

Positional signals establishment in the regulation of female germline specification

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Abstract

The female germline specification process of a single megaspore mother cell (MMC) of ovule primordium (nucellus) is intriguingly complex because it involves the interaction of different pathways tightly linked with positional information. Various Arabidopsis genes, including the stem cell promoting factor WUSCHEL, have already been shown to be involved in this precise regulation process. Recently, there have been some reviews on MMC specialization, mainly from the aspects of epigenetics, microRNAs and gene regulatory networks. However, those reviews have not taken into consideration the function of positional signals in female germline specification. Here, we review major progress in the cell fate control of female germline specification, highlighting the functions of positional cues.

Citation: Cai H, Ma S, Su H, Liu K, Aslam M, et al. 2022. Positional signals establishment in the regulation of female germline specification. *Seed Biology* 1:6 <https://doi.org/10.48130/SeedBio-2022-0006>

Introduction

One hallmark that distinguishes higher plants from animals is the development of germlines within the flowers of adult plants. Thus, unlike in animals, plant germline specification does not occur during embryo development. In the majority of flowering plants, only a single germline precursor cell – the megaspore mother cell (MMC) – is differentiated from the subepidermal somatic cells within the distal ovule tissue giving rise to the female germline^[1,2]. By undergoing meiosis, MMC produces four megaspores. Then, merely one megaspore near the chalazal end develops into the functional megaspore (FM), nevertheless, the remaining three cells degenerate^[1,2]. Subsequently, as FM progress through three mitotic and nuclear divisions, a mature female gametophyte (FG), the embryo sac, is finally formed^[1] (Fig. 1).

In Arabidopsis, the position of the single germline precursor is strictly restrained. The precise spatiotemporal coordination of cell divisions that multiply the somatic cell or switch to the meiotic cell cycle to become the MMC is vital for the acquisition of female reproductive fate, which begins during the formation of the ovule primordium^[3]. Once MMC differentiates, suppression of the core cell cycle machinery is necessary to stabilize its meiotic commitment. However, how the intercellular signaling among somatic cells results in the differentiation of only one hypodermal somatic cell to MMC remains unclear. In this review, we emphasize the role of positional signals in MMC specification.

Establishing an environment in the epidermal cell layer promotes female germline formation

Early in ovule development, the cells in the outermost layer of the nucellus are defined as the epidermal cell layer (also

called L1 cells), where numerous essential genes express in the epidermal cell layer and establish the female germline^[3]. Particularly in the apical epidermal cell layer *SPOROCTELESS/NOZZLE* (*SPL/NZZ*) and *WUSCHEL* (*WUS*) have been identified (Fig. 2a). *SPL/NZZ* encodes a nuclear localization protein similar to the MADS box transcription factors^[4]. As an adaptor-like transcriptional repressor, *SPL/NZZ* recruits *TOPLESS/TOPLESS-RELATED* (*TPL/TPR*) corepressors to repress the activities of *CIN*-like TCP transcription factors to control ovule development in Arabidopsis^[5,6]. In the *spl/nzz* mutant, MMCs cannot form and loss-of-function of *TPL1* and overexpression of *TCP* transcription factors display aborted ovules that resemble the phenotypes of those observed in the *spl/nzz* mutant^[5]. *WUS* encoding a transcription factor is another crucial regulator involved in MMC formation^[7]. Like the *spl/nzz* mutant, the *wus* mutant displays no MMCs, suggesting that *SPL/NZZ* and *WUS* may belong to the same genetic pathway. Since the expression of *WUS* is decreased in the *spl/nzz* mutant, *WUS* was verified to act downstream of *SPL/NZZ* during MMC development. In addition, it has been shown that *WUS* controls MMC formation by indirectly activating the expression of two redundantly acting genes, *WINDHOSE 1* (*WIH1*) and *WIH2*, which encode small peptides in plants^[7]. Although *wih1* or *wih2* single mutants showed no obvious phenotype in MMC development, *wih1 wih2* double mutants showed no MMCs^[7]. The possible partners for *WIH1* and *WIH2* may be *TORNADO 1* (*TRN1*) and *TRN2*, as the twisted organs of *wih1 wih2* have similar phenotypes to *trn1* and *trn2* single mutants^[7]. It has been reported that *TRN1* is a cytosolic protein and *TRN2* is a multi-transmembrane protein^[8], suggesting that *TRN1* or *TRN2* are not the receptors of *WIH1/2*. Altogether, the above reports describe an essential genetic pathway for MMC formation, which involves *SPL/NZZ*, *WUS*, *WIH1/2* and *TRN1/2*.

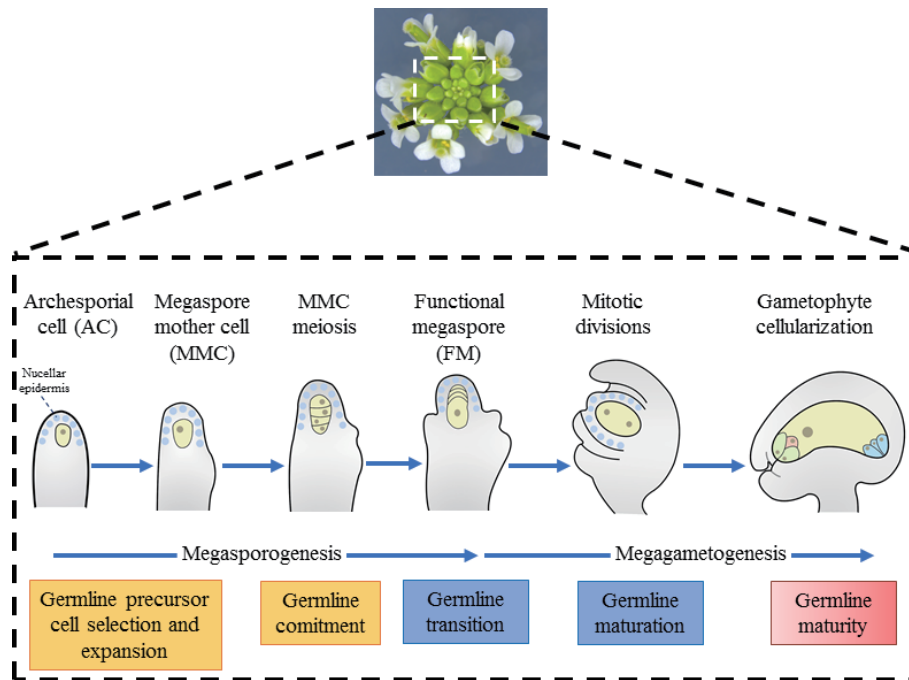


Fig. 1 Female germline development in *Arabidopsis thaliana*. The developmental process of female gametophyte can be divided into two steps, including megasporogenesis and megagametogenesis, in *Arabidopsis*. Megasporogenesis starts from a subepidermal cell at the top of the ovule primordium and the differentiates into MMC. After one meiosis, the cell formed four haploid megaspores. Among them, the three megaspores near the micropyle end degenerated rapidly, and only the one remaining megaspore survives, which is then named as the functional megaspore (FM). Functional megaspores undergo mitosis to form syncytia and then undergo cellularization to form a mature female gametophyte with multicellular structure.

As the key transcription factor or transcription factor-like protein, *WUS* and *SPL/NZZ* must be associated with key genes of other pathways and/or involved in their transcriptional regulation. A recent report showed that loss of function mutant of *nucleolar histone deacetylases 1 (HDT1)* and *HDT2* affects the expression of *WUS* and generates multiple MMC-like cells^[9]. The regulation between plant hormones and *WUS* or *SPL/NZZ* proteins might also be necessary to generate the microenvironment to establish the female germline. Plant hormones play an important role in the physiological processes of plant development, especially by offering location information to support local differentiation events^[10]. Auxin may play an important role in female germline formation since auxin creates a phytohormone gradient in apical epidermal cells (based on the synthetic auxin sensor, *DR5*)^[11–13]. In addition, *PIN-FORMED1 (PIN1)*, which localizes in the epidermal cell layer of the nucellus adjacent to the region where the MMC is formed, can facilitate polar auxin transport in *Arabidopsis*^[14,15]. Although auxin accumulates in the tip of the apical epidermal cell layer, auxin carriers mutants show defects at later stages rather than the initiation of female gametophyte development, suggesting that auxin is essential for female gametophyte development. Still, its role in germline specification remains unclear^[14].

Regulations in the sub-epidermal cell layer control female germline initiation

The cells below the epidermis cell layer of the nucellus can be defined as the sub-epidermal cell layer (also called L2 cells)^[3]. During the ovule initiation stage, the sub-epidermal cell layer functions as a sporogenous tissue full of the nucellar dome.

Only one cell in the sub-epidermal cell layer becomes the generative cell through differentiation, first developing into an archesporial cell (AC) and then into the MMC^[3]. After MMC formation, the sub-epidermal cells can be divided into MMC and companion cells^[3]. Since the MMC is specialized from sub-epidermal cells, the precise regulation of sub-epidermal cells is very important. With the deepening of research, more and more genes expressed in MMC or companion cells have been reported to participate in MMC specialization (Fig. 2b & c).

Once the MMC begins to expand, its epigenetic and transcriptional status changes quickly from that of the circumambient sporophytic tissues, and it involves changes in chromatin content and chromatin reprogramming, including the condensed chromatin or heterochromatin dynamics, the key histone modifications and changes in the core histone variants^[16–18]. Recent studies using non-denaturing whole-mount DNA staining and confocal imaging revealed that the MMC differentiation exhibits chromatin decondensation and a decrease in heterochromatin^[19]. Furthermore, linker histone (H1) regulates chromatin compaction by binding linker DNA and core nucleosomes to stabilize the high-order chromatin structure^[20]. There are three H1 genes in the *Arabidopsis* genome, including H1.1, H1.2, and H1.3^[21]. Compared to the surrounding somatic cells, the levels of H1.1 and H1.2 quickly decrease in the early MMC stage and are undetected at the consecutive stage, indicating that the reduction of H1 might be the result of somatic cell specialization into MMC^[18,19,22]. Similar to H1.1 and H1.2, histone 3 variant H3.1 is evicted in the MMC during MMC differentiation^[23]. Moreover, the gene expression levels also change by distinct levels of histone H3 modifications and active RNA polymerase II^[19]. Besides, histone H3 lysine 4 trimethylation (H3K4me3) and H3K27me3 are two prominent

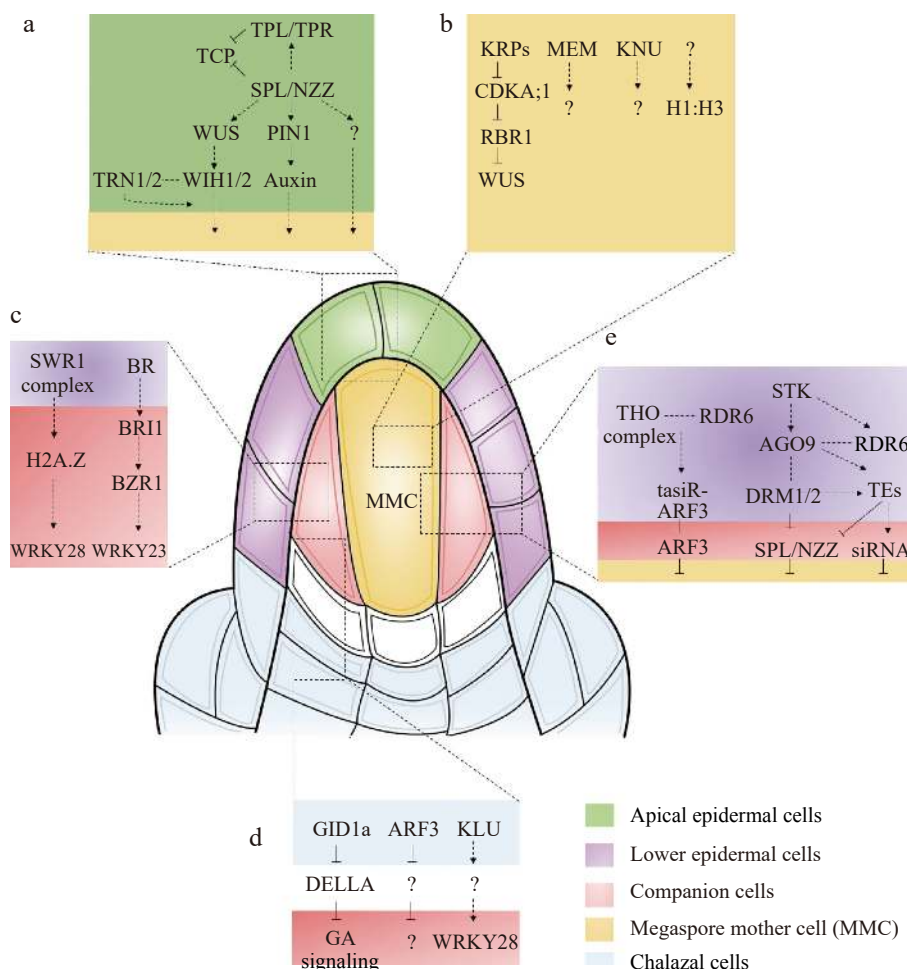


Fig. 2 General mechanisms and key pathways involved in MMC specification. (a) The major signaling pathways of MMC specification in the upper epidermal nucellus. (b) The major signaling pathways of MMC specification in the MMC. (c) The major signaling pathways of MMC specification in the companion cells. (d) The major signaling pathways of MMC specification in the chalaza. (e) The key pathways of ubiquitously expressed genes involved in MMC specification.

histone methylation marks, which act as active and repressive transcription marks, respectively^[24]. Immunofluorescence assays suggest that H3K4me3 increases while H3K27me3 reduces in the MMC in contrast to those in the circumambient cells, suggesting that many genes are required during the MMC specialization process^[19].

Apart from chromatin reprogramming, the changes in expression dynamics of key genes of the sub-epidermal cells (MMC or companion cells) play key roles in female germline initiation. The first type of gene is expressed explicitly in MMC. Among them, the KNUCKLES (KNU) gene, encoding a C2H2 zinc-finger protein, is involved in the initiation of female germline development^[25]. Although KNU has been shown to accumulate in the MMC and is commonly used as a marker of MMC identity, its functions in MMC specialization are still unknown. Another MMC identity marker is ARGONAUTE 9 (AGO9), which localizes in the MMC nucleus^[26]. The localization of the AGO9 protein is also very important because of its vital function in limiting female germline identity. And initial immunolabeling studies showed that AGO9 protein was limited to the epidermal cell of the nucellus^[27]. However, further studies suggested AGO9 protein localization in the nucleus of MMC and the cytoplasm of adjacent L2 ovule cells as well as in the epidermal cell^[27].

Unlike KNU, the mutant of *ago9* displayed approximately 50% multiple MMC-like cells^[26]. Interestingly, mutations in the other AGO proteins (AGO4, AGO6, and AGO8) in the same clade of AGO9 also show multiple MMC-like cells^[28]. In addition, mutations of AGO9 related epigenetic pathway genes, *RNA-DEPENDENT POLYMERASE 6 (RDR6)* and *SUPPRESSOR OF GENE SILENCING 3 (SGS3)*, also promote the L2 cells surrounding the MMC into MMC^[29–31]. The third gene specifically expressed in MMC is *MNEME (MEM)*, which encodes a possibly ATP-dependent RNA helicase and belongs to epigenetic regulators^[32]. Mutations in *MEM* lead to the formation of multiple MMC-like cells in the nucellus^[32]. However, it is still unclear how the activation of *MEM* in the germline regulates somatic cell identity. Furthermore, a ligand-receptor signaling pathway in germline formation has been established in rice. The peptide ligand TAPETUM DETERMINANT-LIKE 1A (*OsTDL1A*) is specifically expressed in MMC, and its receptor MULTIPLE SPOROCTE 1 (*MSP1*), expressed in the somatic cells surrounding the MMC, are required for MMC formation^[33,34]. A similar regulation mechanism has also been reported in maize, where *MULTIPLE ARCHESPORIAL CELLS 1 (MAC1)*, an ortholog of *OsTDL1A*, is specifically expressed in the MMC and represses multiple MMC formation in maize^[35,36].

Relative to L2 cells, the other type is the gene specifically expressed in companion cells. The first reported gene specifically expressed in companion cells is *WRKY28*, a zinc-finger WRKY TF^[37]. In the *wrky28* mutant, multiple MMC-like cells were present in ovules^[37]. Further analysis showed that *WRKY28* is activated by cytochrome P450 gene *KLU* through H2A.Z incorporation mediated by the chromatin remodeling complex SWR1, suggesting that transcription of *WRKY28* is induced by diffusion of the mobile signal generated by *KLU* into sub-epidermal nucellar cells^[37]. It is believed that other local signals in companion cells may also regulate *WRKY28* transcription. The *WRKY23* is another gene specifically expressed in companion cells^[38]. Recently, it was demonstrated that BR signaling restricts multiple sub-epidermal cells in ovule primordia at distal nucellus from acquiring MMC-like cell identity through transient activation of the *WRKY23*^[38]. Since it is derived from the same ancestor cell, it is believed that the characteristics of L2 cells are similar, and the signal exchange may be more frequent. If the gene expressed in companion cells is ectopically expressed in MMC, will it induce multiple MMC phenotypes or restrict MMC formation? To answer this question, *WRKY23* and *WRKY28* were ectopically expressed in MMC using the *KNU* promoter. The results showed that ectopically expressed *WRKY23* by *KNU* promoter causes multiple MMC-like cells in ovules^[38]. However, no obvious abnormal ovules were observed in ectopically expressed *WRKY28* transgenic plants, suggesting that *WRKY23* and *WRKY28* act in different signaling networks.

Non-cell-autonomous pathways in the chalaza regulate female germline formation

It has been reported that the ovule primordium elongates and develops three parts: the nucellus, chalaza and funiculus. The chalaza (the area where the integument and nucellus fuse) is in contact with the placenta in the ovary wall, and the tissue is not clear. The integument originates from the base of the ovule primordium. According to the structural observation, chalaza comprises four layers of cells. Most genes expressed in the chalaza may play key roles in integument development (Fig. 2d), such as *INNER NO OUTER (INO)*^[39], *AINTEGUMENTA (ANT)*^[40] and *ABERRANT TESTA SHAPE/KANADI4 (ATS/KAN4)*^[41]. Since the position of the chalaza is relatively far from the L2 layer cells, how the signaling in the chalaza regulates female germline formation is largely unknown.

Recent reports showed that trans-acting small interfering RNAs known as tasiR-ARFs work non-cell-autonomously to inhibit the expression of *AUXIN RESPONSE FACTOR3 (ARF3)* in Arabidopsis and prevent the formation of multiple MMCs^[42]. The expression of *ARF3* was detected in the central chalazal zone, while the expression of *ARF3m*, which was resistant to tasiR-ARF, diffused from the chalazal region to the distal nucellus except for the MMC, including the cells adjacent to the MMC, resulting in multiple MMC formation^[42]. Furthermore, the expression of *ARF3-GFP* in tasiR-ARFs biogenesis mutant *tex1* extended in the lateral direction to the epidermal cells^[42], which resemble the pattern of *ARF3m-GFP*, indicating that *TEX1*- and *TAS3*-mediated restriction of *ARF3* expression limits supernumerary MMC formation non-cell-autonomously. Moreover, when *WRKY28* promoter was used to express *ARF3m*, it showed *ARF3m* expression in hypodermal cells surrounding

the MMC but not in epidermal cells, leading to more than one MMC formation^[43]. These findings suggest that tasiR-ARFs inhibit ectopic MMC fate by repressing *ARF3* in these hypodermal cells. In addition, *arp6 klu* double mutant and *wrky28* mutant ovules present multiple MMC^[37]. *KLU*, expressed in the inner integument primordia, promotes the expression of *WRKY28* non-cell-autonomously through SWR1-mediated H2A.Z deposition at *WRKY28*, which suggests that *KLU* genetically interacts with SWR1 to suppress germline identity in the sub-epidermal nucellar cells through generating a mobile signal that diffuses from the base of the inner integument to adjacent tissues^[37].

Another gene expressed in the chalaza region is *GIBBERELLIN-INSENSITIVE DWARF1a (GID1a)*, which encodes a gibberellin receptor^[44]. There are three *GID1* genes, *GID1a*, *GID1b* and *GID1c*, in the Arabidopsis genome^[44]. Functional analysis of *gid1a* showed no obvious phenotype in ovule development. However, overexpression of *GID1a* using *35S* promoter and ovule-specific *SEEDSTICK* promoter (*STK*) displayed additional MMC-like cells^[44], suggesting that *GID1a* is involved in MMC differentiation. Further analysis showed that in *pSTK:GID1a* ectopic overexpressing ovules, there are multiple subepidermal nucellus cells that enlarge, but only one of them obtains the germline identity, while the remaining enlarged cells do not develop further.

After the ovule primordium begins to develop, the three PD (Proximal-Distal) pattern elements are successively established during primordium outgrowth, starting from the distal nucellus, then the central chalaza and finally the proximal funiculus. This implies that chalaza may play an important role in the communication between nucellus and funiculus. It is anticipated that more important factors involved in MMC specialization in the chalaza region will be identified and cloned in the future.

Non-cell-type-specific factors are capable of controlling the female germline formation

In addition to the specifically expressed genes, some ubiquitously expressed genes in the early ovules also regulate MMC differentiation (Fig. 2e). Germline specification and subsequent acquisition of meiotic competence require cell cycle regulators. During the cell cycle in Arabidopsis, the inhibitory effect of the Rb homolog RETINOBLASTOMA-RELATED 1 (*RBR1*) is repressed by cyclin-dependent kinase A;1 (*CDKA;1*) when it is phosphorylated^[45]. Several functionally redundant CDK inhibitors of the KIP-RELATED PROTEINs (*KRPs*, also called *ICKs*) repress *CDKA;1*^[46]. Recent studies showed a gene regulatory network (GRN), *KRPs-CDKA;1-RBR1*, restricting MMC formation to a single cell^[45]. All of the genes in this GRN are ubiquitously expressed in the early ovules. *rbr1* mutant and *krp4 krp6 krp7* triple mutant show ectopic MMC-like cells that appear to be caused by mitosis of the MMC^[45]. Interestingly, the expression of *KRP7* under the control of the *KNU* promoter largely rescued the *krp* triple-mutant phenotype, indicating that the *KRPs* expressed in MMC, rather than those ubiquitously expressed in the whole ovule, may be the dominant part of MMC specialization^[45]. At the same time, Cao et al. showed a similar phenotype in the septuple *ick1 ick2 ick3 ick4 ick5 ick6 ick7* (named as *ick1/2/3/4/5/6/7*) mutant^[47]. Surprisingly, *WUS* mutation significantly restored the phenotype of multiple MMCs in the *rbr1* mutant^[45]. Nevertheless, ectopic expression

of *WUS* failed to induce the MMC to enter mitosis^[45]. In contrast, ectopic expression of *SPL/NZZ* induces multiple MMC. A recent report showed a GRN, *STK-AGO9-DRM1/2-SPL/NZZ* is required to restrict the expression of *SPL/NZZ* to ensure a single MMC formation^[48]. The DRM methyltransferase double mutants *drm1drm2* present multiple MMCs, similar to the *stk*, *ago9* and *rdm6* mutants^[48]. Ectopic expression of *SPL/NZZ* was observed in these mutants, indicating that the excessive MMC-like cell development might be due to the ectopic activation of *SPL/NZZ*^[48]. In addition, a series of heterochromatin regulators, including MET1 (a DNA methyltransferase responsible for CG methylation)^[49], ARID1 (ARID domain-containing 1)^[49], and TRAMGaP (TRAF Mediated Gametogenesis Progression)^[50], are ubiquitously expressed during MMC formation. Mutations in these genes exhibit additional MMC-like cells in the ovules.

Transcriptomics application in the quest for germline specification factors

Although many factors involved in megasporogenesis have been identified by genetic screening, the molecular mechanism controlling germline specification and development remains unclear. A transcriptome is dynamic and a distinct representative of the cellular state. In addition, the convenience of genome-wide analysis using sequencing technologies made transcriptome profiling an integral part of almost all genomic studies of biological processes. Transcriptome profiling has identified multiple pathways acting in the MMC specification in Arabidopsis^[32]. Schmidt et al. used a combination of laser-assisted microdissection (LAM) and Affymetrix ATH1 GeneCHIP profiling to analyze the transcriptome of the MMC as well as the surrounding ovule sporophytic tissue and revealed more than 9,000 MMC expressed genes^[32]. Further analysis revealed that *MEM* was exclusively expressed in MMC and more than one MMC was observed in ovules of heterozygous *mem/MEM* plants^[32]. Using the same methods, Tucker and his colleague identified genes expressed in and/or around developing megaspores during the transition to megagametogenesis^[51]. They found that *AGO5* was expressed around reproductive cells during megasporogenesis, and mutants in *AGO5* displayed multiple MMC^[51]. Besides, *spl* mutant is a recessive sporophytic mutation that completely abolishes both male and female sporogenesis. Consistently, a differential gene expression profiling (DEG) using high-throughput Tag-seq analysis was carried out to identify genes with higher expression levels in WT ovules undergoing megasporogenesis compared with those from homozygous *spl* mutants^[52]. This study identified 862 candidate genes and revealed the involvement of *KLU* in female meiosis^[52].

The emerging high-throughput technology of single-cell RNA sequencing (scRNA-seq), which enables the capture of the genetic information of the individual cell, generates extensive genetic profiling of thousands of single cells in a population, reflecting the heterogeneous nature of different cell types and the biological complexity of individual tissues. Hou et al. identified 16,872 single cells of Arabidopsis ovule primordia at three developmental time points during female germline differentiation and observed dynamic waves of gene expression along the developmental trajectory^[53]. Furthermore, the study indicated that *ERECTA* (*ER*) receptor-like kinase family is important in restricting MMC specification to a single cell and promoting MMC differentiation^[53].

Positional signal for the establishment of functional megaspores

After the archesporial cell (AC) expands and specializes in MMC, it undergoes meiosis to produce four megaspores. Callose (beta-1,3-glucan) precedes the initiation of meiosis, and callose deposition/dynamics may play important roles in the plant meiosis process through positional signals and cell-to-cell communication^[54]. However, it is unclear how the callose contribute to the process of meiosis. In most higher plants, such as Arabidopsis, only one megaspore at the chalaza can develop into the FM, whereas the other three megaspores far from the chalaza degenerate, indicating a positional signal from the chalazal megaspore that may promote degeneration of the other spores, resulting in a single embryo sac per ovule. The genetic basis and molecular mechanisms that control the formation, death, or survival of megaspores remain largely unknown. Only a few genes that participate in the specification of the FM have been identified. *ANTIKEVORKIAN* (*AKV*) is the first reported gene involved in the specification of the FM^[55]. In the *akv* mutant, the number and position of surviving megaspores are variable^[55]. Similar to the *akv* mutant, in the *ick1/2/3/4/5/6/7* septuple mutant supernumerary FMs were formed^[47]. Interestingly, the multiple surviving megaspores are capable of developing into mature embryo sacs in both *akv* and *ick1/2/3/4/5/6/7* septuple mutants^[47,55]. Overexpression of *ARABINOGALACTAN PROTEIN 18* (*AGP18*) causes multiple surviving megaspores with an FM identity^[56,57]. However, it is insufficient to promote FM differentiation, suggesting that *AGP18* may act as a positive regulator for the selection of megaspores^[56,57]. By contrast, the FM specification is lost in triple mutants of cytokinin receptor genes (*ahk2 ahk3 cre1*)^[58]. These findings indicate that the positional signals are essential for determining the survival of the megaspore.

Conclusion and perspectives

Since MMC is the first female germline cell lineage in plants, understanding the molecular mechanism of MMC specialization and differentiation is particularly important for plant reproduction. In recent years, some of the key developmental genes that promote the regulation and differentiation of MMC have been identified using genetic screens, such as nuclear localization proteins (*SPL/NZZ*), Cyclin-related proteins (*KRPs*), and transcription factors (*WUS*). More advanced tools, including RNA-seq, single-cell transcriptomic, and CRISPR have been used to investigate MMC specification as research technology has progressed considerably. Currently, the reviews of MMC mainly elaborate on epigenetics, microRNA, developmental processes, etc. This review is the first to summarize MMC formation from the perspective of the positional signal. This provides space for future research on MMC development, which is even more refined than currently described.

The position and the number of the single female germline precursor cell at the distal part of ovule primordia appear to be very precisely controlled. In the early stage of ovule development, two cell types can be distinguished: epidermis layer cells and subepidermal layer cells. The most distal L2 cell will become the precursor of the female germline, first via differentiation into an archesporial cell (AC) and then into the MMC^[59,60]. Although diverse regulatory pathways tightly regulate the initiation of MMC specification at particular

positions within the sporophyte in *Arabidopsis*^[59,60], little is known about the interaction of pathways and the integration of polarity cues in the regulation of these processes. In view of the differential patterns of cell layers, a positional signal was established in the developmental process of female germline cell specification and differentiation. Positional signal regulation of growth and development has been reported in different tissues, including the root epidermis^[61] and embryo sac^[62,63]. Development and cell differentiation in the early *Arabidopsis* ovule are another attractive and appropriate model system for deciphering the mechanism in the regulation of cells and especially germ cell specification by position signaling. Future studies may focus on the positional signal establishment of key genes in plant reproduction via regulating MMC differentiation. Advanced technology may help to find new germline specification factors.

Acknowledgments

We apologize for not being able to refer to some related literature due to the page limit. We acknowledge the funding from the National Natural Science Foundation of China (32170352, 31970333, 32270366), the Excellent Youth Foundation of Fujian Province to H.C. (2022J06014), the Excellent Youth Foundation of Fujian Agriculture and Forestry University to H.C. (xjq202108), the Guangxi Distinguished Experts Fellowship to Y. Q., the Science and Technology Major Project of Guangxi (Gui Ke AA22068096) and the Science and technology innovation project of Pingtan Science and Technology Research Institute (PT2021007, PT2021003).

Conflict of interest

The authors declare that they have no conflict of interest.

Dates

Received 7 August 2022; Accepted 15 September 2022; Published online 2 November 2022

REFERENCES

- Yang W, Shi D, Chen Y. 2010. Female gametophyte development in flowering plants. *Annual Review of Plant Biology* 61:89–108
- Grossniklaus U. 2011. Plant germline development: a tale of cross-talk, signaling, and cellular interactions. *Sexual Plant Reproduction* 24:91–95
- Pinto SC, Mendes MA, Coimbra S, Tucker MR. 2019. Revisiting the Female Germline and Its Expanding Toolbox. *Trends in Plant Science* 24:455–67
- Yang W, Ye D, Xu J, Sundaresan V. 1999. The *SPOROCTELESS* gene of *Arabidopsis* is required for initiation of sporogenesis and encodes a novel nuclear protein. *Genes & Development* 13:2108–17
- Wei B, Zhang J, Pang C, Yu H, Guo D, et al. 2015. The molecular mechanism of sporocyteless/nozzle in controlling *Arabidopsis* ovule development. *Cell Research* 25:121–34
- Chen G, Sun J, Liu M, Liu J, Yang W. 2014. *SPOROCTELESS* is a novel embryophyte-specific transcription repressor that interacts with TPL and TCP proteins in *Arabidopsis*. *Journal of Genetics and Genomics* 41:617–25
- Lieber D, Lora J, Schrempp S, Lenhard M, Laux T. 2011. *Arabidopsis* *WIH1* and *WIH2* genes act in the transition from somatic to reproductive cell fate. *Current Biology* 21:1009–17
- Cnops G, Neyt P, Raes J, Petrarulo M, Nelissen H, et al. 2006. The *TORNADO1* and *TORNADO2* genes function in several patterning processes during early leaf development in *Arabidopsis thaliana*. *The Plant Cell* 18:852–66
- Luo Y, Shi D, Jia P, Bao Y, Li H, et al. 2022. Nucleolar histone deacetylases HDT1, HDT2, and HDT3 regulate plant reproductive development. *Journal of Genetics and Genomics* 49:30–39
- Santner A, Calderon-Villalobos LIA, Estelle M. 2009. Plant hormones are versatile chemical regulators of plant growth. *Nature Chemical Biology* 5:301–7
- Larsson H, Vivian-Smith A, Offringa R, Sundberg E. 2017. Auxin Homeostasis in *Arabidopsis* Ovules Is Anther-Dependent at Maturation and Changes Dynamically upon Fertilization. *Frontiers in Plant Science* 8:1735
- Swarup R, Bhosale R. 2019. Developmental Roles of AUX1/LAX Auxin Influx Carriers in Plants. *Frontiers in Plant Science* 10:1306
- Wang L, Liu Y, Aslam M, Jakada BH, Qin Y, Cai H. 2021. The Glycine-Rich Domain Protein GRDP2 Regulates Ovule Development via the Auxin Pathway in *Arabidopsis*. *Frontiers in Plant Science* 12:698487
- Ceccato L, Masiero S, Sinha Roy D, Bencivenga S, Roig-Villanova I, et al. 2013. Maternal control of PIN1 is required for female gametophyte development in *Arabidopsis*. *PLoS One* 8:e66148
- Pagnussat GC, Alandete-Saez M, Bowman JL, Sundaresan V. 2009. Auxin-dependent patterning and gamete specification in the *Arabidopsis* female gametophyte. *Science* 324:1684–9
- van Zanten M, Koini MA, Geyer R, Liu Y, Brambilla V, et al. 2011. Seed maturation in *Arabidopsis thaliana* is characterized by nuclear size reduction and increased chromatin condensation. *PNAS* 108:20219–24
- Wang H, Dittmer TA, Richards EJ. 2013. *Arabidopsis* CROWDED NUCLEI (CRWN) proteins are required for nuclear size control and heterochromatin organization. *BMC Plant Biology* 13:200
- Jiang T, Zheng B. 2022. Epigenetic regulation of megaspore mother cell formation. *Frontiers in Plant Science* 12:826871
- She W, Grimanelli D, Rutowicz K, Whitehead MW, Puzio M, et al. 2013. Chromatin reprogramming during the somatic-to-reproductive cell fate transition in plants. *Development* 140:4008–19
- Osipova TN, Pospelov VA, Svetlikova SB, Vorob'ev VI. 1980. The role of histone H1 in compaction of nucleosomes. Sedimentation behaviour of oligonucleosomes in solution. *European Journal of Biochemistry* 113:183–88
- Ascenzi R, Gantt JS. 1997. A drought-stress-inducible histone gene in *Arabidopsis thaliana* is a member of a distinct class of plant linker histone variants. *Plant Molecular Biology* 34:629–41
- Hernandez-Lagana E, Mosca G, Mendocilla-Sato E, Pires N, Frey A, et al. 2021. Organ geometry channels reproductive cell fate in the *Arabidopsis* ovule primordium. *Elife* 10:e66031
- Hernandez-Lagana E, Autran D. 2020. H3.1 eviction marks female germline precursors in *Arabidopsis*. *Plants (Basel)* 9:1322
- Guo L, Yu Y, Law JA, Zhang X. 2010. SET DOMAIN GROUP2 is the major histone H3 lysine 4 trimethyltransferase in *Arabidopsis*. *PNAS* 107:18557–62
- Payne T, Johnson SD, Koltunow AM. 2004. *KNUCKLES (KNU)* encodes a C2H2 zinc-finger protein that regulates development of basal pattern elements of the *Arabidopsis* gynoecium. *Development* 131:3737–49
- Olmedo-Monfil V, Durán-Figueroa N, Arteaga-Vázquez M, Demesa-Arévalo E, Autran D, et al. 2010. Control of female gamete formation by a small RNA pathway in *Arabidopsis*. *Nature* 464:628–32
- Rodríguez-Leal D, León-Martínez G, Abad-Vivero U, Vielle-Calzada JP. 2015. Natural variation in epigenetic pathways affects the specification of female gamete precursors in *Arabidopsis*. *The Plant Cell* 27:1034–45
- Hernández-Lagana E, Rodríguez-Leal D, Lúa J, Vielle-Calzada JP. 2016. A multigenic network of ARGONAUTE4 clade members controls early megaspore formation in *Arabidopsis*. *Genetics* 204:1045–56

29. Peragine A, Yoshikawa M, Wu G, Albrecht HL, Poethig RS. 2004. *SGS3* and *SGS2/SDE1/RDR6* are required for juvenile development and the production of trans-acting siRNAs in *Arabidopsis*. *Genes & Development* 18:2368–79
30. Yoshikawa M, Peragine A, Park MY, Poethig RS. 2005. A pathway for the biogenesis of trans-acting siRNAs in *Arabidopsis*. *Genes & Development* 19:2164–75
31. Himber C, Dunoyer P, Moissiard G, Ritzenthaler C, Voinnet O. 2003. Transitivity-dependent and -independent cell-to-cell movement of RNA silencing. *The EMBO Journal* 22:4523–33
32. Schmidt A, Wuest SE, Vijverberg K, Baroux C, Kleen D, et al. 2011. Transcriptome analysis of the *Arabidopsis* megaspore mother cell uncovers the importance of RNA helicases for plant germline development. *PLoS Biology* 9:e1001155
33. Zhao X, de Palma J, Oane R, Gamuyao R, Luo M, et al. 2008. OsTDL1A binds to the LRR domain of rice receptor kinase MSP1, and is required to limit sporocyte numbers. *The Plant Journal* 54:375–87
34. Nonomura KI, Miyoshi K, Eiguchi M, Suzuki T, Miyao A, et al. 2003. The *MSP1* gene is necessary to restrict the number of cells entering into male and female sporogenesis and to initiate anther wall formation in rice. *The Plant Cell* 15:1728–39
35. Sheridan WF, Avalkina NA, Shamrov II, Batyeva TB, Golubovskaya IN. 1996. The *mac1* gene: controlling the commitment to the meiotic pathway in maize. *Genetics* 142:1009–20
36. Sheridan WF, Golubeva EA, Abrahams L, Golubovskaya IN. 1999. The *mac1* mutation alters the developmental fate of the hypodermal cells and their cellular progeny in the maize anther. *Genetics* 153:933–41
37. Zhao L, Cai H, Su Z, Wang L, Huang X, et al. 2018. *KLU* suppresses megasporocyte cell fate through *SWR1*-mediated activation of *WRKY28* expression in *Arabidopsis*. *PNAS* 115:E526–E535
38. Cai H, Liu L, Huang Y, Zhu W, Qi J, et al. 2022. Brassinosteroid signaling regulates female germline specification in *Arabidopsis*. *Current Biology* 32:1102–1114.E5
39. Villanueva JM, Broadhvest J, Hauser BA, Meister RJ, Schneitz K, et al. 1999. *INNER NO OUTER* regulates abaxial-adaxial patterning in *Arabidopsis* ovules. *Genes & Development* 13:3160–69
40. Klucher KM, Chow H, Reiser L, Fischer RL. 1996. The *AINTEGUMENTA* gene of *Arabidopsis* required for ovule and female gametophyte development is related to the floral homeotic gene *APETALA2*. *The Plant Cell* 8:137–53
41. Kelley DR, Skinner DJ, Gasser CS. 2009. Roles of polarity determinants in ovule development. *The Plant Journal* 57:1054–64
42. Su Z, Zhao L, Zhao Y, Li S, Won S, et al. 2017. The THO Complex Non-Cell-Autonomously Represses Female Germline Specification through the *TAS3-ARF3* Module. *Current Biology* 27:1597–1609.E2
43. Su Z, Wang N, Hou Z, Li B, Li D, et al. 2020. Regulation of Female Germline Specification via Small RNA Mobility in *Arabidopsis*. *The Plant Cell* 32:2842–54
44. Gallego-Giraldo C, Hu J, Urbez C, Gomez MD, Sun T, et al. 2014. Role of the gibberellin receptors *GID1* during fruit-set in *Arabidopsis*. *The Plant Journal* 79:1020–32
45. Zhao X, Bramsiepe J, Van Durme M, Komaki S, Prusicki MA, et al. 2017. *RETINOBLASTOMA RELATED1* mediates germline entry in *Arabidopsis*. *Science* 356:eaaf6532
46. De Veylder L, Beeckman T, Beemster GTS, Krols L, Terras F, et al. 2001. Functional analysis of cyclin-dependent kinase inhibitors of *Arabidopsis*. *The Plant Cell* 13:1653–68
47. Cao L, Wang S, Venglat P, Zhao L, Cheng Y, et al. 2018. *Arabidopsis* *ICK/KRP* cyclin-dependent kinase inhibitors function to ensure the formation of one megaspore mother cell and one functional megaspore per ovule. *PLoS Genetics* 14:e1007230
48. Mendes MA, Petrella R, Cucinotta M, Vignati E, Gatti S, et al. 2020. The RNA-dependent DNA methylation pathway is required to restrict *SPOROCTELESS/NOZZLE* expression to specify a single female germ cell precursor in *Arabidopsis*. *Development* 147:dev194274
49. Li L, Wu W, Zhao Y, Zheng B. 2017. A reciprocal inhibition between *ARID1* and *MET1* in male and female gametes in *Arabidopsis*. *Journal of Integrative Plant Biology* 59:657–68
50. Singh SK, Kumar V, Srinivasan R, Ahuja PS, Bhat SR, et al. 2017. The *TRAF Mediated Gametogenesis Progression (TRAMGaP)* gene is required for megaspore mother cell specification and gametophyte development. *Plant Physiology* 175:1220–37
51. Tucker MR, Okada T, Hu Y, Scholefield A, Taylor JM, Koltunow AM. 2012. Somatic small RNA pathways promote the mitotic events of megagametogenesis during female reproductive development in *Arabidopsis*. *Development* 139:1399–404
52. Zhao L, He J, Cai H, Lin H, Li Y, et al. 2014. Comparative expression profiling reveals gene functions in female meiosis and gametophyte development in *Arabidopsis*. *The Plant Journal* 80:615–28
53. Hou Z, Liu Y, Zhang M, Zhao L, Jin X, et al. 2021. High-throughput single-cell transcriptomics reveals the female germline differentiation trajectory in *Arabidopsis thaliana*. *Communications Biology* 4:1149
54. Lora J, Yang X, Tucker MR. 2019. Establishing a framework for female germline initiation in the plant ovule. *Journal of Experimental Botany* 70:2937–49
55. Yang W, Sundaresan V. 2000. Genetics of gametophyte biogenesis in *Arabidopsis*. *Current Opinion in Plant Biology* 3:53–57
56. Acosta-García G, Vielle-Calzada JP. 2004. A classical arabinogalactan protein is essential for the initiation of female gametogenesis in *Arabidopsis*. *The Plant Cell* 16:2614–28
57. Demesa-Arévalo E, Vielle-Calzada JP. 2013. The classical arabinogalactan protein *AGP18* mediates megaspore selection in *Arabidopsis*. *The Plant Cell* 25:1274–87
58. Cheng CY, Mathews DE, Schaller GE, Kieber JJ. 2013. Cytokinin-dependent specification of the functional megaspore in the *Arabidopsis* female gametophyte. *The Plant Journal* 73:929–40
59. Vijayan A, Tofanelli R, Strauss S, Cerrone L, Wolny A, et al. 2021. A digital 3D reference atlas reveals cellular growth patterns shaping the *Arabidopsis* ovule. *eLife* 10:63262
60. Enugutti B, Oelschner M, Schneitz K. 2013. Microscopic analysis of ovule development in *Arabidopsis thaliana*. In *Plant Organogenesis. Methods in Molecular Biology*, ed. De Smet I. Vol. 959. Totowa, NJ:Humana Press. pp. 127–35 https://doi.org/10.1007/978-1-62703-221-6_7
61. Costa S. 2016. Cell identity: a matter of lineage and neighbours. *The New Phytologist* 210:1155–58
62. Gross-Hardt R, Kägi C, Baumann N, Moore JM, Baskar R, et al. 2007. *LACHESIS* restricts gametic cell fate in the female gametophyte of *Arabidopsis*. *PLoS Biology* 5:e47
63. Sun Y, Wang X, Pan L, Xie F, Dai B, et al. 2021. Plant egg cell fate determination depends on its exact position in female gametophyte. *PNAS* 118:e2017488118



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