygote from the sperm after fertilization and translated into SSP proteins (Bayer et al., 2009). Together with the central cell-derived peptide EMBRYO SURROUNDING FACTOR1, SSP activates the YDA signaling cascade by yet to be discovered mechanisms (Costa et al., 2014). ZGA is not only involved in the activation of the aforementioned genes, ZGA itself is also required for both zygote elongation and asymmetric division (Zhao et al., 2019), and further analyses will reveal which genes among those activated during ZGA are responsible for the initiation of repolarization and the direction of zygote elongation.

7 | CONCLUDING REMARKS

During land plant evolution from green algae to bryophytes and flowering plants, drastic changes in the mode of sexual plant reproduction occurred (Figure 3). One example is sperm

differentiation. Early-diverging green algae of the land plant lineage (e.g., *Mesostigma* and *Klebsormidium*) do not differentiate sperm (McCourt, Delwiche, & Karol, 2004), By contrast, stoneworts (Charophyceae) produce motile sperm and the neofunctionalized MYB domain transcription factor DUO1 was recently identified as the key regulatory factor for sperm differentiation in the land plant lineage (Higo et al., 2018; Hisanaga et al., 2019). In land plants, from bryophytes to some gymnosperms (i.e., *Ginkgo* and cycads), sperm motility has been retained (Figure 3). Other gymnosperms (i.e., conifers and *Gnetum*) and flowering plants have lost centrioles and sperm motility (Southworth & Cresti, 1997). Interestingly, both gymnosperms and flowering plants generate the pollen grain/tube, yet it is not clear how these traits (i.e., centriole loss, sperm motility, and acquisition of the pollen grain/tube) are linked to each other and evolved during seed plant evolution (Hackenberg & Twell, 2019). Nevertheless, the pollen grain/tube allowed plant fertilization to become completely independent from water as is now seen in flowering plants (siphonogamy; Figure 3). *Ginkgo* and cycad gymnosperms generate motile sperm with pollen grain/tube (Hackenberg & Twell, 2019), and these species possibly represent the transition of the mode of sexual reproduction in seed-bearing plants.

Centrioles are essential not only for flagella formation as basal bodies, but also for microtubule-based sperm nuclear migration. Interestingly, in early-diverging land plants, such as the liverwort, *Marchantia polymorpha*, blepharoplasts consisting of centrioles appear only in the sperm mother cells (Carothers & Kreitner, 1968). The absence of centrioles in somatic cells of the early-diverging land plants indicates that land plant cells were already capable of centriole-independent cellular dynamics. Although it is not still clear, this systematic change might have enabled and/or accelerated the shift from microtubule-based to F-actin-based gamete nuclear migration as well as the complete loss of centrioles in flowering plants. The biological significance of the complete loss of centrioles in flowering plants remains unknown. The investigation of sperm nuclear migration in gymnosperms will provide us with further insights into the evolution of the mode of sexual reproduction in land plants.

Cytological investigations of the female gametophyte and seed in early-diverging flowering plants have shed light on the evolution of flowering plant sexual reproduction (Baroux & Grossniklaus, 2019; Friedman & Williams, 2004; Gasser & Skinner, 2019). The genomes of freshwater green algae (Charophytes), the relatives of land plants, have been sequenced and compared with those of land plants, highlighting the genetical origin of the adaptions to the terrestrial environment of ancient land plants (Hori et al., 2014; Nishiyama et al., 2018). Together with these findings, the integration of the identified mechanisms of molecular and cellular dynamics at fertilization and genome and transcriptomic data from a range of land plants should provide further insights into the evolution of sexual reproduction of land plants such as the shift from motile to nonmotile sperm, centriole loss, gamete nuclear migration, double fertilization, and MZT and ZGA in the zygote.

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