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Signaling and Transcriptional Control of Reproductive Development in *Arabidopsis*

Review

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Plant reproductive development is a complex process with diploid and haploid phases, including male and female organogenesis, meiosis, gametogenesis, pollination and fertilization. A number of regulatory mechanisms control both diploid and haploid cell division and differentiation, especially cell–cell signaling pathways mediated by receptor-linked protein kinases with prominent roles in early male development, and hormonal signaling pathways crucial for later events in male and female reproductive development. Furthermore, transcriptional networks control the proper formation of specific cell layers and embryo sac cell specification.

Introduction

Angiosperms (flowering plants) are morphologically diverse, ecologically widespread, and among the most successful of living organisms [1]. This success is largely due to reproductive innovations, including colorful floral organs such as petals, the nutritious nectar and pollen that attract pollinators, and the protective fruits that are used as food by seed dispersers [2]. Angiosperms are also characterized by double fertilization, which leads to an embryo and a nutritional organ called the endosperm [3,4]. The development of complex reproductive organs involves many cellular and molecular processes, thereby offering great opportunities for understanding plant development. The nutritious reproductive structures (flowers, pollen, fruits and seeds) have been recognized by animals and humans and utilized as foods. Thus, the study of angiosperm reproductive development is relevant to both plant–animal interactions and agriculture.

Angiosperm reproductive development, as in other plants, involves diploid (sporophytic) and haploid (gametophytic) phases. The sporophytic phase contains male and female reproductive organs, the stamen and pistil, with somatic tissues and the germline. The latter consists of microspore (male) or megaspore (female) mother cells, each of which undergoes meiosis to produce four microspores or one functional megaspore, respectively. The haploid spores then divide to form multicellular haploid gametophytes, called the pollen grain (male) and embryo sac (female). Unlike mosses and ferns, the haploid phase of angiosperms is extremely reduced, consisting of only three (male) or seven (female) cells that are dependent on the sporophytic generation for nutrition and protection.

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Typically, as in *Arabidopsis thaliana* and rice (*Oryza sativa*), the stamen has two parts: the anther and the filament (Figure 1A). The anther has four lobes, each containing the microspore mother cells (Ms) and somatic tissues (Figure 1B). The filament connects the anther to the rest of the flower, providing physical support, water, nutrients, and signals. Anther development has been divided into 14 stages [5–7]. Stages 1–5 involve cell division and differentiation that form the four lobes, and stages 6–8 are marked by meiosis, spore formation, and their release, respectively. Stages 9–12 are characterized by pollen development, whereas stages 13 and 14 are defined by pollen release and collapse of the anther. The first pollen mitosis results in two cells, with the large vegetative cell subsequently surrounding the small generative cell. The vegetative cell will produce the pollen tube later during pollination, whereas the generative cell divides again to form two sperm cells.

The female reproductive organ, the pistil, is usually located centrally in the flower (Figure 1A) and contains the stigma, style and ovary. The *Arabidopsis* ovary has approximately 50 ovules, which are initiated from the placental tissue [8,9]. At the distal end of the ovule, a subepidermal cell becomes the megaspore mother cell (Figure 1D), which undergoes meiosis to produce one functional megaspore and three other haploid cells that degenerate. The female gametophyte (embryo sac) is embedded in the ovule and surrounded by the integuments (Figure 1E).

In more than 70% of plant species, including *Arabidopsis* and major crops such as soybean and cereals, female gametophyte development is of the Polygonum type, first described in the plant *Polygonum divaricatum* [8]. In the Polygonum-type female gametophyte, the megaspore nucleus goes through three rounds of mitosis to produce 8 nuclei. Cellularization then results in 7 cells: an egg cell and two adjacent synergids forming the egg apparatus at the distal or micropylar end of the embryo sac, a large central cell with two of the nuclei, and three antipodals occupying the proximal or chalazal end (Figure 1E).

Pollination begins when pollen lands on the stigma and produces a pollen tube, which then grows through the transmitting tissues of the pistil to deliver two sperm cells to the embryo sac [10] (Figure 1F), guided by the female sporophytic tissues and the female gametophyte. The pollen tube then enters the embryo sac through the micropyle and bursts to release two sperm cells [11]. In double fertilization, one sperm cell fertilizes the egg cell to form the embryo, and the other one fuses with the central cell to form the endosperm. After fertilization, the embryo and endosperm develop into a seed [12].

Major progress has been made recently in understanding the molecular mechanisms of plant reproductive development using the model plants *A. thaliana* and rice. Several comprehensive reviews have been published describing earlier studies on the male and female development processes, respectively [7,8,13–18]. And several excellent reviews have also discussed recent advances in the understanding of gene functions in the male gametophyte and

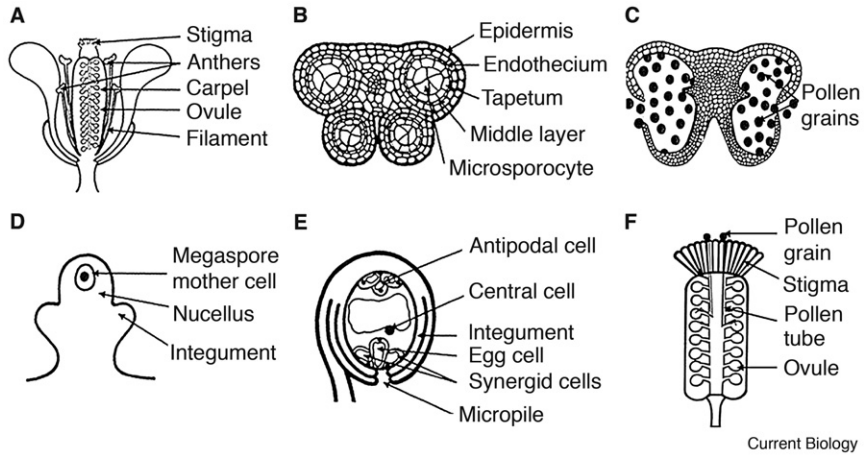

Figure 1. A schematic depiction of *Arabidopsis* floral structure, male and female gametophytes, and the pollination process.

(A) A flower showing floral organs. (B) An anther cross section at stage 5 with the epidermis, endothecium, middle layer, tapetum, and microsporocytes. (C) A mature anther showing dehiscence (release of pollen). (D) An early stage ovule with integument, nucellus, and the megaspore mother cell. (E) A mature female gametophyte at stage 6 with seven cells: one egg cell, two synergids, a central cell, and three antipodals. (F) Pollen tubes grow toward the ovules for fertilization.

for ovule development [16,19–26]. In this review, we will focus on the signaling and transcriptional regulation underlying anther development and embryo sac cell specification and the interaction between male and female gametophytes, emphasizing recent advances in *Arabidopsis*.

Stamen Cell Division and Differentiation

During stamen development, the anther primordium initiates from the floral meristem at stage 1, then the L2 archesporial cells, the cells that give rise to spores, divide to form the typical four-lobed anther by stage 5 [7]. Normal anther development requires temporal and spatial coordination of different cell types, being regulated by signals and regulatory molecules, including hormones, kinases, transcription factors and microRNAs [5,16–18,27–34] (Figure 2).

Control of Stamen Initiation and Identity

The stamen primordia are generated from the floral meristem, whose stem cell activity is maintained by the antagonistic actions of *CLAVATAs* (*CLVs*) and *WUSCHEL* (*WUS*) [35]. WUS activates the expression of the MADS-box gene *AGAMOUS* (*AG*) in the presence of *LEAFY* (*LFY*) at the center of the floral apex [36,37], whereas AG represses *WUS* by activating a repressor of *WUS*, KNUCKLES (KNU), thereby terminating the floral meristem [38] (Figure 2). *AG* further cooperates with the MADS-box genes *APETALA3* (*AP3*), *PISTILLATA* (*PI*), and *SEPALLATA1/2/3/4* to specify stamen identity [7] (Figure 2). In addition, *UFO* (*UNUSUAL FLORAL ORGAN*), and *ASK1* (*ARABIDOPSIS SKP1-LIKE1*) also play critical roles in regulating stamen development [7]. Recent studies have further revealed that the phytohormone auxin is essential for the correct stamen number [39,40].

Regulation of Anther Cell Layer Formation

During early anther development, the activity of the archesporial cells results in the eventual formation of four anther lobes. Recent studies have revealed that *ERECTA* (*ER*), *ER-LIKE 1* and *2* (*ERL1,2*) genes encoding leucine-rich repeat receptor-like kinases (LRR-RLKs) and MPK3/6 MAP kinases are important for lobe formation [41]. Mutations in these genes cause defects in anther lobe formation and aberrant cell patterning [41–43], suggesting that ER/ERL1/2 and MPK3/6 mediate cell–cell communication in this process. The RLKs might receive extracellular signals and transduce them inside the cell, possibly through the MAPK cascade, thereby coordinating the development of multiple cell layers. In addition, auxin also plays a role in regulating the number of anther lobes [39,40], but the relationship between auxin and the ER/ERL1/2 pathway is not known.

The archesporial cells divide at stage 3 to produce the primary sporogenous and primary parietal cells (PSC and PPC). The *SPOROCYTELESS/NOZZLE* (*SPL/NZZ*) gene, which encodes a putative transcription factor, is essential for this process. The *spl/nzz* mutations block the formation of both male and female sporocytes (i.e; the germline) [44–46]. *SPL/NZZ* is one of the earliest anther genes to be activated directly by AG [44] and regulates the expression of over 1,900 anther genes, which encode receptor-like protein kinases, transcription factors, and other proteins [47]. Two genes positively regulated by SPL/NZZ are the *BARELY ANY MERISTEM1* (*BAM1*) and *BAM2* genes encoding RLKs, which regulate the formation of anther somatic cell layers [48,49]. The *bam1 bam2* double mutant is male sterile, with many pollen mother-like cells but lacking the three subepidermal somatic cell layers [48,49]. SPL/NZZ and BAM1/2 form a feedback loop (Figure 2), with SPL/NZZ positively regulating the expression of *BAM1/2* but BAM1/2 restricting *SPL/NZZ* expression to sporogenous cells [48]. This relationship is similar to that between WUS and CLV1/3 [50], suggesting that *SPL/NZZ* maintains the sporogenous activity and that *BAM1/2* promote somatic growth, providing a balance between reproductive and somatic cells in the anther [48].

Specification of the Tapetal Layer

The tapetum is well known for its important function in supporting pollen development [7], such as providing lipids and other molecules for pollen wall formation, and is regulated by several genes, including *EXCESS MICROSPOROCYTES1* (*EMS1*)/*EXTRA SPOROGENOUS CELLS* (*EXS*), which encodes a putative LRR-RLK that is localized to the cell surface and possesses kinase activity *in vitro* [51,52]. The *ems1/exs* mutations cause a lack of tapetal cells and concurrent production of extra microsporocytes (i.e., microspore mother cells) [51,52], and induction of the expression of *EMS1* restored the *ems1* mutant to normal [53], suggesting EMS1 is required for specifying the tapetal cell fate. Interestingly, reduced expression of two other *Arabidopsis*

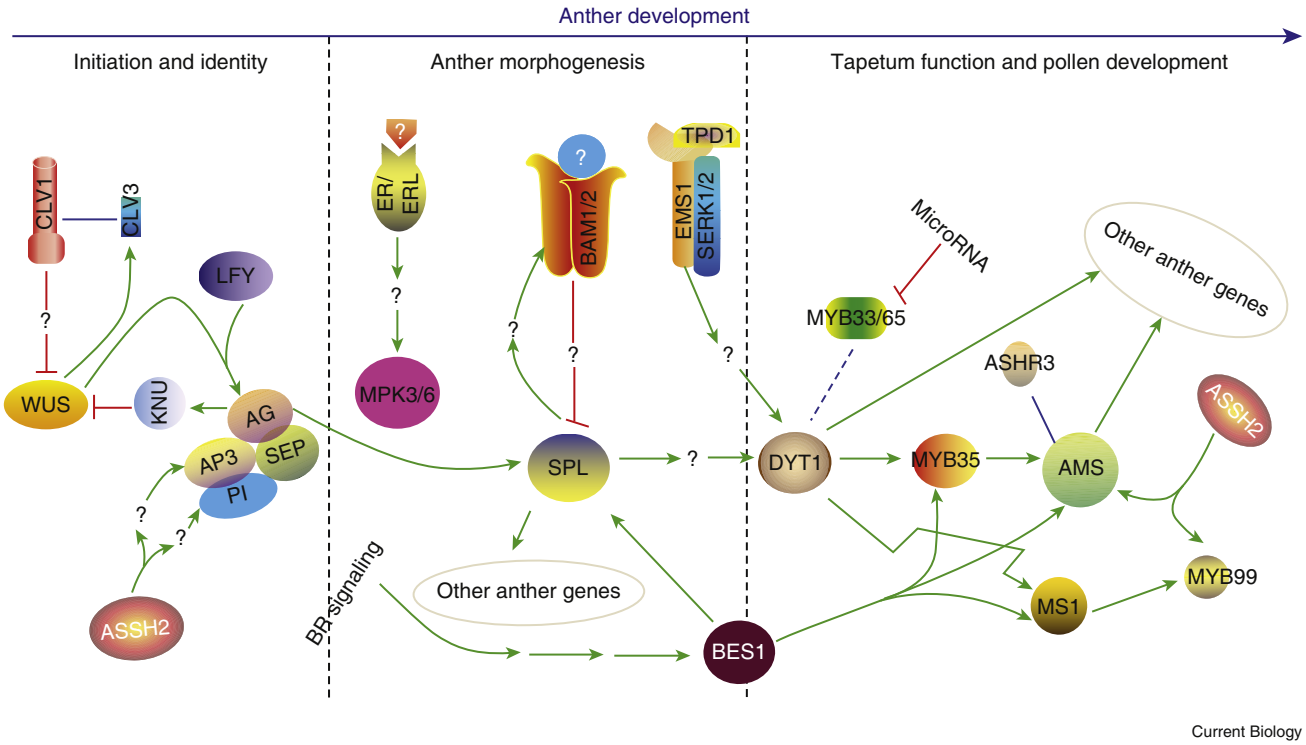

Figure 2. Genetic interactions during anther development.

Combined summary diagram showing the known genetic interactions between genes involved in stamen initiation and identity, anther morphogenesis, tapetum function and pollen development. The genetic interactions are not necessarily direct and include: feedback regulation between CLV and WUS, and between WUS and AG factors, which promote the initiation and identity of the anther; a postive feedback loop between BAM1/2 and SPL, regulation between ER and MPK3/4, and EMS1 and DYT1, all key pathways essential for the formation of the five-layered anther lobe morphology; and genetic interactions among DYT1, MYB35, AMS1, MS1, MYB99 and others, genes that are crucial for tapetum function and pollen development. Interactions between genes are shown by green arrows for positive regulation, red T-bars for negative regulation, and blue lines for protein interaction (solid lines for those with experimental evidence, and dashed line for interaction lacking experimental support). Question marks indicate the unknown components in the pathways.

LRR-RLKs, Somatic Embryogenesis Receptor-like Kinase1 (SERK1) and SERK2, caused phenotypes similar to those in *ems1/exs* mutants [54,55], suggesting that SERK1/SERK2 act in the same pathway as EMS1/EXS, even forming a receptor complex. Additionally, a putative secreted peptide, TPD1, might act as a ligand for EMS1/EXS, or the puative EMS1–SERK1/2 receptor complex [56–58] (Figure 2). The fact that loss of EMS1/EXS, SERK1/2 and TPD1 functions all resulted in the lack of the tapetal layer and extra meiotic cells strongly supports the idea that the microsporocyte is a default cell fate, and that cell–cell signaling triggers the differentiation of tapetal cells. Recent studies also discovered that mutations in *ems1* and *tpd1* homologues in both rice and maize result in similar phenotypes [59–61]; thus, the role of cell–cell signaling in determining the anther cell fate is conserved in flowering plants. Another RLK-encoding gene, *RECEPTOR-LIKE PROTEIN KINASE2* (*RPK2*), is essential for the formation of both the tapetum and middle layer [62], suggesting that additional cell–cell signaling pathways are important.

Transcriptional Regulation of Tapetal Function and Pollen Development

Gene functions in both the tapetum and pollen are crucial for normal pollen development. Given that the molecular regulation of male gametophytic functions from microspores to the mature pollen has recently been reviewed [24,26], the focus here is on sporophytic gene functions for tapetum and pollen development (Figure 2).

DYT1 encodes a member of the basic helix-loop-helix (bHLH) transcription factor family [63]. The *dyt1* mutants produce an abnormal tapetum and lack microsporocytes [63]. *DYT1* expression is dramatically reduced in *spl* and *ems1* mutants, suggesting that *DYT1* acts downstream of these genes (Figure 2); similarly, DYT1 is a positive regulator of several anther genes, including *MYB35/TDF1, MYB103/80, AMS* and *MS1* [63,64] (Figure 2). Another bHLH gene important for tapetum function and microspore development is *AMS* [65], a likely direct target of the transcription factor MYB35, which in turn is downstream of DYT1 [63,64]. AMS itself then regulates the expression of many floral genes [66], possibly in part via its interaction with ASHR3, a putative histone methylase and transcriptional regulator involved in anther development [67].

Two related MYB factors, AtMYB33 and AtMYB65, function redundantly in regulating tapetum and pollen development [68], as the *atmyb33 atmyb65* double mutant produces abnormal tapetal cells and no pollen [68] but the single mutants are normal. The *atmyb33 atmyb65* phenotypes are partially similar to those of the *dyt1* mutant; however, AtMYB33/65 act in a DYT1-independent manner. Because bHLH and MYB proteins can potentially form heterodimers, it is possible that DYT1 and AtMYB33/65 might form complexes with each other (Figure 2) or other

bHLH/MYB proteins to regulate tapetum development and function [63,69].

The *MS1* gene encoding a PHD-finger protein is also downstream of DYT1 and supports tapetum and pollen development. Although the *ms*1 anther can undergo meiosis to produce microspores, the tapetum subsequently becomes defective and pollen wall formation is abnormal [70,71]. The expression of numerous anther genes were altered in the *ms1* mutant, including those required for pollen wall formation, programmed cell death (PCD) of the tapetum, and hormone biosynthesis/signaling [47,70–72]. Thus, *MS1* is a key regulator of post-meiotic tapetal functions and pollen wall biogenesis.

Other genes important for tapetum and/or pollen development include *AtMYB99*, *AtMYB103* (also known as *AtMYB80*), *AtMYB32*, *AtMYB4,* and *AtMYB26.* Also, the ASH1 HOMOLOG2 (ASSH2) histone H3 methyltransferase is required for normal tapetum and pollen development, in part by regulating the expression of several anther genes, including *AP3*, *PI*, *AMS*, and *MYB99* [73] (Figure 2). Therefore, anther and pollen development is also regulated by chromatin structure and histone modifications; future analysis will likely uncover additional components of transcriptional regulation of anther development.

Very recently, the plant hormone brassinosteroid (BR) was shown to be important for anther and pollen development [74]. Mutants defective in BR synthesis or response are abnormal in male fertility with reduced pollen number and viability. Molecular analyses revealed that a transcription factor that functions in BR signaling pathways, BES1, binds to the promoter regions of several anther regulatory genes, including *SPL/NZZ*, *MYB35/TDF1*, *AMS*, and *MS*1 (Figure 2), uncovering a mechanistic connection between BR signaling and anther gene expression [74].

Hormonal Control of Pollen Maturation, Filament Elongation, and Anther Dehiscence

Plant hormones regulate many aspects of development, including anther and pollen development. The hormones auxin, ethylene, gibberellins, jasmonate (or jasmonic acids), and brassinosteroids have been demonstrated to regulate pollen maturation, filament elongation, and anther dehiscence in *Arabidopsis* [74–78]. Recently, studies discovered that genes involved in auxin biosynthesis and perception, and those responsive to auxin, are all expressed in late stages of anther development. Furthermore, mutants that are not auxin-responsive exhibited precocious or heterochronic pollen maturation and anther dehiscence [77]. The regulation of anther development by auxin is at least in part mediated by two Auxin Response Factors, ARF6 and ARF8, and a microRNA, MiR167, that targets the *ARF6/8* genes [79,80]. In addition, blocking auxin flow through the filament suppressed filament elongation and affected pollen mitosis, indicating the importance of polar auxin transport [77,81].

The lipid-derived signal molecule jasmonate or jasmonic acid (JA) also plays an important role in stamen development [33,76,82,83]. Mutants deficient in JA biosynthesis are male sterile, with defects in filament elongation, pollen maturation, and anther dehiscence [76,82,83]. Recent studies have found that JA acts downstream of several MYB factors (MYB108, MYB24) in regulating anther maturation [84]. Mutations affecting gibberellin (GA) synthesis cause similar phenotypes to those of JA mutants, suggesting that GA might regulate similar aspects of stamen development. Recent studies demonstrated that GA regulation of anther development is mediated by DELLA proteins, GID1 (GA INSENSITIVE SWARF 1), MYB factors, and microRNAs [75,78,85–88]. In addition, pollen maturation and anther dehiscence is also modulated by RPK2, which regulates the expression of genes for metabolic enzymes and several MYB genes [62].

The action of multiple hormones in regulating anther development is integrated at several points. For instance, auxin positively regulates JA biosynthesis via auxin response factors ARF6 and ARF8 [89], whereas GA promotes JA signaling by facilitating DELLA degradation [78]. Moreover, JA and ethylene act together with abscisic acid to promote the expression of *QRT2*, which is required for pollen grain separation and anther dehiscence [90]. Furthermore, hormonal pathways can regulate known anther transcription factors, thereby controlling anther development [78,91].

Cell Specification in the Female Gametophyte

In the developing ovule, the megaspore undergoes three rounds of mitosis and subsequent cellularization, resulting in the mature embryo sac with four cell types arranged in a precise spatial pattern: two synergids at positions closest to the micropyle, an egg cell slightly away from the micropyle, a diploid central cell at the center, and three antipodals at the chalazal end [8,9] (Figure 1E). Molecular and genetic analyses have found that the hormone auxin serves as a morphogenic signal for embryo sac patterning and that several genes play important roles in the cell specification process of the female gametophyte [92–95].

Regulation of Female Gametophyte Patterning by Auxin

Differential auxin distribution is often important for plant organogenesis, such as lateral root initiation and gynoecial apical–basal patterning [96–98]. Recently, it was proposed that an auxin gradient determines female gametophyte patterning [93], and this is supported by the finding that two *YUCCA* (*YUC*) genes encoding flavin monooxygenases crucial for local auxin biosynthesis [99,100] are expressed in the ovule in a pattern consistent with auxin acting as a cell-fate determinant [93]. According to this model, all embryo sac cells are competent to differentiate into any of the four cell types, but the auxin gradient determines the cell fate (Figure 3). The nuclei nearest to the micropyle receive the highest concentration of auxin and cellularize into synergids, the next nucleus receives less auxin and becomes that of the egg cell, and the nuclei perceiving the lowest amount of auxin develop into those of the antipodal cells. This model is also supported by the phenotypes of the maize *indeterminate gametophyte* (*ig1*) and *Arabidopsis retinoblastoma* (*rbr1*) mutant embryo sacs, which produce extra nuclei that cellularize into cell types consistent with their positions in the embryo sac [101,102].

However, the attributes of the central cell and antipodal cells are relatively stable even when auxin levels are abnormal, suggesting that auxin-independent mechanisms might contribute to central and antipodal cell specification. It is also possible that other hormones in addition to auxin are involved in female gametophyte patterning, including GAs and cytokinins, according to some indirect evidence [103,104]. Further studies are needed to ascertain their functions in embryo sac cell specification.

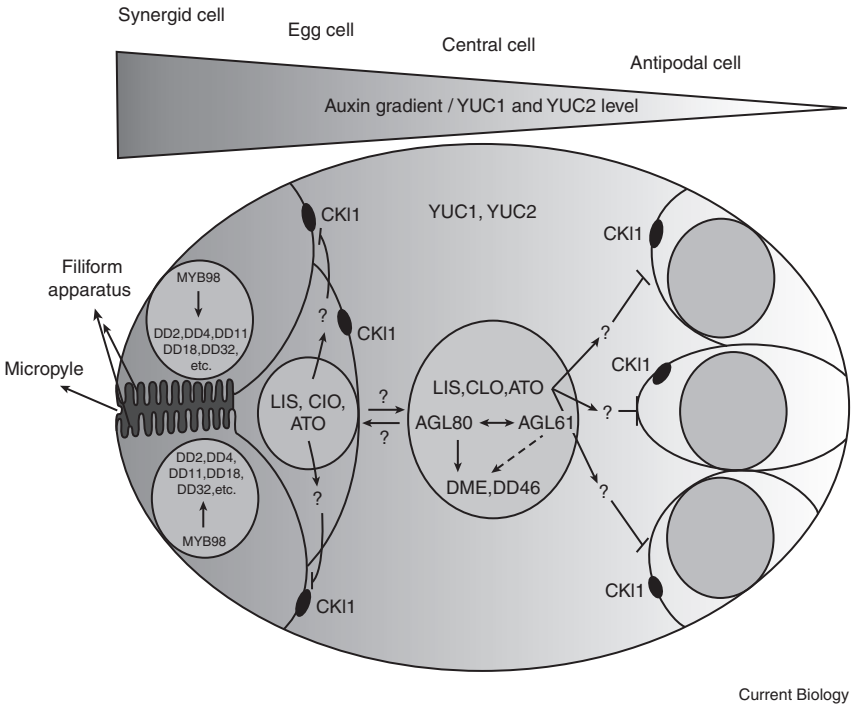

Figure 3. Signaling mechanisms that regulate cell specification processes in the female gametophyte.

Four cell types, including two synergid cells, one egg cell, one fused central cell and three antipodal cells, are shown. Genes shown are involved in the cell specification process of the female gametophyte. Arrows suggest direct relationships: solid arrows indicate experimental results and the dashed arrow indicates a proposed relationship. An auxin gradient and YUC1/YUC2 levels are represented by the intensity of the grey color. Nuclei are in grey, but not related to the chemotactic gradient.

Control of Embryo Sac Cell Differentiation by Signaling and Other Mechanisms

The spatially and temporally coordinated cell differentiation in the embryo sac suggests that cell–cell communication is likely important for normal development. One possible signaling mechanism was suggested by the high density of cytoplasmic bridges called plasmodesmata connecting cells within the embryo sac, allowing for the passage of RNAs, proteins and small molecules [105]. An alternative mechanism involves the binding of extracellular signal molecules to cell surface receptors [106]. A histidine kinase gene, *CYTOKININ INDEPENDENT 1* (*CKI1*), is expressed throughout embryo sac development (Figure 3) and a mutation in *CKI1* causes abortive cell specification and female gametophyte degeneration after mitosis [106,107]. Histidine kinases are part of two-component signaling systems, which receive a variety of signals, such as those from plant pathogens [108]. Like other cytokinin receptor histidine kinases, CKI1 can phosphorylate downstream ARABIDOPSIS HISTIDINE PHOSPHOTRANSFER PROTEINS (AHPs), thus activating the cytokinin signaling cascade. However, it does not appear to bind to cytokinin [107]. Whether CKI1 binds to other endogenous ligands in the embryo sac remains to be determined.

Additional regulation of embryo sac cell specification was suggested by a genetic screen for regulators of the egg cell fate, identifying three mutants, *lachesis* (*lis*), *clotho* (*clo*) and *atropos* (*ato*) [94,95], that exhibit a conversion from synergids and central cell to egg cells, and from the antipodal cells to the central cell fate. An explanation for this phenotype is that a lateral inhibitory signal that normally prevents non-gametic cells from adopting gametic cell fates is disrupted in these mutants. The corresponding genes encode the putative core spliceosome components PRP4, SNU114 and SF3a60, respectively [94,95], suggesting that RNA splicing is involved in establishing or maintaining cell-specific attributes in the embryo sac (Figure 3). Other mutants also exhibit conversion of non-gametic cells into gametic cells [15,92], but no mutant has been found to convert gametic cells into non-gametic cells. This is analogous to the situation in *ems1/exs* and other anther mutants that form additional sporogenous cells at the expense of somatic cells. It is possible that gametic cell fate is the default cell type, whereas somatic cell types require additional regulatory steps. Alternatively, redundant mechanisms may promote the gametic cell fates, making the genes that determine gametic fates difficult to uncover using forward genetic approaches.

Transcriptional Regulation in Cell Specification

Distinct cell types in the female gametophyte with unique cellular features likely correspond to differential gene expression programs. Gene expression profile analyses of female gametophytic cell types have revealed that many genes are exclusively or predominantly expressed in one of the four cell types [109,110]. The genes with exclusive expression patterns also serve as markers for studies of cell-fate determination. Additionally, the regulation of these genes provides entry points for understanding the cell specification process.

One such regulator is MYB98, which is a transcription factor exclusively expressed in synergid cells and is required for synergid cell differentiation and pollen tube guidance, with the *myb98* mutant showing a defective filiform apparatus, which is a subcellular structure important for pollen tube guidance [111]. MYB98 likely regulates the expression of many genes (such as *DD11* and *DD18*; Figure 3) in the synergid cell by binding to the *cis*-element GTAACNT [112], forming a MYB98-dependent regulatory network. Some of the putative MYB98 target genes might be important for the formation of the filiform apparatus and others may encode secreted proteins that might play a role in cell signaling, such as pollen tube attraction [113].

Another cell-type-specific transcription factor is the MADS-box protein AGAMOUS-LIKE80 (AGL80/FEM111), which is required for proper nucleolus and vacuole maturation in the central cell [114]. AGL80 regulates two central cell expressed genes, *DEMETER* and *DD46,* which are important for normal embryo sac development [114]. AGL80 can form a heterodimer with another MADS-box transcription factor, AGL61/DIANA, which also regulates central cell development [115].

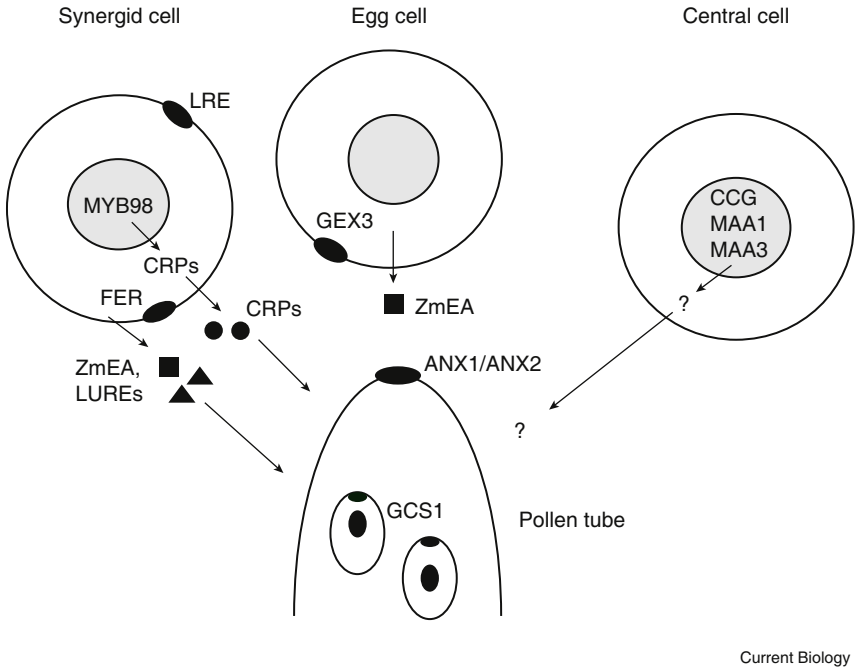

Figure 4. Genes expressed in male and female gametophytes that are involved in pollen tube guidance and male–female interaction.

This figure shows an embryo sac and an approaching pollen tube, with pollen attractive signals identified from different species. The secreted peptide ZmEA and the LUREs are found in maize and *Torenia fournieri*, respectively, whereas the other proteins are from *Arabidopsis*. The cellular localizations of these proteins are marked at the corresponding positions.

In the *agl61* mutant, the polar nuclei fail to fuse and degenerate before fertilization. Consistent with their functions, both *AGL61* and *AGL80* are expressed exclusively in the central cell within the embryo sac [114,115]. Moreover, dimerization with AGL80 is necessary for AGL61 to localize to the nucleus.

These studies likely have only uncovered a small fraction of the total number of regulatory genes involved in female gametophyte development, with many more yet to be discovered. For example, the maize *ZmDSUL* gene coding for a small ubiquitin-related modifier (SUMO) was recently shown to regulate nuclei positioning and cell specification [116]. Therefore, identification of additional cell-type regulators and investigation of the related networks will provide new insights into the control of cell specification in the embryo sac.

Male–Female Interactions

When the pollen tube germinates on the stigma and grows toward the female gametophyte, both sporophytic and gametophytic signals provide guidance [10]. These include arabinogalactan proteins (AGPs), triacylglycerides, lipid transfer proteins, and γ-aminobutyric acid (GABA), which guide pollen tube growth before arrival at the placenta [117–119]. Subsequent signals from the female gametophyte attract the pollen tube along the funiculus and then into the micropyle [120], such as a species-specific pollen tube attractant secreted from the synergids [121]. The filiform apparatus, a subcellular structure which consists of invaginated synergid cell wall, is important for both secreting pollen tube attractants and recognizing the arriving pollen tube [122]. When the female gametophyte and the pollen tube are compatible, the pollen tube penetrates the filiform apparatus of one synergid, terminates growth and ruptures to release two sperm cells [122,123]. The cytoskeleton of the penetrated synergid somehow reorganizes and facilitates the migration of the two sperm cells to fertilize the egg and central cells [105,111]. After fertilization, the female gametophyte ceases to attract pollen tubes or might even produce repulsive signals to prevent other pollen tubes from approaching [11,120,124].

In addition to synergid specification, the synergid-specific transcription factor MYB98 also plays an important role in female–male signaling (Figure 4), as indicated by a *myb98* defect in pollen tube guidance [113,122]. *MYB98* positively regulates the expression of dozens of genes, many of which encode cysteine-rich proteins (CRPs) [113], including DD2, DD4, DD11, DD12, and DD32, secreted by the filiform apparatus (Figure 3). Specifically, DD2 is a member of the defensin-like family and is similar to the *Torenia fournieri* Cys-rich peptides termed LUREs, which are pollen tube attractants secreted by synergids [125]. Therefore, MYB98 might regulate the expression of pollen tube attractants in the synergid.

The egg cell is also critical for micropylar pollen tube attraction (Figure 4). In maize, *EGG APPARATUS 1* (*ZmEA1*) is necessary for micropylar pollen tube guidance and encodes a small protein that is secreted into the extracellular matrix by the egg and synergids [126]. The ZmEA1 protein disappears after fertilization; this timely absence of the attractant is important for prevention of polyspermy. Another egg cell expressed gene called *GEX3* that encodes a plasma membrane localized protein [127] is also required for proper micropylar pollen tube guidance.

Recent evidence showed that the central cell also participates in pollen tube attraction/guidance (Figure 4). The central cell specific *CCG* (*CENTRAL CELL GUIDANCE*) gene encodes a nuclear protein and a *ccg* mutation causes defects in micropylar pollen tube guidance but not in female gametophyte morphology [127]. Therefore, the failure in pollen tube guidance could be a primary consequence caused by the loss of a central cell protein. The contribution of the central cell in attracting the pollen tube is further supported by the *mma* (*magatama*) mutants, which fail in polar nuclei fusion and pollen tube guidance [128,129].

After entering the micropylar hole, the pollen tube contacts the female gametophyte. Two allelic female gametophytic mutants, *feronia* (*fer*) and *sirene* (*sir*), have excess pollen tube growth and fail to release sperm cells in the embryo sac [11,124]. FER/SIR is a receptor-like kinase localized to the filiform apparatus portion of the synergid cell membrane. Its extracellular domain was thought to be recognized by a pollen tube ligand that might trigger a signaling cascade inside the synergid cell, which then signals back to the pollen

tube, causing pollen tube growth arrest and rupture to release sperm cells [130]. Another *Arabidopsis* mutant, *lorelei* [131], has a very similar phenotype to the *fer/ser* mutant. *LORELEI* encodes a small glucosylphosphatidylinositol-anchored protein that is highly expressed in synergid cells and might function in the same pathway as FER/SIR for regulating sperm delivery.

In addition, male signals can also play important roles in the fertilization process. Two related paralogs of FER/SIR, ANX1/2 [132], are expressed at the pollen tube tip and prevent premature pollen tube rupture but trigger pollen tube discharge when stimulated by the egg apparatus [133], indicating that receptor-like kinases are involved in mutual signaling between male and female. Furthermore, mutations in the *GENERATIVE CELL SPECIFIC 1* (*GCS1*) (also *HAP2*) gene encoding a transmembrane protein result in male sterility due to a failure in gamete fusion, even though sperm cells are released [134,135]. GCS1 is a sperm-specific protein and its homologs are widely distributed in angiosperms and nonangiosperms, suggesting an evolutionarily conserved male–female recognition mechanism [135].

In short, various signaling and regulatory molecules from different embryo sac cells play very important roles in male–female gametophytic interactions, especially pollen tube guidance, culminating in fertilization.

Conclusion and Perspectives

Recently, dramatic progress has been made in unraveling the signaling and regulatory mechanisms underlying plant reproductive development. It is clear that receptor-like protein kinases and other signaling molecules play crucial roles in both anther cell fate determination and male–female interactions. Additionally, hormones are important for both late anther development and female gametophytic cell specification. Furthermore, transcriptional control is employed frequently during male and female development. Therefore, even though male and female reproduction in flowering plants have several distinct morphological features, similar or common mechanisms are important for both processes.

Although there is still a long way to go before the complete regulatory machinery for reproductive development is uncovered, rapid advances in analytical methods will continue to reveal the mystery and complexity of plant reproduction. The laser microdissection technique has been successfully used for studying the development of plant reproductive tissues or organs [136,137], especially for analyzing the transcriptomes of different cell types or layers. These and other approaches will facilitate further understanding of gene functions and regulatory mechanisms for plant reproduction.

In the past twenty years, studies of *Arabidopsis* reproduction have provided valuable knowledge and contributed to understanding flowering plant reproduction in general. However, most major crops are monocots with possible differences in reproductive processes. Thus, studies of crops such as rice and maize have received more attention in recent years. With the aid of genome sequences and novel research tools, analyses in these plants will be dramatically accelerated, revealing secrets related to the conservation and divergence of reproductive development, especially the regulatory mechanisms that control this complex process.

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