Male gametogenesis in flowering plants

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The life cycles of plants are characterized by significant alternations between the diploid sporophytic and the haploid gametophytic generations. Meiosis and fertilization are the prerequisites for achieving the alternation of generations. Diploid sporophytes undergo meiosis to produce haploid gametes, and male−female gametes fuse (double fertilization) to revert to the diploid sporophyte. The formation and development of male gametophytes are crucial for the alternation of generations in higher plants. During the long evolution of plants from aquatic to terrestrial, the way of sexual reproduction has undergone subversive innovations. From sperm swimming for fertilization to relying on the precise delivery of pollen tubes to female gametes for the fusion of the male−female gametes, higher requirements are placed on the male gametophytes' development and fertilization process. The formation of male gametophytes has undergone significant changes to meet the needs for delivery and transportation of the male gametes. With the emergence of more and more evidence, the molecular mechanism underlying male gametophytes' development, especially the initiation and specialization of germline cells has been better understood. Focusing on the latest studies, we reviewed and elucidated the critical proteins and factors involved in male gametophyte formation, highlighting the decisive role of auxin in archesporial cell specialization and the importance of microspore maturation in pre-mitosis, and analyzed the molecular mechanisms underlying male gametogenesis, with a view to providing insights for further exploration of male gametophytes formation in the future.

KEYWORDS

male gametogenesis, meiosis, microgametogenesis, microsporogenesis, mitosis

Highlights

- We reviewed the latest advance in male gametophyte formation, highlighting the vital discovery of auxin in the specialization of archesporial cells and the importance of microspore maturation for microgametogenesis.

1 Introduction

The formation of viable pollen grains is crucial for the double fertilization of angiosperms. In flowering plants, the ontogeny of anther, especially the formation of male gametophytes, is tightly governed, and the processes are highly conservative and programmed. The understanding of male gametophyte development in most angiosperms

on genetic and molecular knowledge comes from the model plants *Arabidopsis thaliana* (Sanders et al., 1999), *Oryza sativa* (rice) (Zhang et al., 2011), and *Zea mays* (maize) (van der Linde and Walbot, 2019). Male gametophyte development refers to a series of programmed developmental processes, including the differentiation of male germ cells, the development of microspores, and the formation of male gametophytes. The development of male gametophytes can be divided into two phases according to the morphological changes of tissue and cells. In *Arabidopsis*, during phase I, composed of flowering stages 1–7, the anther primordium differentiates and divides into archesporial cells to establish the anther morphology (Sanders et al., 1999). The microspore mother cells undergo meiosis to give rise to haploid microspores. During phase II, containing stages 8–14, the anther grows and extends upward through the filaments. Free microspores undergo two rounds of mitosis to produce mature male gametophytes, and anthers dehisce to release pollen grains. About 70% of angiosperms undergo only one mitosis to form bicellular pollen when their pollen grains are released. In the growing pollen tubes, the reproductive cells undergo a second mitosis to form two sperm cells (Brewbaker, 1967). In the anther ontogeny, the specification of pre-meiotic cell fate in germ cells and the differentiation of somatic cell layers surrounding archesporial cells, are essential for male gametocyte formation and development. The formation of male gametophytes is a complicated process that has been accurately monitored in the spatiotemporal window. They undergo two consecutive processes: microsporogenesis and microgametogenesis. Microsporogenesis is the differentiation and development of archesporial cells into microsporocytes. Subsequently, meiosis occurs, and the microsporocyte gives rise to haploid microspores within the microsporangia (or pollen sac). Depending on the species, microgametogenesis refers to the process during which the microspore undergoes one or two mitotic divisions to develop into bicellular or tri-cellular pollen (Brewbaker, 1967).

We will review these two phases of pollen formation, focusing on discoveries in the formation of male gametophytes. Pollen grains that land on the stigma germinate to generate pollen tubes that grow in the pistil. The pollen tube carries two sperm cells and grows toward the ovule, where it fuses with two female gametes to complete double fertilization. Frequent cell–cell communication between the male (pollen or pollen tube) and the female tissues occurs at various stages, such as pollen adhesion and recognition, pollen germination, pollen tube elongation, and fertilization (Yu et al., 2022). Dresselhaus and Franklin-Tong (2013) gave a comprehensive overview (reviewed in Mol Plant), and we would not mention these sections.

2 The male gametophyte formation

2.1 Microsporogenesis

The formation of the male gametophyte begins with the differentiation of stamen primordium generated by the floral meristem. The pollen development pattern of *Arabidopsis* is shown in Figure 1 (Sanders et al., 1999). At stage 1, the anther primordium derived from the division and differentiation of the floral meristem generally contains three cell layers, from outside to inside, the outer epidermis layer (L1), the middle layer (L2), and the inner layer (L3). At stage 2, cell division and proliferation lead to an increase in each

layer of cells and expansion of anther primordia. Subsequentially, L1 cells differentiate and form the epidermis layer. L2 cells rapidly divide and develop into archesporial cells and L2-derived cells at four corners of the anther, where four anther lobes will be formed subsequently. In the center of each anther lobes, archesporial cells are surrounded by the somatic cell layer differentiated by L2-derived cells. L3 cells divide and give rise to vascular and connective tissue. At stage 3, archesporial cells undergo periclinal division to produce the outer primary parietal cells and inner primary sporogenous cells, which belong to germline cells. At stage 4, the periclinal division of primary parietal cells gives rise to outer endothecium and inner secondary parietal cells. The secondary parietal cells divide to form the primary tapetum and the middle layer. The primary sporogenous cells divide periclinally again to generate the secondary sporogenous cells. At stage 5, after the formation of the anther walls, the secondary sporogenous cells divide continuously and differentiate into many microsporocytes (microspore mother cells), filling the entire interior of the microsporangium. The diploid microspore mother cells are interconnected, with a prominent feature of a large nucleus located at the center. The four anther lobes each comprise five-layer cells in a pre-meiotic anther. The cells are microspore mother cells, tapetum, middle layer, endothecium, and epidermis from inside to outside. At stage 6, callose deposition occurs to isolate microsporocytes and the microspore mother cells initiate the process of meiosis to produce four haploid microspores embedded by a unique callose envelope. The tapetum undergoes endomitosis and the cytoplasm becomes dense. The volume of the locules becomes large, the middle layer becomes crushed and degenerated, and the cytoplasm of endothecium and epidermal cells almost disappears. The tapetum is tightly associated with the anther locule (Quilichini et al., 2014). At stage 7, after meiosis is completed, and tetrads of microspores are surrounded and separated by the callose wall. The tapetal cells become two-nucleate as a result of endomitosis, with numerous expanded vacuoles (Quilichini et al., 2014). Remnants of the middle layer are present. At stage 8, the surface of the microspore membrane becomes undulating, and the development of primexine initiates between the callose layer and the microspore membrane. The callose surrounding the microspores is degraded by the callase enzyme synthesized and secreted by the tapetal cells. The precursors of sporopollenin secreted by the tapetal cells accumulate at the top of the undulated microspore membrane, forming the bacula and tectum. Subsequently, the microspores are released from the tetrads (Sanders et al., 1999). The exine of microspores has been attached with sporopollenin, lipase, and glycine-rich proteins. Up to the early tricellular stage of pollen development, a large amount of liquid fills the space surrounding the microspore instead of the space between the tapetum and the microspore, as previously thought (Quilichini et al., 2014).

2.2 Regulation of microsporogenesis

2.2.1 Initiation of anther primordium and specialization of archesporial cells

In angiosperms, the development of male gametophytes is uniform and synchronized before the tetrad stage. The sporophyte portion of the stamen primordia, including four anther locules, the central vascular bundle or connective tissue, and the anther wall, presents a butterfly-shaped feature in the transverse section. The four
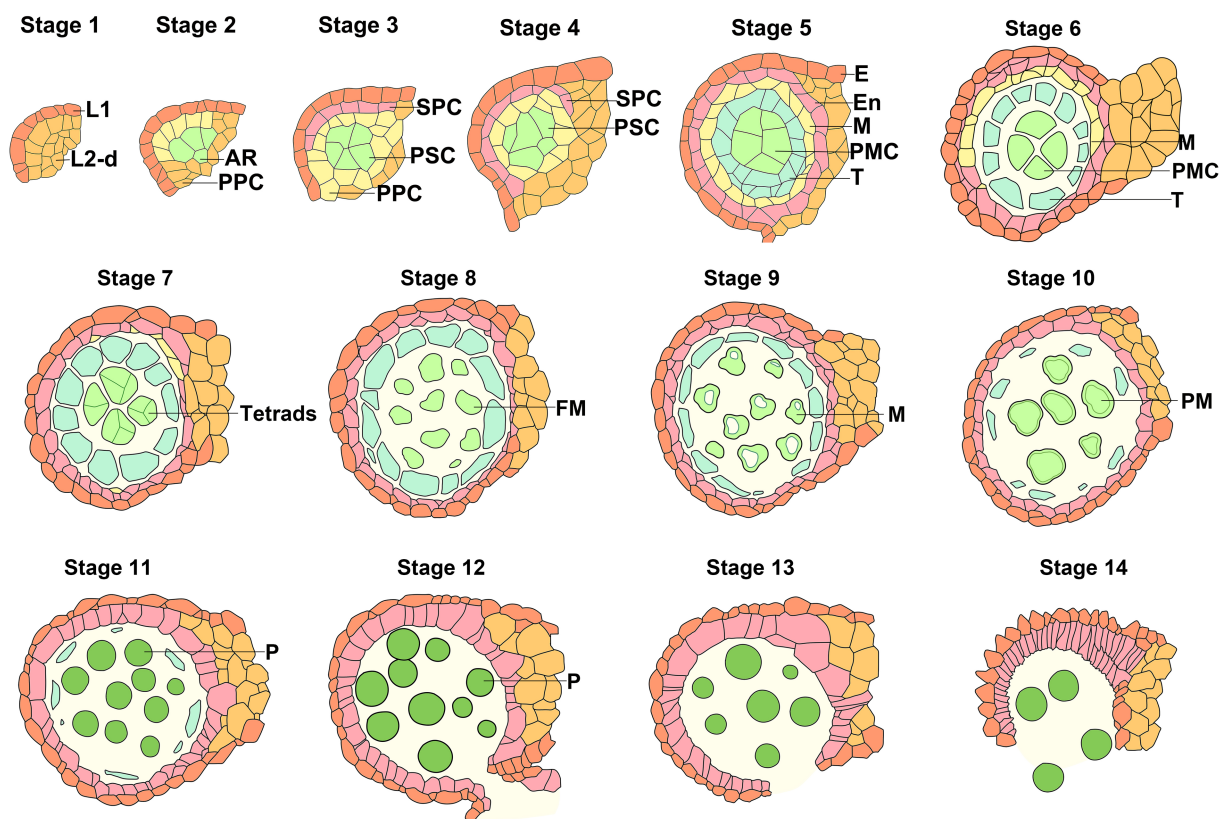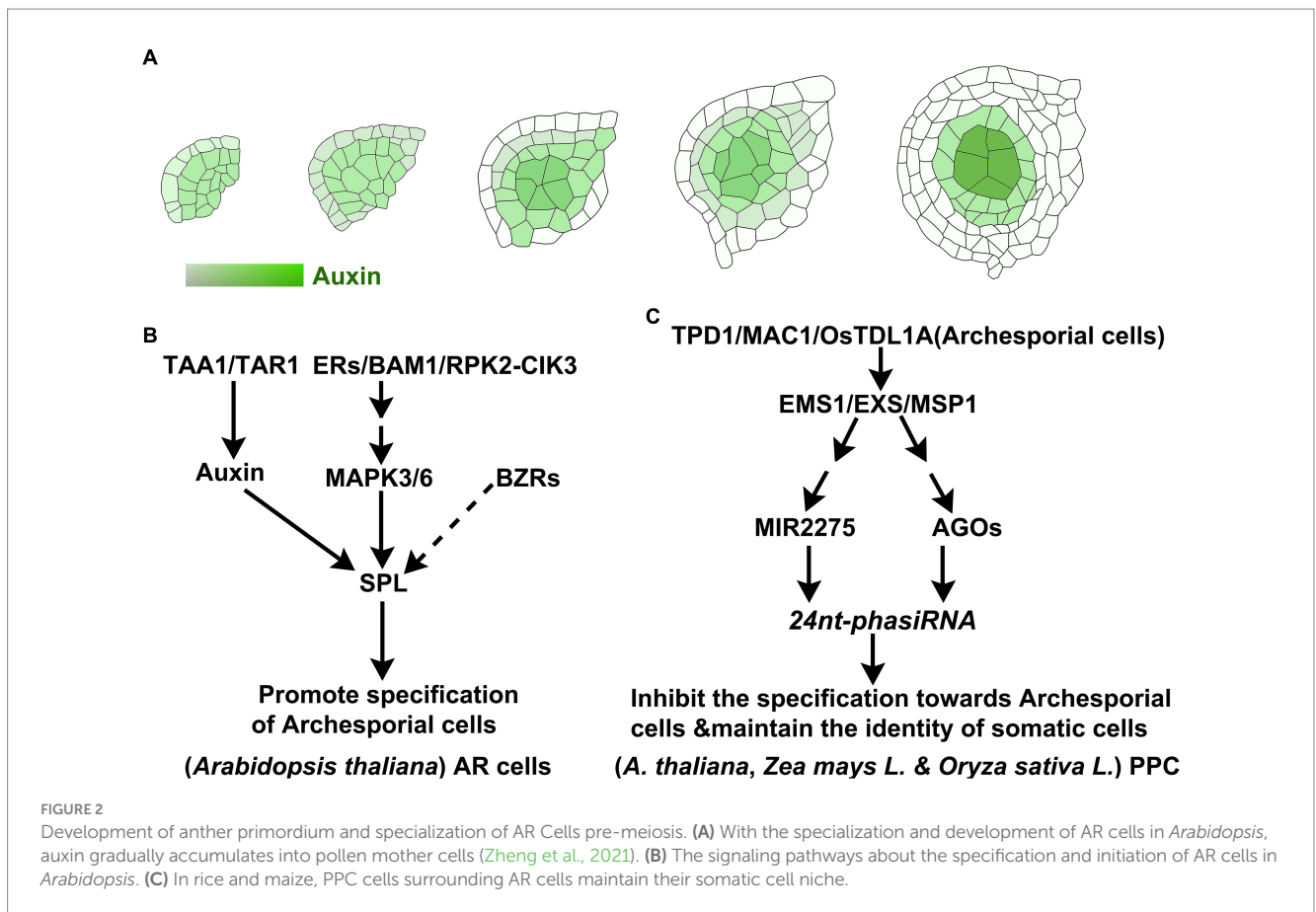
FIGURE 1
Schematic diagram of anther development stages in *Arabidopsis*. Schematic diagram of anther development from Stage 1 to Stage 14 in Arabidopsis (Sanders et al., 1999). E, epidermis (orange); En, endothecium (Pale Mauve); M, middle layer (yellow); PMC, pollen mother cell or microsporocyte (light green); T, tapetum (teal); FM, free microspore (light green); M, microspore (light green); PM, polarized microspores (light green); P, pollen (green). Color coding displays cells are identical types at the same stage or derived cells at different stages.

identical lobes, two adaxial and two abaxial, surround the central connective and vascular tissues. However, the stamen lacks meristem, and all parts of the stamen can be traced back to the floral meristem. In *Arabidopsis*, the formation of the stamen primordia relies on the division activity of the L1, L2, and L3 layers in the floral meristem. In the subsequent patterning establishment of anther primordium, only L1 and L2 meristem layers contribute to forming anther lobes; L3 participates in developing the vascular bundle in filament and connective tissue at the center of each four-lobed anther. In *Arabidopsis*, during the cell type specialization of anther lobes, L2 cells undergo the periclinal division to form two-layer cells: the outer primary parietal cells and the internal subsets endowed with the fate of archesporial cells. The primary parietal cells carry out the periclinal division, producing the secondary parietal cells and an external subset that subsequently differentiates into the endothelium. The secondary parietal cells divide again to form two-layer cells that differentiate into the tapetum and middle layer, respectively. Except that the epidermis is derived from L1 cells, the other cells result from the development and specialization of L2 cells. The tapetum, middle layer, endothecium, and epidermis cells comprise the locular wall and surround the inner archesporial cell, which develops into microsporocytes. In *Arabidopsis*, the specialization of early archesporial cells is closely related to the patterning establishment of the anther primordium as shown in Figure 1. It is of great significance to elucidate and understand the molecular mechanisms underlying

the initiation, differentiation, and development of the germline cell, archesporial cells.

To the knowledge, most of the previous studies and discoveries have focused on meiosis. The underlying molecular mechanism initiating the specialization of male germline cells from the somatic cell of the floral meristem is not well understood. The latest studies suggested that auxin may play an important role in determining the fate of AR cells during male gametophyte formation. It is proposed that archesporial cell initiation is governed by numerous external and internal factors, among which the phytohormones are the key controllers. In *Arabidopsis*, the dynamic distribution of auxin in pre-meiotic anther was consistent with the initiation and development of the germ cells (Zheng et al., 2021). In *Arabidopsis*, detected by immunostaining and biosensor, the auxin was uniformly distributed in three layers of cells of anther primordium at stage 1. From stage 3, the auxin distribution gradually accumulated to the center and formed an inconspicuous auxin gradient. At stage 5, auxin was concentrated in the microsporocytes (microspore mother cells) and an auxin gradient was gradually established in the anther primordium. Auxin signals could hardly be detected in the outer endothecium and epidermis. The local auxin maximum was detected in germ lineage cells compared with the somatic cells from other layers of the anther lobes. With the development and differentiation of anther primordium, auxin was dynamically converged in the germ lineage cells, such as archesporial cells, primary sporogenous cells, and

FIGURE 2
Development of anther primordium and specialization of AR Cells pre-meiosis. **(A)** With the specialization and development of AR cells in *Arabidopsis*, auxin gradually accumulates into pollen mother cells (Zheng et al., 2021). **(B)** The signaling pathways about the specification and initiation of AR cells in *Arabidopsis*. **(C)** In rice and maize, PPC cells surrounding AR cells maintain their somatic cell niche.

microsporocytes (Figure 2A). These results collectively demonstrate that the local auxin maximum orchestrates the specification of male germline cells, and the auxin gradient governs the stamen primordium formation.

TRYPTOPHAN AMINOTRANSFERASE OF ARABIDOPSIS 1 (TAA1) and TRYPTOPHAN AMINOTRANSFERASE RELATED2 (TAR2) of *Arabidopsis* are involved in tryptophan-dependent auxin biosynthesis (Stepanova et al., 2008; Ma et al., 2014). In *Arabidopsis*, *TAA1* and *TAR2* are initially expressed in three layers of cells of the floral meristem. With the specialization of the anther primordium, they gradually concentrate in the germline cells, microspore mother cells, at stage 5. The loss function of *TAA1/TAR2* showed dysfunction in the initiation of anther primordium. In the abaxial locules of the *taa1 tar2-2* weak allele mutant, microsporocytes displayed malformations, and the somatic layer was disordered. Most of the cells in adaxial locules were highly vacuolated. It was suggested that the expression pattern of *TAA/TAR2* and auxin's dynamic distribution were highly consistent and closely correlated with the specialization of the anther primordium. *Arabidopsis* transcription factor SPOROCYTELESS (SPL) (Yang et al., 1999) is one of the early regulators that is responsible for the differentiation of microspore mother cells and the determinant of sporogenous identity. AGAMOUS directly interacts with the CArG-box-like motif in the 3′ region of the *SPL* to regulate its expression in *Arabidopsis* (Ito et al., 2004). However, *SPL* was downregulated in the *taa1 tar2-2* double mutant. The ectopic expression of *SPL* in archesporial cells partially rescued the sterile phenotype of the *taa1 tar2-2* mutant (Zheng et al., 2021). The transcripts of *TAA1/TAR2* were over-accumulated in *spl* mutants. The

above results indicated that TAA1/TAR2 directly participates in the local auxin biosynthesis and accumulation, leading to activating *SPL* expression, which plays a critical role in the initiation and specialization of archesporial cells (Figure 2B). Taken together, SPL/NZZ serves as an adaptor-like transcriptional repressor to precisely inhibit the biosynthesis of auxin through a feedback pathway (Yuan and Sundaresan, 2015; Zhang et al., 2015; Zheng et al., 2021).

Van der Linde and Walbot (2019) speculated that the identity determination of archesporial cells might depend on environmental conditions rather than phytohormones after initiating the development of the stamen primordium (van der Linde and Walbot, 2019). Kelliher and Walbot (2012) found that archesporial cells could be differentiated from hypoxia-treated L1 cells (Kelliher and Walbot, 2012). MALE STERILE CONVERTED ANTHER1 (MSCA1) in *Maize*, termed glutaredoxin, participates in coordinating hypoxic homeostasis, which triggers the specialization and initiation of archesporial cells in anther lobes (Kelliher and Walbot, 2012). *Maize MULTIPLE ARCHESPORIAL CELLS1* (*MAC1*), encoding a small secreted peptide, is initially expressed throughout the anther lobes and the central connective tissue before AR cells specification. After differentiation, the expression of *MAC1* is significantly increasing and is limited to AR and AR-derived cells (Wang et al., 2012). *MULTIPLE SPOROCYTE1* (*MSP1*) is expressed in cells surrounding microsporocytes (Microspore mother cells, MMC) rather than in microsporocytes (male germ lineage cells). MAC1 secreted from archesporial cells or MMC, is recognized by the receptor (MSP1) on the cell membrane of neighboring somatic cells (van der Linde et al., 2018), inhibiting the self-specification toward MMC fate and

promoting the establishment and self-renew of somatic cell niche. The loss function of *MAC1* resulted in generating excessive archesporial cells and missing the endothelium and the secondary parietal cells (Wang et al., 2012). These data indicated that the MSP1 of somatic cells recognized MAC1 secreted from archesporial cells (or MMC) and mediated cell–cell communication to maintain the specific identity of somatic cells (Figure 2C). *Maize* MSP1 and MAC1 were proposed to be homologs of rice OsMSP1 (Nonomura et al., 2003) and TDL1A (TAPETUM DETERMINANT1-like 1A) (Zhao et al., 2008), respectively. Similarly, they are homologs of *Arabidopsis* EMS1/EXS1 (EXCESS MALE SPOROCYTES1/EXTRA SPOROGENOUS CELLS) (Zhao et al., 2002) and TPD1 (TAPETUM DETERMINANT1) (Yang et al., 2003), respectively. *Oryza sativa* MSP1, as the receptor of MMC-secreted-OsTDL1A, is specifically expressed in somatic cells neighboring MMCs, preventing the excessive somatic cells from differentiating into MMCs (Nonomura et al., 2003; Zhao et al., 2008). OsMSP1-OsTDL1A orchestrates the precise differentiation between MMCs and their neighbors, maintaining the identity specification of somatic cells neighboring MMCs and suppressing the over-proliferation of MMCs (Figure 2C). Analysis of miRNA data in wild-type rice, *msp1*, and *ostdl1a* showed that *MIR2275* was not expressed in the mutants, resulting in the depletion of 24 nt-phasiRNAs derived from PHAS loci (Phased small interfering RNA (phasiRNA) generating loci, PHAS loci) (Fei et al., 2016). The spatiotemporal profile of *Argonaute* expression (*OsAGO1d*, *OsAGO2b*, and *OsAGO18*) positively correlated with the stage-specific accumulation of *phasiRNA*, which is essential for specifying cell fate in the early anther at the appropriate spatiotemporal windows (Fei et al., 2016; Dhaka et al., 2020). These data suggested that MIR2275 and AGOs function downstream of OsMSP1-OsTDL1A to maintain the specific cell identities of anther primordium through small RNA-mediated gene silencing at the pre-meiosis stage. On the other hand, this small RNA-mediated gene silencing was a prerequisite for switching from a mitotic to a meiotic program in MMCs. Rice MEIOSIS ARRESTED AT LEPTOTENE1(MEL1), one of the members of the AGO family, is specifically expressed in MMCs and involved in repressing the somatic cell fate via a siRNA-mediated gene silencing (Nonomura et al., 2007). It is unclear whether the intercellular movement of small interfering RNA (siRNA) contributes to determining the cell identities of anther primordium.

Similarly, the TPD1-EMS1/EXS receptor-ligand pairs regulate the specialization of germ cells and somatic cells during anther pattern development in *Arabidopsis*. The *tpd1*, *ems1/exs*, and *serk1 serk2* mutants exhibited the defect of the tapetum and the over-proliferation of MMCs (Zhao et al., 2002; Yang et al., 2003; Albrecht et al., 2005; Colcombet et al., 2005; Chen W. et al., 2019). The EMS1/EXS receptor, localized at the secondary parietal cells, recognizes TPD1 secreted by microsporocytes to be activated, and its kinase activity is enhanced by the trans-phosphorylation with SERK1/SERK2 coreceptors, specifying the tapetum cell fate and limiting the over-production of microsporocytes (Chen W. et al., 2019; Gou and Li, 2020).

In *Arabidopsis*, ERECTA (ER)/ERECTA-LIKE1 (ERL1)/ERL2 (Hord et al., 2008), BARELY ANY MERISTEM 1/2 (BAM1/2) (Hord et al., 2006), and RECEPTOR-LIKE PROTEIN KINASE2 (RPK2) (Mizuno et al., 2007) have been proposed to participate in the initiation and specification of anther primordium. CLAVATA3-insensitive receptor kinases (CIKs) (Cui et al., 2018) serve as co-receptors and are involved in early cell fate specification of anther

primordium orchestrated by BAM1/2 and RPK2. Mitogen-activated protein kinase 3/6 (MPK3/6) (Hord et al., 2008) might function downstream of leucine-rich receptor kinases and co-receptors. It phosphorylates SPL to trigger the formation of archesporial cells and primary parietal cells (Zhao et al., 2017; Cui et al., 2018). In the loss function of the BRASSINAZOLE-RESISTANT1 family *bzr-h* (*bzr* hextuple, *bzr-h*) mutant, the anther primordium had only the L1 layer, failed to generate archesporial cells and hardly detected the expression of SPL (Chen L. G. et al., 2019). However, in the quintuple mutant of the BES1 (BZRs) family mutant, *bes1-1 bzr1-1 beh1-1 beh3-1 beh4-1* (*qui-1*), the increased number of microspore mother cells squeezed or encroached on the space of the tapetum, resulting in the defective or missed tapetal cell layer (Chen W. et al., 2019). These data support that BZRs transcription factors participate in the anther primordium's cell fate specialization (Figure 2B). So far, the small peptides or ligands, which are perceived by BAM11/2-ER/ERL1/ERL2-RPK2-CIKs receptor/co-receptor complex to coordinate the cell fate specification of the anther primordium, are still mysteries.

2.2.2 Meiosis of microsporocytes

Meiosis plays a vital role in maintaining genomic stability and genetic diversity for sexually reproductive organisms (Mercier et al., 2015). Two critical events, meiosis and mitosis, take place in male gametogenesis. Meiosis occurs after the primary sporogenous cells in the anther locules develop into microsporocytes. During microsporogenesis, the diploid microsporocytes undergo one meiosis to generate four haploid microspores. It requires the cell division machinery to be reprogrammed at the entry into meiosis and be paralyzed at the termination of meiosis. Each microspore contains half of the genetic content of microsporocytes, which is attributed to a single duplication of the genome followed by two rounds of chromosome separation in meiosis. Genetic exchange and recombination mainly occur in the first division of meiosis (meiosis I) (Mercier et al., 2015). The prophase I can be divided into leptotene, zygotene, pachytene, diplotene, and diakinesis, according to the dynamic morphology of chromosomes. Nelms and Walbot (2019) used single-cell sequencing and found that during the leptotene and pre-zygotene of meiosis, the transcriptome of microsporocytes underwent two sharp transitions in *Zea mays* male gametogenesis. These two transcriptome re-organizations are consistent with the meiotic cytological process. During zygotene, programmed double-strand breaks (DSBs) and the homologous chromosome pairing appear, and the synaptonemal complex (SC) forms (Imai et al., 2021). Homologous recombination repairs a subset of DSBs to form the crossovers (CO) that result in the reciprocal exchanges of genetic information between the homologous non-sister chromatids, creating new allelic combinations, and generating genetic diversity (Mercier et al., 2015). However, recombination does not occur randomly on chromosomes, and the numbers of COs on each chromosome are limited. Of course, the prerequisite for recombination is the production of DNA double-strand breaks (DSBs) (Willems and De Veylder, 2022). Mutations of meiosis-related genes and cytokinesis-associated genes will lead to abnormal microspore formation, which ultimately results in pollen abortion.

DNA topoisomerase VI (Topo VI), a highly conservative heterotetramer composed of two TopVIA and two TopVIB subunits (Robert et al., 2016), alters DNA topology structure via catalyzing transient DNA double-strand breaks to form the cleavage complex,

TABLE 1 Proteins are involved in the meiosis of microsporocyte.

Protein	Stage	Function	Species	References
OsAM1	Leptotene	leptotene-zygotene transition	*Oryza sativa*	Zhang et al. (2015)
AtSPO11-11/AtSOP11-2	Zygotene	Formation of DNA double-strand breaks	*Arabidopsis thaliana*	Grelon et al. (2001) and Hartung et al. (2007)
MTOPVIB		Formation of DSBs	*Oryza sativa*	Fu et al. (2016), Robert et al. (2016), and Vrielynck et al. (2016)
OsRAD51D	Zygotene	Homologous pairing and recombination	*Oryza sativa*	Zhang et al. (2020)
HCR1	Zygotene	Limit the number of crossovers during repairing DSBs	*Arabidopsis thaliana*	Nageswaran et al. (2021)
OsAGG1	Zygotene	Promote the COs (crossover) formation	*Oryza sativa*	Chang et al. (2020)
DSNP1	Zygotene	Formation of the COs and synaptonemal complex	*Oryza sativa*	Ren et al. (2021)
GEM3/AUG6-1	Metaphase	Organization of microtubule nucleation to promote spindle localization or arrangement	*Arabidopsis thaliana*	Oh et al. (2016)
MS1/GmNACK2a	Metaphase I	Promote phragmoplast expansion and cell plate formation	*Glycine max* (L.) *Merr.*	Fang et al. (2021)
CDM1	Anaphase I	Cytokinesis division of meiosis	*Arabidopsis thaliana*	Lu et al. (2014)

guiding other intact DNA strands to pass. Three Spo11/TopVIA homologs and one TopVIB homolog have been studied in *Arabidopsis*. AtSPO11-1 interacts with AtSOP11-2 to form a heterodimer catalytic core for breaking double-strand DNA, functioning in meiotic recombination (Grelon et al., 2001; Hartung et al., 2007). *Arabidopsis* MEIOTIC TOPOISOMERASE VIB-LIKE (MTOPVIB) stabilizes the catalytic structure and participates in ATP binding and hydrolysis (Fu et al., 2016; Robert et al., 2016; Vrielynck et al., 2016). AtSOP11-1/2 and TOPVIB are indispensable for relaxing DNA super-helixes, inducing the formation of DNA double-strand breaks and playing essential roles in meiotic recombination (Fu et al., 2016; Robert et al., 2016; Vrielynck et al., 2016). Five Spo11/TopVIA homologs and one TopVIB have been identified in *Oryza sativa*. In the *Ossop11-1*, *Ossop11-2*, *Ossop11-4*, *AtmtopVIB*, *ZmmtopVIB*, and *OsmtopVIB* mutants, the pairing of homologous chromosomes was blocked, and univalents were randomly arranged in the nuclei of microsporocytes rather than aligned at the metaphase plate. With the asymmetrical separation of chromosomes, aneuploid chromosomes were generated in the aneuploid mono-microspores (Grelon et al., 2001; Hartung et al., 2007; An et al., 2011; Fu et al., 2016; Vrielynck et al., 2016). OsAM1, encoding coiled-coil domain protein, is critical for the leptotene-zygotene transition during rice meiosis. The *Osam1* mutant displayed dysfunction in sister chromatid cohesion, homologous recombination, and telomere bouquet formation. The meiosis was arrested at the leptotene stage (Zhang et al., 2015). These findings collectively suggest that topoisomerase and other components involved in DNA replication, recombination, and repair machinery play crucial functions in the meiotic progress (Table 1).

In meiosis, DSBs are induced to occur naturally, and repaired by Nonhomologous End joining (NHEJ), homologous Recombination (HR), and Microhomology mediated End joining (MMEJ). The homologous recombination depends on homologous sequences, including chain invasion of homologous chromatids, and synthesis

based on homologous templates, then leading to gene conversion or crossovers formation. Most DSBs are repaired via sister chromatids without the formation of crossovers; crossover occurrence only accounts for an extremely low proportion of DSB repairs (Mercier et al., 2015). Single-strand binding proteins, RecA and DMC1(Disrupted Meiotic cDNA1)–RAD51 (Radiation sensitive 51), mediate the inter invasion of homologous chains to form the crossovers for repairing DSBs (Tarsounas et al., 2004). RAD51D has previously been verified to be critical for the repair of double-strand breaks in mammals (Tarsounas et al., 1999). Rice OsRAD51D combines telomere to inhibit non-homologous linkage and promote homologous pairing and recombination. In the *Osrad51d* mutants, non-homologous pairing and interaction occurred in the repair of double-strand breaks during meiosis, leading to male sterility (Zhang et al., 2020). *Arabidopsis HIGH CROSSOVER RATE1* (*HCR1*) encoding PROTEIN PHOSPHATASE X1 is a member of the PP4 (Ser/Thr-specific phosphoprotein phosphatase) complex (Nageswaran et al., 2021). HCR1 interacted with crossover-associated protein HEI10 (homologous of human enhancer of invasion 10), PTD (homologous of Par Ting Dancer), MSH5 (homologous of MutSγ), and MLH1(homologous of MutLγ) (Mercier et al., 2015; Pyatnitskaya et al., 2019; Nageswaran et al., 2021; Willems and De Veylder, 2022). HCR1 also interacted with chromosome axis protein, DSB factor, and recombinant enzyme in the Y2H assay (Nageswaran et al., 2021). The loss of HCR1 resulted in enormously increasing numbers of crossovers and significantly decreased the interference intensity of distal euchromatic regions (Nageswaran et al., 2021). It was suggested that the HCR1/PPX1–PP4 phosphatase complex might antagonize or equibalance the function of pre-recombinant kinase to limit the number of crossovers in *Arabidopsis*. However, the loss of *ABERRANT GAMETOGENESIS1* (*OsAGG1*) led to the reduction of crossovers in meiosis (Chang et al., 2020). A similar phenotype was found in the mutants involved in crossover formation, including

ZMM loss-of-function mutants, *Osmsh4*, *Osmsh5*, *Oszip4*, *Osrad17*, and *Oshei10* (Ishimaru et al., 2005; Zhang et al., 2014; Wang et al., 2016; Hu et al., 2018). Rice OsAGG1 could interact with ZMM-associated proteins, including OsHEI10, OsZIP4, and OsMSH5, which were involved in the formation of class I crossover (Chang et al., 2020). In rice, loss of DESYNAPSIS1 (DSNP1), an E3 ubiquitin ligase, generated aberrant synaptonemal complex-like polymers. Loss of ZMM class proteins, including HEI10, ZIP4, and MER3, were reduced for distribution on meiotic chromosomes, resulting in a decrease in the formation of crossovers (Ren et al., 2021). These indicated that DSNP1 inhibited the formation of the synaptonemal complex-like polymer to stabilize homologous chromosome pairing and promote the formation of crossovers (COs) during meiosis (Ren et al., 2021). DSNP1 and OsAGG1 were involved in forming class I COs in rice. How DSNP1 and OsAGG1 coordinate and jointly promote the formation of COs is required to further explore.

Gemini Pollen 3 (GEM3)/AUGMIN subunit 6 (AUG6-1), encoding a conservative component of the AUGMIN complex, mediated AUGMIN-dependent microtubule nucleation (Oh et al., 2016). In the *gem3* mutant, the bivalents were scattered at different locations rather than aligned at the metaphase plate. The organization of microtubule nucleation in meiotic spindle formation was defective, resulting in spindle position or structural abnormality (Oh et al., 2016). In soybean, loss of *MS1/GmNACK2a* resulted in an insufficient phragmoplast expansion to influence the cell plate formation. MS1 interacted with GmMAPKKK to activate microtubule-associated protein 65 (GmMAP65) via the MAPK cascade signaling pathway, promoting the dissolution of overlapping microtubules and recruitment of Golgi-derived vesicles toward the middle region of the phragmoplast to form the new cell plates (Fang et al., 2021). *Arabidopsis* Callose Defective Microspore1(CDM1), a CCCH-type zinc finger protein, is required for cytokinesis division of meiocytes and plays an important role in the regulation of callose metabolism during microsporogenesis (Lu et al., 2014; Pandey et al., 2021). The assembly of the cytoskeleton and cytokinesis associated with the formation of the cell plate might affect both the meiosis and mitosis processes.

After meiosis, the haploid microspores terminate the progress of meiosis, which involves the synergies of multiple complexes. In *Arabidopsis*, the previously discovered SUPPRESSOR WITH MORPHOGENETIC EFFECTS ON GENITALIA7 (SMG7) and THREE DIVISIONMUTANT1 (TDM1)/ MS5/POLLENLESS3 play essential functions in the termination and exit of meiosis (Bulankova et al., 2010; Cifuentes et al., 2016). TDM1 is incorporated into PROCESSING BODY (P-bodies) via interaction with SMG7. Similarly, the main translation initiation complex, elF4F, is recruited by TMD1 into TDM1-containing P-bodies to inhibit the translation (Cairo et al., 2022). A suppressor screen to reveal that the inactivity of cyclin-dependent kinase D;3 (CDKD;3) would inhibit the multiple cycles of nuclear division in *smg7* mutant (Tanasa et al., 2023). A centromeric histone H3 (CENH3) mutation promotes meiotic exit and partially restores fertility in SMG7-deficient *Arabidopsis* (Capitao et al., 2021). The reduced level of CENH3 alleviates the SMG-defective phenotype, which displayed excessive chromosome aggregation and spindle assembly, exiting meiosis. Collectively, temporary inhibition of translation governed by TDM1-SMG7-elF4F-containing P bodies downregulates the

level of cyclin-dependent kinases and CENH3 and promotes the termination of meiotic processes in *Arabidopsis*.

2.3 Microgametogenesis

Microgametogenesis means the microspore undergoes mitosis to produce the bicellular or tri-cellular pollen grains depending on the species (Shin et al., 2021). In *Arabidopsis*, the first asymmetric division gives rise to a vegetative and a generative cell with different morphological characteristics and cell fate. The generative cell is enclosed within the vegetative cell, forming a cell-in-celled structure. Subsequently, the generative cell goes through symmetric mitosis to produce two sperm cells with the identity properties. The schematic diagram of *Arabidopsis* pollen development is shown in Figure 1. At stage 9, the small vesicles aggregate to form vacuoles and surround the central nucleus in free microspores (Sanders et al., 1999). The emergence of baculae and tecta marks the initiation of the microspore exine (Quilichini et al., 2014). The intine wall appears between the exine and the microspore plasma membrane. At stage 10, within the microspore, small vesicles coalesce into a large central vacuole that squeezes the nucleus to the edge. Microspores are highly polarized. The exine wall of the microspore is gradually formed. The sporopollenin polymers consist of a mixture of unbranched amylose monomers and branched amylopectin monomers (Ariizumi and Toriyama, 2011; Lallemand et al., 2013; Ghelli et al., 2023). These sporopollenin precursors are synthesized by ER-related enzymes or cytoplasmic enzymes in the tapetum, secreted into the locules through the secretory pathways or ABC transporter localized on the plasma membrane, reaching the surface of microspores, and finally assembling into the exine wall of microspores (Huang et al., 2013). The cytoplasmic contents fill the entire intracellular space enclosed by the microspore wall. At stage 11, the microspores undergo an asymmetric mitotic division to produce bi-cellular pollen, a smaller germ cell engulfed in the cytoplasm of a larger vegetative cell, forming unique cell-in-celled structures. The bicellular pollen contains many small vesicles, and the large vegetative cell is rich in subcellular organelles such as lipid droplets, mitochondria, and endoplasmic reticulum. The large vacuoles are broken into many small vesicles in the vegetative cell (Bai et al., 2023). The darkly stained organelles appear in tapetum cells, which are likely elaioplasts and tapetosomes in size and shape. The tapetum becomes thin and remains disintegrated during the bicellular pollen. At stage 12, the generative cell undergoes the second mitosis to form two identical sperm cells housed in the vegetative cell. *Arabidopsis* NOT-LIKE-DAD (NLD) is a phospholipase that is localized to the endo-membrane of vegetative cells to enclose the two sperm cells (Gilles et al., 2021). The nucleus of the vegetative cell keeps close physical contact with two sperm cells for the delivery of sperm cells during fertilization (Lalanne and Twell, 2002; McCue et al., 2011). The tapetal remnants derived from the rupture and degeneration of tapetal cells (mainly composed of lipids and proteins) are gradually deposited on the pollen surface, facilitating the formation of the pollen coat (Quilichini et al., 2015). At stage 13, the pollen coat deposition is completed, and the pollen grains mature after dehydration. Vegetative cells store lipids and carbohydrates during pollen maturation in order to supply the germination and growth of the pollen tube. The anther dehisces and the septal cells rupture to release tri-cellular pollen grains (Zhao and Ma, 2000). The
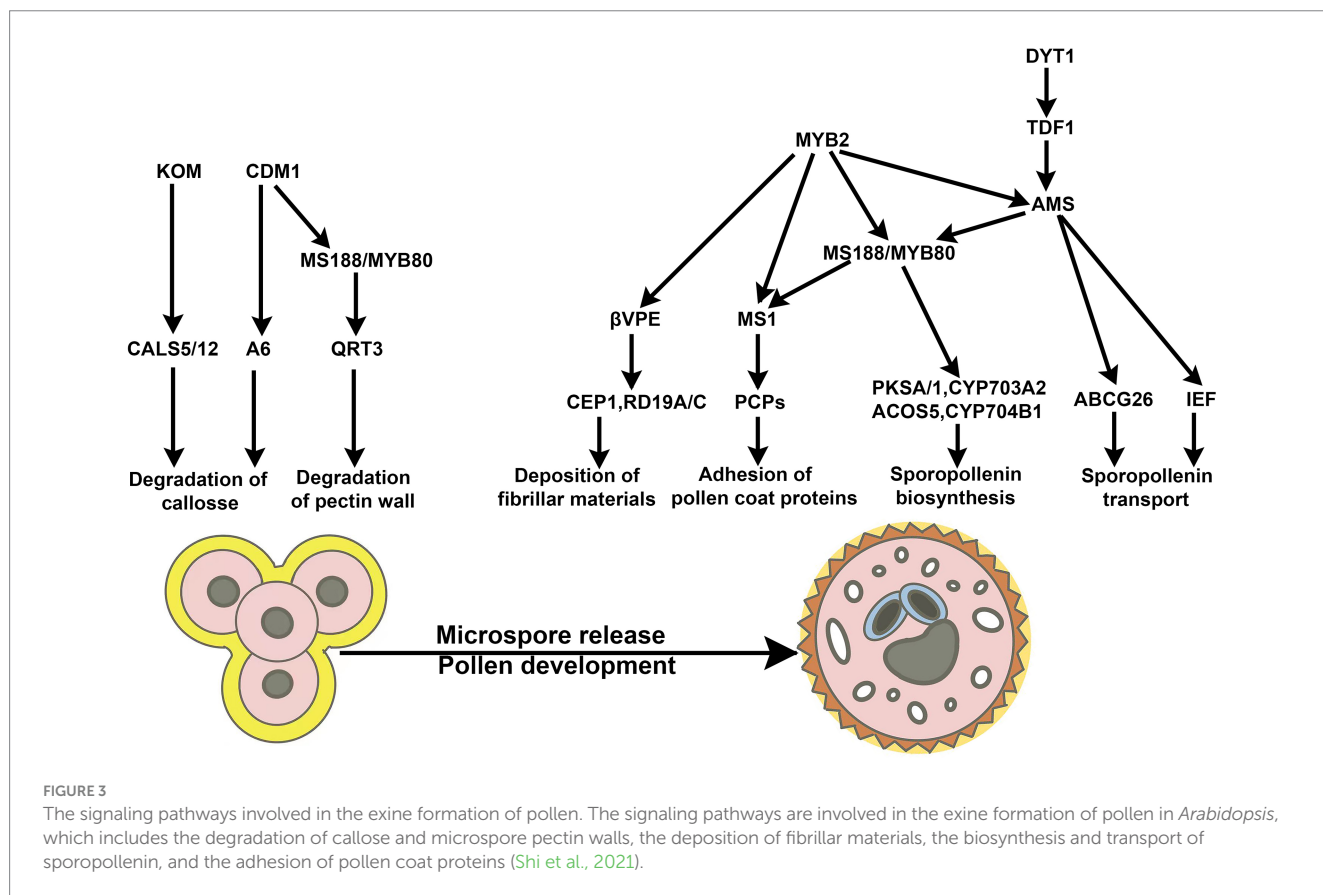
stamens begin to senesce at stage 14, and the anther structures shrink (Sanders et al., 1999).

2.4 Regulation of microgametogenesis

2.4.1 Microspores mature in pre-mitosis

At pre-mitosis of *Arabidopsis*, the development and maturity of mononuclear microspores are vital for the microgametogenesis initiated by subsequent mitosis. In *Arabidopsis*, from the initiation of meiosis, the apoplastic gating structure at the tapetum-middle layer interface appears, which promotes the tapetum and the developing pollen forming the symplastic isolation, separating from other sporophytic tissues (Truskina et al., 2022). The pollen wall is composed of the exine layer and the intine layer. Its formation is an extremely complex process involving various biological events regulated by genes from sporophytes and gametophytes (Ariizumi and Toriyama, 2011). The exine layer is mainly composed of sporopollenin, and its formation is accompanied by the maturation of microspores, containing the development and degradation of the callose wall, the biosynthesis and transport of sporopollenin in the tapetum, the programmed cell death of the tapetum, and the deposition of pollen coat (Shi et al., 2015). The formation mechanism of the inner layer is different from that of the exine layer. The main components of the inner layer are pectin and cellulose, which are synthesized in the microspore and transported to the surface for the assembly of the intine of the microspore wall (Ariizumi and Toriyama, 2011). The active expression of *Arabidopsis* genes in the generative cells regulates the formation of inner layers (Figure 3).In angiosperms, the types and degradation of tapetum exhibit a high degree of diversity, which endows them with various mechanisms for the formation of the exine layer. In *Arabidopsis*, pollen coat and sporopollenin are secreted by tapetal cells and adhere to the surface of microspores, mediating the exine layer biogenesis. Callose synthesis and punctual degradation are essential for the exine formation of microspore mother cells, microspores, and pollen. On the other hand, the remnants of the degenerated tapetal cells (mainly composed of lipids and proteins) are deposited on the microspores' surface. They are involved in the formation of the cuticle and the exine layers. During microsporogenesis, microspores are surrounded and separated by the callose wall to form the tetrad structure, the tapetal cells subsequently synthesize and secrete callase (β-1,3-D-glucanase) complex to degrade the callose wall, leading to the release of microspores. It has been previously discovered that rice callose synthase GSL5 (GLUCAN SYNTHASE-LIKE5) (Shi et al., 2015) and UGP1 (UDP-GLUCOSE PYROPHOSPHORYLASE 1) (Chen et al., 2007) is predominantly expressed in the meiotic MMCs and participate in the callose deposition of microsporocytes. *Arabidopsis* CALS5 (CALLOSE SYNTHASE 5) is required for callose formation around developing microspores, which is critical for the exine formation during microgametogenesis (Dong et al., 2005). *Arabidopsis* KOM (KOMPEITO), localized to the Golgi apparatus, is a member of atypical rhomboid-serine protease that lacked activity. KOM plays an essential role in the regulation of the activity and function of CALS5 in *Arabidopsis* (Kanaoka et al., 2022). *Arabidopsis* CALLOSE DEFECTIVE MICROSPORE1 (CDM1) promotes the biogenesis of

FIGURE 3

The signaling pathways involved in the exine formation of pollen. The signaling pathways are involved in the exine formation of pollen in *Arabidopsis*, which includes the degradation of callose and microspore pectin walls, the deposition of fibrillar materials, the biosynthesis and transport of sporopollenin, and the adhesion of pollen coat proteins (Shi et al., 2021).

the exine by regulating the expression of callose synthesis genes (*CALS5*, *CALS12*) and callase-related genes (*MYB80* and *A6*). *Arabidopsis* MYB80/MS188 controls the secretion of the callase by promoting the expression of the *A6* in the tapetum (Zhang et al., 2007). MYB80 coordinates the development between the tapetum and microspore, mediating the programmed cell death in the tapetum. The precise coordination of the timing between the degeneration of the tapetum and the development of microspores is critical for male gametogenesis.In rice, the ETERNAL TAPETUM 1 (EAT1) transcription factor regulates the programmed cell death of the tapetum by activating the aspartic protease of OsAP25 (ASPARTOC PROTEASES 25) and OsAP37 in rice (Niu et al., 2013). Two bHLH transcription factors, TDR (TAPETUM DEGENERATION RETARDATION) and TIP2/bHLH142 (TDR INTERACTING PROTEIN2) could combine and function upstream of EAT1 (Ko et al., 2021). EAT1 was recently found to interact with OsUDT1 (UNDEVELOPED TAPETUM1). The loss of UDT1 resulted in lagging the tapetal degradation and the abortion of microspores (Jung et al., 2005; Ko et al., 2021). During early meiosis, UDT1 directly interacts with TIP2 to form a heterodimer, activating the expression of EAT1 which is enhanced by the dimerization complex of EAT1/UDT1. UDT1 indirectly regulates the expression of TIP2/bHLH142 in the tapetum and meiocytes and plays a role in the meiosis of microspores and the PCD of the tapetum (Ko et al., 2021). However, it has previously been proved that TDR and TIP2 act upstream of EAT1. There should be a feedback cycle pathway between UDT1-EAT1 and TDR-TIP2, it needs solid experimental data to be supported. On the other hand, GIBBERELLIN MYB GENE (GAMYB) specifically binds to the MYB motif in the promoter of TIP2/bHLH142 to regulate the expression of bHLH142 in the tapetum. TDR combines the E-box near the MYB motif in the bHLH142 promoter to repress the module of GAMYB-regulated bHLH142 (Ko et al., 2021). Rice UDT1 and PTC1 (PERSISTENT TAPETAL CELL1) activate GDSL esterase/lipase RMS2 (RICE MALE STERILE 2) to degrade lipids and promote the anther cuticles formation and lipid deposition on the exine layer of microspores (Zhao et al., 2020). These data indicate that TIP2/bHLH142 functions as the hinge, which integrates and orchestrates the UDT1-EAT1 and GAMYB-TDR pathways in *Oryza sativa*. Finally, they coordinate the meiotic process of microsporocytes, the programmed cell death of the tapetum, and lipid transport, achieving microspore maturity and microsporogenesis.In *Arabidopsis*, transcription factor AMS (ABORTED MICROSPORE) regulates membrane transporters IEF (IMPERFECTIVE EXINE FORMATION) and ABCG26 (ATP-BINDING CASSETTE TRANSPORTER G26) that are involved in the transport of sporopollenin/tetraketide sporopollenin precursors, promoting the formation of the exine layer in two parallel pathways (Choi et al., 2011; Lou et al., 2018; Wang et al., 2021). TEX2 (THIN EXINE2) is a nucleotide sugar transporter that transports the sporopollenin precursors for the formation of the primexine and the assembly of the exine structures (Wang and Dobritsa, 2021) in *Arabidopsis*. Fatty Acid Export 1 (FAX1) exports fatty acids from the tapetum to regulate ROS homeostasis to activate the DYT1-TDF1-AMS-MS188 signaling pathway in the tapetum and is involved in the exine biogenesis of microspores in *Arabidopsis* (Zhu et al., 2020). *Arabidopsis QUARTET 3 (QRT3)* encodes a polygalacturonase that plays a critical role in the degradation of the tetrad pectin wall (Shi et al., 2021). In *Arabidopsis*, the coat protein complex II (COPII) composed of five cytoplasmic components for the transportation of vesicles mediates the anterograde trafficking from

the endoplasmic reticulum (ER) to the Golgi apparatus. Sar1, a small G protein, is activated by the guanine nucleotide exchange factor SEC12 anchored in the endoplasmic reticulum. Subsequently, Sar1 assembles Sar1–SEC23–SEC24 by interacting with SEC23 and integrates SEC13-SEC31 via the interaction between SEC23 and SEC31 (Movafeghi et al., 1999; Saito-Nakano and Nakano, 2000; Lederkremer et al., 2001; Futai et al., 2004). *Arabidopsis* Sar1B/C is involved in the targeted delivery of sporopollenin to the outer surface of microspores (Liang et al., 2020). SEC31A and SEC31B are coat proteins found in COPII vesicles, which promote the formation of larger central vacuoles in microspores (Liu X. et al., 2021). *Arabidopsis* Hyp-galactosyltransferase (GALT2-GALT9) activates the Hyp-O-galactosylation of Hyp-arabinogalactan (AG). Highly glycosylated arabinogalactan proteins (AGPs) play a crucial role in male gametogenesis. In the *galt2galt5galt7galt8galt9* (Hyp-galactosyltransferase) quintuple mutant, mature microspores displayed membrane blebbing, and many lytic vacuoles and their pollen exine and inner membrane layers showed malformations (Kaur et al., 2022). *Arabidopsis* βVPE (β VACUOLAR PROCESSING ENZYME) is directly activated by MYB2 to play critical roles in the maturation of CEP1, RD19A, and RD19C proteases (Guo et al., 2022). The loss of βVPE resulted in little fibrillar materials attaching to the exine of the microspores, and microspores were shrunken and degraded (Cheng et al., 2020). These data indicate that proteins, which regulate or participate in the secretory/transportation of sporopollenin and lipids, play essential roles in exine biogenesis and pollen coat formation. Proteins involved in the tapetum development and apoptosis are important for the maturity of microspores or pollen (Figure 3).The pattern formation of the intine is established during the microspore development stage, later than the initiation of the exine layer, and is mainly regulated by the male gametophyte genes. The intine layer of rice pollen is initiated by microspores after releasing it at the later tetrad stage and is predominantly completed at the end of the binuclear pollen stage. Similarly, *Arabidopsis thaliana* Microgametophyte Defective Mutant 1(AtNMDM1) interacts with AtNMDM2 in pollen nuclei to activate the expression of pollen intine-related Arabinogalactan proteins (AGPs) and pectin methylesterases (PEM), mediating cellulose distribution, and promoting intine layer formation (Mi et al., 2022). These data indicate that the genes derived from germ cells regulate the intine development and the vesicle transport of microspores, and are also crucial for microspore maturity.Microspore maturation also contains the generation and transport of vesicles within the microspore. In *Arabidopsis*, the adaptor protein (AP) complex is the major coat protein that connects membrane proteins with the clathrin molecules that form the lipid vesicle coat. *Arabidopsis* AP1G2 is an adaptor protein complex 1 (AP-1) γ subunit and interacts with PICALM5A/B and PICALM9A/B, mediating endocytosis in transporting insoluble enzymes and membrane proteins from the trans-Golgi network (TGN) to endosomes and lysosomes. In the *ap1g2–1$^{+/-}$* mutant, nearly half of the microspores failed to undergo mitosis due to defective polarization (Zhou et al., 2022). *Arabidopsis* importin β, KETCH1 (karyopherin enabling transport of cytoplasmic HYL1), is located in the nucleus of the reproductive cells at unicellular and bicellular pollen (Xiong et al., 2020). It specifically interacts with RPL27a and other RPs (ribosome proteins) to protect RPs from 26S proteasome-mediated degradation and maintain the nuclear accumulation of RPs. It plays a vital role in ribosome biogenesis and translational efficiency during microspore maturity in *Arabidopsis* (Xiong et al., 2020). Intracellular membrane fusion undergoes dynamic assembly and disassembly of soluble

TABLE 2 Proteins are involved in the mitosis of microspores.

Protein	Stage	Function	Species	References
Myb81	Microspore polarization	Polarization of mononuclear microspores	*Arabidopsis thaliana*	Oh et al. (2020)
VLG	Microspore polarization	Vesicular fusion and formation of the large central vacuole	*Arabidopsis thaliana*	D'Ippolito et al. (2017)
SEC6	PM I	Vesicular fusion and formation of cell plate during PMI	*Arabidopsis thaliana*	Tan et al. (2022)
AtTMS	PM I	Vesicle secretion and membrane fusion	*Arabidopsis thaliana*	Li et al. (2019)
SYP31/SYP32	PM I	Cell plate formation and intine deposition	*Arabidopsis thaliana*	Rui et al. (2021)
YKT61	PM I	Cell plate formation	*Arabidopsis thaliana*	Ma et al. (2021)
CAF-1	PM II	Chromatin assembly	*Arabidopsis thaliana*	Chen et al. (2008)
DUO1/3	PM II	Regulation of cell cycle	*Arabidopsis thaliana*	Brownfield et al. (2009a,b)
REF6	PM II	Determine the identity of the generative cell	*Arabidopsis thaliana*	Huang and Sun (2021), Pan et al. (2022), and He et al. (2022)

N-ethylmaleimide-sensitive factor (NSF) attachment protein (SNAP) receptors (SNAREs). Assembly and disassembly of SNARE complexes are essential for maintaining microspore membrane homeostasis (Ma et al., 2021; Rui et al., 2021; Liu X. et al., 2021; Tan et al., 2022). *Arabidopsis* SEC17/18 promotes the formation of large central vacuoles after the disassembly of the SNAREs (Liu F. et al., 2021). In *Arabidopsis asnap-1/+* mutant, the cytoplasmic contents of the mononuclear microspore were leaked, and the internal tissue was disintegrated (Liu F. et al., 2021). The inability of microspores to maturity is prevented from undergoing mitosis. During the development of the microspore, any proteins that participate in its assembly, disassembly, transportation of vesicles, and ribosomal biogenesis will affect the maturity of the microspore, resulting in developmental stagnation before mitosis.

2.4.2 Mitosis of microspores

In *Arabidopsis*, a mature mononuclear microspore characterized by a thick cell wall and uniform dense cytoplasm has a larger nucleus located at the center with small growing vesicles surrounding the central nucleus. Subsequently, the microspore's volume becomes larger with growth, the scattered small vacuoles in the cytoplasm gather to form a larger vacuole, and the nucleus gradually migrates to the location of the germinal pore at the reproductive pole. The highly polarized microspore undergoes an asymmetric mitosis I (pollen mitosis I, PM I), producing a larger vegetative cell and a smaller generative cell. In approximately 30% of angiosperms, including *Arabidopsis*, the reproductive cells undergo direct division to produce tri-cellular pollen. In approximately 70% of angiosperms, the division of germ cells is suspended, and pollen remains in the bicellular morphology until germination. In the growing pollen tube, reproductive cells undergo mitosis to form mature tri-cellular pollen (Brewbaker, 1967). Therefore, mitosis is an important process and a distinct mechanism for microgametogenesis.

Gametophyte proteins are essential for vesicle transport, organelle reprogramming, microspore polarization, asymmetric mitotic division, and cytokinesis (Table 2). *Arabidopsis* MYB81 is a microspore-specific member of the GAMYB transcription factor family and is specifically expressed in the nucleus of mononuclear microspores. In the *myb81* mutant, the mononuclear microspore with the large central vacuole is arrested at the microspores polarization stage and fails to enter PM I (Oh et al., 2020). *Arabidopsis* VACUOLELESS GAMETOPHYTES (VLG) is a cysteine/histidine-rich zinc finger protein and interacts with proteins in

multivesicular bodies or pre-vacuolar compartments, mediating a large central vacuolar fusion before microspore polarization (D'Ippolito et al., 2017). *Arabidopsis* RANs, the small GTPases, play fundamental roles in pollen mitosis I. Both the dominant negative and constitutively active variants of RAN1 can lead to the developmental stagnation of microgametogenesis at PM I (Qin et al., 2022). SEC6, an exocyst subunit of octameric tethered complexes, is involved in vesicle trafficking to promote cell plate formation in pollen mitosis I in *Arabidopsis* (Tan et al., 2022). The mutation of *SEC6* failed to form the cell plate during the mitosis I and caused the accumulation of vesicles in the cytoplasm. The cytokinesis-specific syntaxin KNOLLE failed in being recruited to the cell plate, but was localized in the cytosol (Tan et al., 2022). RAN1 and SEC might facilitate tethering the vesicles before fusion and participate in cell plate formation during cytokinesis in *Arabidopsis*. The C-terminal R-SNARE domain of *Arabidopsis* Tomosyn protein (TOMOSYN, AtTMS) bound to SYP1s (SYP111, SYP124, SYP125, SYP131, and SYP132) which are the members of Qa-SNAREs, activating the vesicle secretory and negatively regulating SNARE-dependent membrane fusion (Li et al., 2019). Syntaxin of Plants 31/32 (SYP31/SYP32) interacts with COG3 (Conserved Oligomeric Golgi, COG) localized on Golgi, guiding the transport of SYP31/32 within the Golgi. They might maintain the anterograde and retrograde transport in Golgi-to-ER and be involved in cell plate formation and the deposition of the intine during pollen mitosis I (PM I) in *Arabidopsis* (Rui et al., 2021). R-SNARE protein YKT61 interacts with many SNAREs derived from different endomembrane components, including the cell plate-associated KNOLLE during PM I (Ma et al., 2021), which is involved in vesicular trafficking to maintain the biogenesis and dynamics of endomembrane compartments in *Arabidopsis* (Rui et al., 2021). However, in phospholipid metabolism, the production of phosphatidylinositol 3,5-bisphosphate [PI (3,5) P2] is regulated by FAB1A/B/C/D, PI (3,5) P2-converting kinase, and *Arabidopsis* VAC14 which is a homolog of the yeast and metazoan VAC14s that are responsible for the production of PI (3,5) P2. The mutations of *FAB1A/B/C/D* and *VAC14* resulted in the inability of the central vacuoles to fission into small vesicles after pollen mitosis I in *Arabidopsis*. These demonstrated that phospholipids, as membrane components, play a crucial role in the dynamic vacuolar organization (Zhang et al., 2018). *Arabidopsis* PM I is accompanied by the dynamic patterns of vacuolar organization, involving the formation of cell plates, the maintenance of the endomembrane system, and the intra-Golgi trafficking. The deposition of callose is critical for the *de novo* cell plate

formation in mitotic cell division and pollen maturity. In cotton, callose synthase gene GhCalS4/GhCalS8 is generally repressed by transcriptional factor GhWRKY15. After mitosis, pollen-specific SKS-like protein (PSP231) interacted with the RNA-binding protein GhRBPL1 to disarm the repression, promoting pollen maturity (Li et al., 2020). *Arabidopsis* transcription factor TFIIB-related protein BRP4 mediated the subunit 6 of the Origin Recognition Complex (ORC6) to participate in regulating the PM I cell cycle process (Qin et al., 2014).

During pollen mitosis II (PM II), the generative cell encysted in the vegetative cell undergoes symmetric mitosis to produce two identical sperm cells, forming tricellular pollen in *Arabidopsis*. *Arabidopsis H + -ATPase (AHA)* localized on the plasma membrane facilitates H+ efflux depending on the ATP hydrolysis and is essential for maintaining pH homeostasis during PM II (Robertson et al., 2004). CAF-1 (CHROMATIN ASSEMBLY FACTOR 1), a histone chaperone, interacts with replisome and mediates the formation of H3-H4 tetramers through DNA replication-dependent chromatin assembly in *Arabidopsis* (Chen et al., 2008). *Arabidopsis* DUO POLLEN1(DUO1) and DUO3 have redundant and distinct molecular mechanisms for regulating generative cell mitosis and sperm cell specification (Brownfield et al., 2009b). The loss of DUO1 and DUO3 led to the failure of germ cells to enter the M phase of cell cycles after undergoing S-phase DNA replication. AtCycB1;1, the G2/M checkpoint protein in the cell-cycle progression, was defective in the *duo1* mutant to block generative-cell division. However, another mechanism was suggested to block the generative cell entry into the M phase since AtCycB1;1 was detected in the *duo3* mutant. A-type cyclin-dependent kinase;1 (CDKA;1) acts as a crucial regulator of both the G1/S and the G2/M phase transitions of cell cycles, and also plays vital roles in PM II (Brownfield et al., 2009a).

During organismal development, two distinct molecular mechanisms underlying epigenetics coordinate developmental and environmental cues to determine cell fate. They regulate chromatin structure by DNA methylation and histone modification to control gene output and N6A mRNA modification that regulates mRNA metabolism. Epigenetics regulates the cell fate determination of generative and vegetative cells via DNA methylation and covalent modification of histones (Huang and Sun, 2021; Huang and Sun, 2022). Arabidopsis RELATIVE OF EARLY FLOWERING 6 (REF6), a specific lysine(K)-H3K27me3 demethylase, recognizes and binds CTCTGYTY motif, negatively regulating DNA non-CG methylation and playing essential roles in epigenetic reprogramming during male gametogenesis (Huang and Sun, 2021; Pan et al., 2022). When the LAT52 promoter drove it to erase H3K27me3 methylation modifications in the vegetative cell of transgenic *Arabidopsis*, leading to chromatin remodeling and triggering the identity transition from the vegetative cell to the germline one (He et al., 2022; Pan et al., 2022). It suggests that the H3K27me3 paradigm is critical for determining the identity of vegetative cells during male gametogenesis. Quantitative proteomic analysis of vegetative nuclei (VNs) and germ cell nuclei (GNs) derived from germinated lily pollen have identified 29 transcription factors (TFs) and ten cell fate determinants. The 29 TFs derived from VN are mainly involved in the development of pollen tubes. Ten germ cell-specific TFs primarily participate in cell fate differentiation and male gametogenesis (You et al., 2021). In *Arabidopsis* pollen grains, the transposable elements (TEs) of sperm cells are methylated to disfunction by the mechanism of RNA-directed DNA methylation (Martinez et al., 2016). The mobile siRNAs derived from pollen vegetative nuclei enhance the transposon methylation of the reproductive cells, resulting in TEs silencing of offspring in an epigenetic inheritance manner (Long et al., 2021). Three excellent reviews highlight how the cell cycle has influenced DNA methylation dynamics during male gametogenesis and shed light on the epigenetic regulation of cell fate (Huang and Sun, 2021; Liu and Wang, 2021; Papareddy and Nodine, 2021).

3 Conclusion

Male gametophyte development in angiosperm is a complex and continuous biological process. Each critical step is independent and closely related to each other. In the past few years, many functional proteins required for the male developmental program have been found, including those that functioned in microspore and tapetal development, meiosis and mitosis, the dynamic state of callose and vacuole, the specification of cell fate, and the differentiation of cell layers. The proteins involved in male gametophyte formation are classified and reviewed here. However, the molecular mechanism underlying this progress and the regulatory network is required for further elucidation. With gene editing technologies and high-throughput approaches, more and more functional proteins will be discovered during male gametogenesis. In the future, we should integrate molecular biology, biochemistry, and multi-omics techniques to find and elucidate many functional proteins in male gametophyte development. The next challenge is exploring the systematic and high-throughput approaches to construct a more rigorous regulatory network diagram for male gametophytic development.

Author contributions

D-LC: Investigation, Visualization, Writing – original draft. C-XX: Investigation, Resources, Visualization, Writing – original draft. PW: Investigation, Writing – review & editing. T-YG: Funding acquisition, Investigation, Writing – review & editing. BW: Writing – review & editing. T-YY: Conceptualization, Data curation, Funding acquisition, Investigation, Project administration, Resources, Supervision, Visualization, Writing – original draft, Writing – review & editing.

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Conflict of interest

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