© 2015. Published by The Company of Biologists Ltd | Development (2015) 142, 229-241 doi:10.1242/dev.102103

REVIEW

# Plant germline formation: common concepts and developmental flexibility in sexual and asexual reproduction

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## ABSTRACT

The life cycle of flowering plants alternates between two heteromorphic generations: a diploid sporophytic generation and a haploid gametophytic generation. During the development of the plant reproductive lineages – the germlines – typically, single sporophytic (somatic) cells in the flower become committed to undergo meiosis. The resulting spores subsequently develop into highly polarized and differentiated haploid gametophytes that harbour the gametes. Recent studies have provided insights into the genetic basis and regulatory programs underlying cell specification and the acquisition of reproductive fate during both sexual reproduction and asexual (apomictic) reproduction. As we review here, these recent advances emphasize the importance of transcriptional, translational and post-transcriptional regulation, and the role of epigenetic regulatory pathways and hormonal activity.

**KEY WORDS: Cell fate acquisition, Gene regulation, Germline development, Plant reproduction, Polarity**

## Introduction

In higher plants, diverse and versatile strategies have evolved to ensure reproductive success. During gametogenesis (see Glossary, Box 1), the male (pollen) and female (embryo sac) gametophytes, which harbour the male (sperm) and female (egg and central cell; see Glossary, Box 1) gametes, respectively, form in specialized reproductive tissues of the flower: the anther and ovule (Fig. 1). The multicellular gametophytes are formed following meiosis of spore mother cells (see Glossary, Box 1), thus producing reduced gametes that harbour half the chromosome number of the maternal sporophyte (haploid in case of diploid plants). During sexual reproduction (see Glossary, Box 1), sperm cells fuse with both the egg and the central cell in the process of double fertilization, giving rise to the embryo and endosperm, respectively, the major components of the seed (Fig. 1). The embryo constitutes the next sporophytic generation, while the endosperm is a terminal nourishing tissue for the embryo and also provides the majority of calories for human and animal consumption. Haploid plants can also form directly from male and female gametes. While this process occurs at very low frequencies in nature, it can be induced in culture and by mutation, and hence is being exploited to accelerate plant breeding (Germanà, 2011). By contrast, during vegetative reproduction (see Glossary, Box 1) and somatic embryogenesis (see Glossary, Box 1), which are two distinct types of asexual reproduction (see Glossary, Box 1), new plants develop without the formation of gametes and seeds. However, plants can also produce seeds via asexual reproduction, avoiding the need for fertilization, in a process known as gametophytic apomixis (hereafter referred to as apomixis, see Glossary, Box 1). Apomixis occurs in more than 400 plant species belonging to ~40 genera.

Both sexual reproduction and apomixis have distinct advantages for natural plant populations and agricultural applications. Sexual reproduction leads to genetically and phenotypically variable offspring, thus forming the basis for plant adaptation to changing environments and allowing for the breeding of new varieties. By contrast, apomixis produces clonal offspring that are genetically identical to the mother plant, thus fixing complex genotypes. Although apomixis is rare among crop plants, the engineering of apomictic crops promises great potential and economical value for crop production and for other applications in agriculture (Koltunow et al., 1995; Vielle-Calzada et al., 1996; Grossniklaus et al., 1998a,b; Spillane et al., 2004).

Over the past decade, plant sexual and apomictic germline formation has attracted the attention of scientists for a number of reasons: (1) the transition from sporophytic to reproductive fate by

**Box 1. Glossary**

**Apomeiosis.** The omission or abortion of meiosis during sporogenesis
**Apomictic initial cell (AIC).** The first cell in the apomictic female germline that omits or aborts meiosis
**Apomixis.** Asexual reproduction via seed formation
**Apospory.** The formation of an unreduced female gametophyte from an apomictic initial cell (AIC) developing adjacent to the sexual germline in the ovule
**Archesporial cell.** The cell giving rise (with or without division) to the spore mother cell
**Asexual reproduction.** Reproduction without the fusion of gametes
**Central cell.** The female gamete giving rise to the endosperm
**Diplospory.** The apomeiotic formation of an unreduced female gametophyte from an AIC at the position of the megaspore mother cell
**Egg cell.** The female gamete giving rise to the embryo
**Functional megaspore (FMS).** The cell that develops into the female gametophyte
**Gametogenesis.** The development of gametophytes from spores
**Parthenogenesis.** The formation of an embryo from an unfertilized egg cell
**Pseudogamy.** The fertilization-dependent formation of endosperm from a central cell in apomicts
**Sexual reproduction.** The mode of reproduction whereby female (egg) and male (sperm) gametes fuse to form a zygote
**Somatic embryogenesis.** The formation of an embryo from a sporophytic cell without gamete and seed formation
**Sporogenesis.** The formation of spores from spore mother cells
**Spore mother cell.** The first cell of the reproductive lineage, formed from sporophytic cells in female and male reproductive tissues of the flower
**Synergid cells.** Accessory cells of the mature female gametophyte that are important for pollen tube guidance and reception
**Vegetative reproduction.** A form of reproduction in which a new plant is formed without the formation of an embryo

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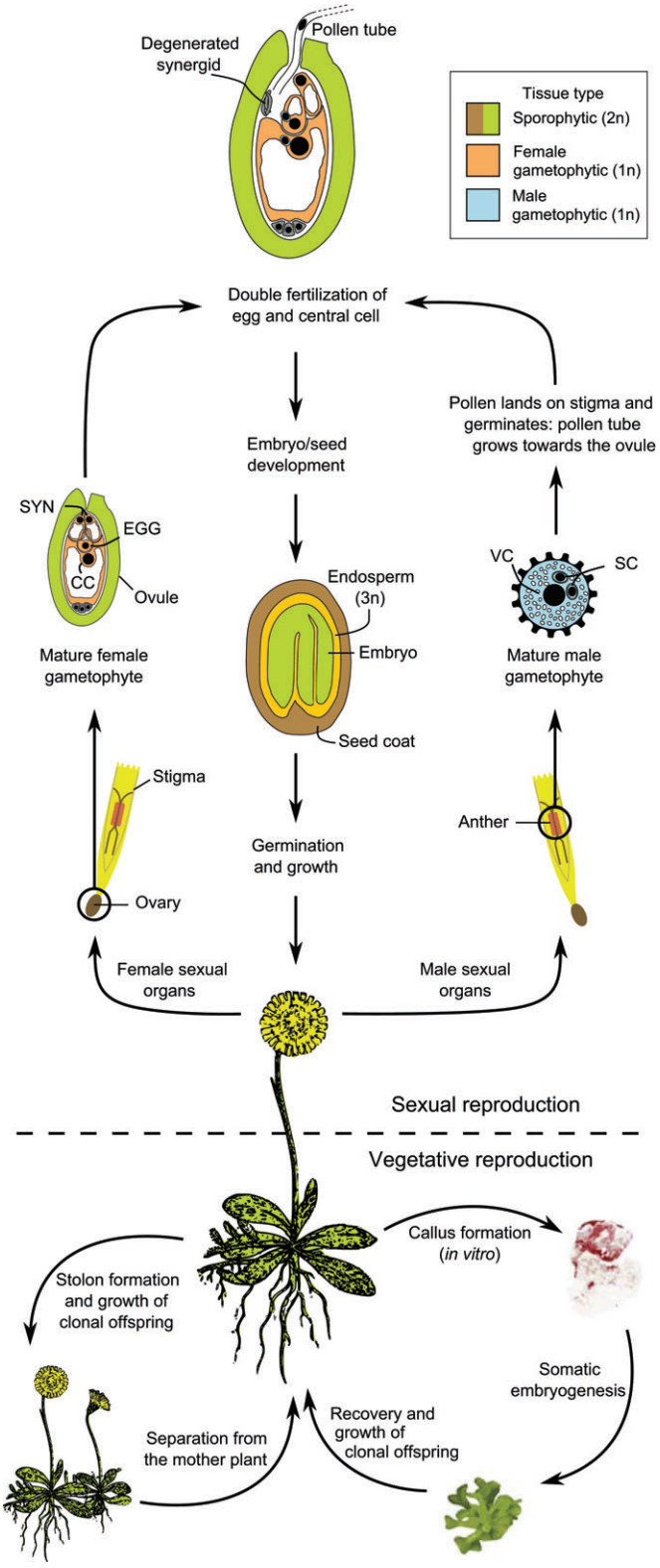


**Fig 1. The life cycle of a plant.** Plants have a more complex life cycle than animals, alternating between two heteromorphic generations: the sporophyte and the gametophyte. In the diploid sporophyte, distinct cells undergo meiosis and produce haploid spores. These give rise to multicellular haploid gametophytes, which produce gametes through mitotic divisions. The fusion of a male (sperm) and a female (egg) gamete results in the formation of a zygote, which constitutes the sporophyte. The example depicted, *Hieracium pilosella*, follows the common life cycle of angiosperms. The anthers of the flower produce the male gametophyte (called the pollen or microgametophyte), which consists of three cells: two sperm cells (SC) and one vegetative cell (VC). The female gametophyte (called the embryo sac or megagametophyte) is embedded in maternal sporophytic tissues of the ovule. The latter is enclosed in the carpels of the flower. For sexual reproduction, the pollen needs to germinate on the stigma and to deliver the two sperm cells to the female gametophyte. Fertilization, the transition from the gametophytic to the sporophytic generation, occurs within the ovule. In addition to sexual reproduction, plants can frequently reproduce asexually, e.g. by stolon outgrowth (vegetative reproduction) or via the formation of calli in culture, followed by somatic embryogenesis and development into an adult plant. CC, central cell; EGG, egg cell; SC, sperm cell; SYN, synergid cell; VC, vegetative cell.

reprogramming a somatic cell is a key step in the plant life cycle; (2) during gametogenesis, a few rounds of mitosis and cellularization lead to the formation of functionally distinct cell types that are all derived from a single spore, a process ideally suited to address fundamental questions in developmental biology; and (3) understanding the molecular mechanisms that determine sexual or asexual fate decisions is a precondition for the targeted manipulation of plant reproduction for agricultural use and crop improvement. Accordingly, many studies have focussed on determining the gene expression profiles, epigenetic mechanisms and regulatory pathways involved in germline development (reviewed by Drews and Koltunow, 2011; Sprunck and Gross-Hardt, 2011; Schmidt et al., 2012; Gutierrez-Marcos and Dickinson, 2012; Wüest et al., 2013). Here, we focus on recent studies that have elucidated the molecular mechanisms underlying the acquisition of reproductive fate in sexual and apomictic species, the determination of meiosis versus apomeiosis (see Glossary, Box 1), and the polar development of the female gametophyte.

## Development of the plant reproductive lineages

The formation of the plant reproductive lineages proceeds in two distinct phases: during sporogenesis (Glossary, Box 1), spores are formed by sporophytic (somatic) cells, whereas during gametogenesis the spores develop into mature gametophytes that harbour the male or female gametes (Fig. 2). During the course of evolution, the gametophytic phase of the plant life cycle, which is dominant in bryophytes (i.e. liverworts, hornworts and mosses), has been dramatically reduced to only a few cells in the angiosperms (flowering plants). Thus, unlike in most animals, where the germline is set aside early in embryogenesis, the plant germline is determined only late in development, during floral organ formation. Here, we consider the spore mother cells to be the first cells of the germline, as the lineage of the gametes can unambiguously be traced back to them (Grossniklaus, 2011). However, it should be noted that, because gametophytes consist of both gametic and non-gametic accessory cells and the germline is defined as the cell lineage that differentiates into gametes, some authors place the determination of the germline later during gametophyte development to the immediate precursors of the gametes (e.g. Berger and Twell, 2011; Twell, 2011).

The formation of the male reproductive lineage begins with the differentiation of a microspore mother cell (MiMC) in the developing anthers; the periclinal division of archesporial cells (see Glossary, Box 1) gives rise to outer parietal cells and inner sporogenous cells, and the MiMCs differentiate from the latter. The MiMC undergoes meiosis to give rise to a tetrad of microspores (Fig. 2), each of which undergoes an asymmetric division (termed pollen mitosis I, PMI) to form a vegetative and a generative cell (Borg et al., 2009). During pollen mitosis II (PMII), the generative cell forms two sperm cells (male gametes), while the vegetative cell does not divide again. The

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sperm cells are then delivered to the female gametes by the pollen tube, which forms via growth of the vegetative cell. The timing of PMII varies in different species; in most plant species, PMII takes place in the growing pollen tube but in some species, including *Arabidopsis* and maize, the generative cell divides before the pollen is released from the anther (Boavida et al., 2005).

During formation of the female sexual reproductive lineage, typically a single somatic cell per ovule acquires reproductive fate and differentiates to form an archesporial cell. It can be distinguished from the surrounding cells by its subepidermal localization and its enlarged size. In the sexual model species *Arabidopsis*, as in most species, the archesporial cell directly differentiates into a megaspore mother cell (MMC) without intervening divisions. The MMC is defined by its commitment to the meiotic fate and gives rise to a tetrad of megaspores (Fig. 2). Typically, only one functional megaspore (FMS; Glossary, Box 1) survives while the others degenerate (Fig. 2). Interestingly, the FMS occupies a defined position in the ovule, suggesting that this is important for its survival and cell fate acquisition. A role for signalling from sporophytic ovule tissues during the selection of the FMS has been discussed (Koltunow, 1993; Grossniklaus and Schneitz, 1998; Koltunow and Grossniklaus, 2003) and, in maize, the accumulation of callose in the cell walls of the degenerating megaspores has been hypothesized to play a role in shielding these cells from such signals (Russell, 1979). The FMS, in turn, typically undergoes three mitotic divisions to form a syncytial female gametophyte (Fig. 2). In most species, cellularization results in an eight-nucleate, seven-celled mature gametophyte (embryo sac), referred to as a *Polygonum* type embryo sac. It harbours the two female gametes, the synergid cells (see Glossary, Box 1), which are important for pollen tube guidance and reception, and three antipodal cells (Fig. 2). Although the role of the antipodal cells remains unclear, they might be involved in transferring nutrients from the surrounding sporophytic tissues to the embryo sac (Raghavan, 1997). The *Polygonum* type embryo sac occurs in ~70% of all angiosperms, including the model systems *Arabidopsis thaliana* (mouse ear cress), *Zea mays* (maize) and *Oryza sativa* (rice), and many apomictic species (Drews and Koltunow, 2011).

From the beginning of its development, the female gametophyte is highly polarized, suggesting that positional information may play a role in cell fate acquisition (Grossniklaus and Schneitz, 1998; Lituiev and Grossniklaus, 2014). The exact position of nuclei within the syncytium may thus be an important factor for cell specification during cellularization (Sundaresan and Alandete-Saez, 2010; Sprunck and Gross-Hardt, 2011). It is also evident that variations in this developmental pattern exist: while megasporogenesis typically leads to a single surviving one-nucleate FMS (monosporic megasporogenesis), failures in cell plate formation after meiosis I or after both meiotic divisions can lead to two- or four-nucleate FMSs, developmental patterns referred to as bisporic or tetrasporic megasporogenesis, respectively (Maheshwari, 1950; Willemse and Went, 1984; Haig, 1990; Huang and Russell, 1992; Drews and Koltunow, 2011). Other developmental variations concern the number of mitoses during megagametogenesis before cellularization, the possibility of additional mitoses after cellularization, and the timing of the fusion of the polar nuclei in the central cell (Maheshwari, 1950; Drews and Koltunow, 2011).

### Sexual reproduction and apomixis are interrelated

Compared with sexual reproduction, apomixis differs only in three key developmental steps (Fig. 2). First, female meiosis is circumvented, in a process referred to as apomeiosis, leading to the formation of unreduced megaspores and, consequently, unreduced female gametes. The first cell of the apomictic lineage is termed an apomictic initial cell (AIC; see Glossary, Box 1). The AIC is either formed at the position of the MMC and omits or aborts meiosis (diplospory; see Glossary, Box 1) to give rise to an unreduced FMS, or is derived from a somatic cell in close proximity to the MMC that directly differentiates into an unreduced FMS (apospory; see Glossary, Box 1) (Bicknell and Koltunow, 2004). Usually, male meiosis is unaffected but unreduced pollen can also be produced in some apomicts (Bicknell and Koltunow, 2004). Second, the egg develops into an embryo in the absence of fertilization in a process known as parthenogenesis (see Glossary, Box 1). Currently, the molecular mechanisms that activate the egg cell and initiate embryogenesis are unknown. Third, the central cell can form endosperm either autonomously or after fertilization (pseudogamy; see Glossary, Box 1). Functional endosperm formation in pseudogamous apomicts requires adaptations in either megagametogenesis (the production of a four-nucleate embryo sac), microgametogenesis (the formation of unreduced sperm cells) or double fertilization to ensure a balanced endosperm with the correct 2:1 ratio of maternal to paternal genomes crucial for seed development in many species (Grossniklaus, 2001; Koltunow and Grossniklaus, 2003; Spillane et al., 2004). For example, in maize *indeterminant gametophyte1* (*ig1*) mutants, abnormal numbers of nuclei are formed in the female gametophyte, leading to an aberrant maternal to paternal genome ratio in the endosperm, which results in seed abortion (Lin, 1984; Huang and Sheridan, 1996). In autonomous apomicts, the requirement for a balanced endosperm is alleviated, likely also depending on specific adaptations that are under genetic control.

### The acquisition and restriction of reproductive fate

During sexual reproduction, only one somatic cell per ovule is usually committed to the reproductive fate. However, it is not fully understood what determines the commitment of this somatic cell to initiate germline development and what prevents the formation of additional germline cells in the same ovule. In higher plants, it has been hypothesized that the MMC represses the formation of additional MMCs and thus restricts germline formation to only one cell per ovule (Grossniklaus and Schneitz, 1998). In support of the hypothesis that the germline itself suppresses the formation of additional germline lineages, the formation of multiple female gametophytes per ovule has been described in *Trimenia*, an ancient angiosperm taxon, where tip growing female gametophytes compete to reach the site of fertilization (Bachelier and Friedman, 2011).

Initial insights into the signalling pathways that regulate the restriction of germline fate came from the analyses of mutants in maize, rice and *Arabidopsis* (summarised in Table 1). In rice carrying mutations in *MULTIPLE SPOROCYTE* (*MSP1*) and in *Arabidopsis* plants carrying mutations in the orthologue *EXTRA SPOROGENOUS CELLS*/*EXCESS MICROSPOROCYTES1* (*EXS*/*EMS1*) or mutations in *SOMATIC EMBRYOGENESIS RECEPTOR KINASE1* and *2* (*SERK1*/*2*), more MiMCs develop per anther in comparison to the wild type (Canales et al., 2002; Zhao et al., 2002; Nonomura et al., 2003; Albrecht et al., 2005; Colcombet et al., 2005; Jia et al., 2008). These genes encode leucine-rich receptor kinases (*MSP1* and *EXS*/*EMS1*) and LRR receptor-like serine threonine kinases (*SERK1*/*2*) (Canales et al., 2002; Zhao et al., 2002; Nonomura et al., 2003; Albrecht et al., 2005; Colcombet et al., 2005; Jia et al., 2008). Similar phenotypes have been described in mutant in which the genes *TAPETUM DETERMINANT1* (*TPD1*) in

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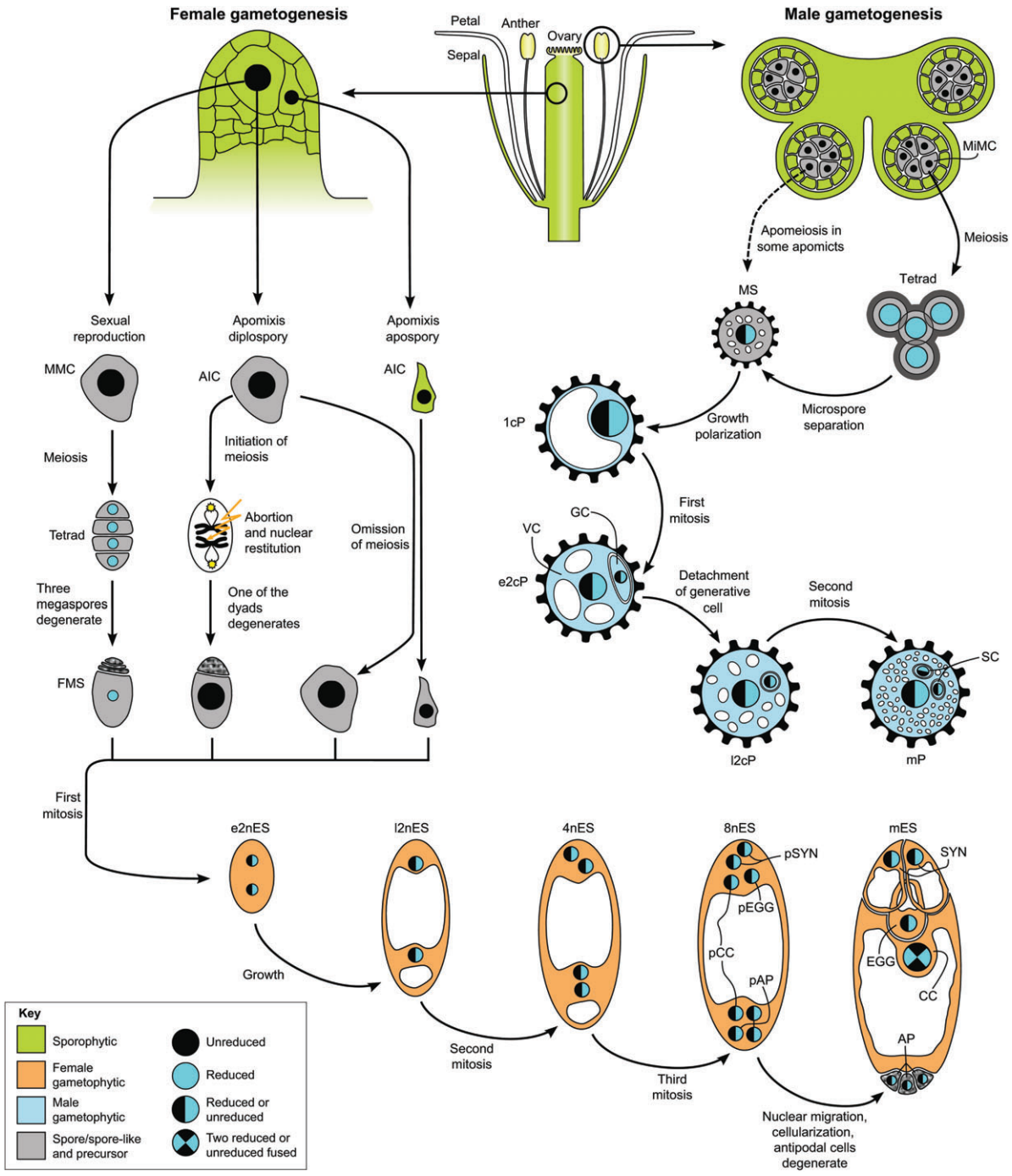


**Fig. 2. Male and female gametophyte development in sexually reproducing and apomictic plant species.** Germline development starts with the differentiation of sporophytic cells into spore mother cells (female, megaspore mother cell, MMC; male, microspore mother cell, MiMC) that, in sexually reproducing species undergo meiosis to give rise to four haploid spores. During male gametogenesis, the four spores separate and form unicellular microspores (MS), which grow by cell expansion to form unicellular pollen (1cP). The first asymmetric mitosis produces bicellular pollen (e2cP) containing a large vegetative cell (VC) and a small generative cell (GC). The GC detaches from the cell wall and becomes engulfed by the VC. Sperm cells (SC) are formed during the second mitosis of the GC. The mature pollen (mP) consists of a VC, which will form the pollen tube, and two SCs that mediate double fertilization. During female gametogenesis, three of the four spores degenerate, leaving one functional megaspore (FMS), which undergoes three mitotic divisions in a syncytium to give rise to the early/late two-nucleate, four-nucleate and then eight-nucleate embryo sac (e/l2nES, 4nES, 8nES). Nuclear migration and concomitant cellularization eventually lead to the formation of a mature embryo sac (mES), a highly polarized structure that contains four distinct cell types: two synergid cells (SYN), the egg cell (EGG), the central cell (CC) and antipodal cells (APs), which degenerate prior to fertilization. In apomictic species, different mechanisms can lead to the formation of unreduced gametes. In diplospory, the apomictic initial cell (AIC) may initiate meiosis but restitution results in the formation of two unreduced AICs, one of which degenerates. By contrast, aposporous apomicts form a FMS-like cell at a different position in the ovule. The unreduced AICs then develop into unreduced female gametophytes. Meiosis on the male side is usually normal in apomicts. Female gametophyte stages (FG) are according to Christensen et al. (1997). p, precursor of.

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*Arabidopsis*, *MULTIPLE ARCHESPORIAL CELLS* (*MAC1*) in maize and its rice orthologue *OsTDL1A* are disrupted. These genes encode small secreted proteins identified as the putative ligands of the MSP1 or EXS/EMS1 receptor kinases (Sheridan et al., 1996, 1999; Yang et al., 2003, 2005; Zhao et al., 2008; Wang et al., 2012; Kelliher and Walbot, 2012). Unlike in *Arabidopsis*, in rice and maize this pathway also affects female sporogenesis, indicating differences in the mechanism of repression of additional sporocytes (Sheridan et al., 1996; Zhao et al., 2008; Nonomura et al., 2003). In maize and rice, excess archesporial cells were observed, leading to the formation of more sporogenous cells (Zhang and Yang, 2014). In *Arabidopsis*, however, the pathway plays a role in cell fate decisions and cell specification after the periclinal division of the archesporial cell. As recently demonstrated, EXS/EMS1 forms complexes with SERK1/2 to control the proliferation of tapetal cells in the anther (Albrecht et al., 2005; Colcombet et al., 2005; Feng and Dickinson, 2010). Interestingly, partially complementary expression patterns have been reported for *TPD1* and *EXS*/*EMS1*, which are predominantly expressed in sporogenous cells and tapetal cells, respectively, at the developmental stages at which the mutant phenotypes are first established, indicating signalling between cell types (Yang et al., 2003).

In aposporous apomicts, such repression of additional germline lineages is not active, as both an enlarged somatic AIC and the MMC can initiate reproductive lineages (Fig. 1). Formation of the AIC in *Hieracium pilosella* even depends on differentiation and meiosis of the sexual MMC (Koltunow et al., 2011). Thus, as suggested by the signalling pathways described above, communication between cell types during sporogenesis seems to be involved in cell type specification and the acquisition or restriction of germline fate. It remains unclear whether this is achieved by overcoming the mechanism that usually represses the development of additional germline cells or by an alternative signalling pathway that induces reproductive fate in an additional somatic cell, or whether a combination of both of these mechanisms is involved. However, it should be noted that, once established, the apomictic lineage often suppresses the further development of the sexual female gametophyte (Koltunow et al., 2011), suggesting that distinct control mechanisms exist at these developmental steps.

In diplosporous species, the AIC omits or aborts meiosis producing an unreduced FMS (Fig. 2; Bicknell and Koltunow, 2004). As in sexual species, diplosporous apomicts typically develop only one germline lineage per ovule, suggesting that the processes by which gametophytic fate is acquired in apospory and diplospory follow distinct developmental principles. It is unknown whether apomictic fate is regulated by related or alternative molecular mechanisms in diplosporous and aposporous species. Investigations into this question have proved to be technically challenging as the female germline is deeply embedded in maternal floral tissues. Nevertheless, recent methodological advances have allowed the transcriptional profiling of such rare cell types by combining laser-assisted microdissection or micromanipulation with microarray and/or RNA-Seq analyses. These studies have provided novel insights into the transcriptional basis of germline specification and development (Wüest et al., 2010; Schmidt et al., 2011, 2012, 2014; Schmid et al., 2012; Okada et al., 2013; Wüest et al., 2013; Abiko et al., 2013; Anderson et al., 2013; Chettoor et al., 2014).

New insights into the issue of how cell specification is regulated during diplospory, when compared with sexual or aposporous reproduction, were recently provided by cell type-specific transcriptome analyses of the reproductive lineage in *Boechera gunnisoniana*, a diplosporous apomict that is related to sexual *A. thaliana* (Schmidt et al., 2014). Comparative transcriptome analyses detected a number of commonalities between the sexual MMC and the diplosporous AIC (Schmidt et al., 2014). Importantly, significant differences in the activities of a number of regulatory pathways were also observed, including differences in cell cycle regulation, hormonal pathways, signal transduction and epigenetic regulatory pathways (Schmidt et al., 2014). Through comparisons with a transcriptome dataset of the AIC of *Hieracium praealtum* (Okada et al., 2013), this study suggests interesting differences between the regulatory mechanisms specifying a diplosporous or an aposporous AIC (Schmidt et al., 2014). Importantly, the *H. praealtum* AIC seems to have already adopted a gametophytic fate (Okada et al., 2013). In agreement with this acquisition of a FMS fate without meiotic division, a number of meiotic genes are not expressed in the *H. praealtum* AIC (Okada et al., 2013). By contrast, the majority of 25 core meiotic genes are expressed in the AIC of

**Table 1. Genes involved in the restriction of additional sporogenous cells during plant reproductive development**

| Gene | Species | Restricts number of MiMCs, MMCs or both | Type/function of protein encoded | Reference(s) |
|---|---|---|---|---|
| *MSP1* | *O. sativa* | Restricts the number of sporocytes in anther and ovule | Leucine-rich repeat receptor-like kinase; orthologue of *Arabidopsis EXS*/*EMS1* | Nonomura et al., 2003 |
| *EXS*/*EMS1* | *A. thaliana* | Restricts the number of microsporocytes | Leucine-rich repeat receptor-like kinase involved in regulating the proliferation of tapetal cells during anther development | Canales et al., 2002; Zhao et al., 2002; Feng and Dickinson, 2010 |
| *SERK1*/*2* | *A. thaliana* | Restricts the number of microsporocytes | Leucine-rich repeat receptor-like kinases; forms complexes with EXS/EMS1 in tapetal cells | Albrecht et al., 2005; Colcombet et al., 2005 |
| *TPD1* | *A. thaliana* | Restricts the number of microsporophytes | Small secreted protein; interacts with EXS/EMS1 | Yang et al., 2003; Yang et al., 2005 |
| *MAC1* | *Z. mays* | Restricts the number of archesporial cells in anther and ovule | Small secreted protein; orthologue of Os*TDL1A* | Sheridan et al., 1996; Sheridan et al., 1999; Wang et al., 2012 |
| Os*TDL1A* | *O. sativa* | Restricts the number of sporocytes in anther and ovule | Small secreted protein; putative ligand of MSP1 | Zhao et al., 2008 |

*A. thaliana*, *Arabidopsis thaliana*; *EXS*/*EMS1*, *EXTRA SPOROGENOUS CELLS*/*EXCESS MICROSPOROCYTES1*; *MAC1*, *MULTIPLE ARCHESPORIAL CELLS1*; MiMCs, microspore mother cells; MMCs, megaspore mother cells; *MSP1*, *MULTIPLE SPOROCYTE1*; *O. sativa*, *Oryza sativa*; Os*TDL1A*, orthologue of *MAC1*; *SERK1*/*2*, *SOMATIC EMBRYOGENESIS RECEPTOR KINASE1* and *2*; *TPD1*, *TAPETUM DETERMINANT1*; *Z. mays*, *Zea mays*.

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the diplosporous species *B. gunnisoniana* before first division restitution (Schmidt et al., 2014). This supports the notion that diplospory results from a modification of the meiotic pathway in an MMC-like cell, while the aposporous AIC becomes directly determined to a gametophytic fate without prior activation of the meiotic program.

### Mutations in meiotic genes can lead to diplospory-like modifications of meiosis

Over recent years, investigations into the regulatory processes governing meiosis have allowed the identification of meiotic mutants that generate unreduced gametes (Table 2) (Brownfield and Köhler, 2011; Crismani et al., 2013). For example, mutations in the gene encoding *DYAD*/*SWITCH1* (*SWI1*) lead to apomeiosis and to the formation of rare triploid offspring that retain full parental heterozygosity (Ravi et al., 2008). In *MiMe-1* and *MiMe-2* triple mutants, a diplospory-like division also leads to the formation of unreduced gametes. *MiMe-1* and *MiMe-2* are combinations of *sporulation11-1* (*spo11-1*), *omission of second division1* (*osd1*) and *recombination8* (*rec8*), and *spo11-1*, *osd1* and *cyc1;2*/*tardy asynchronous meiosis* (*tam*), respectively (d'Erfurth et al., 2009, 2010). Using these *Arabidopsis* mutants, synthetic clonal seeds have been produced by manipulating the expression of the centromere-specific histone 3 variant CENH3, which leads to paternal genome elimination, in the *dyad/swi1* or *MiMe* mutant background (Marimuthu et al., 2011).

### Meiosis and the acquisition of germline fate are affected by abiotic and oxidative stress

Although these mutations in meiotic genes lead to a deregulation of the meiotic program and, eventually, to a switch to a diplospory-like process, little is known about the control of diplospory and meiotic restitution in natural apomicts. Interestingly, abiotic stress can lead to alterations in meiotic cell division (de Storme and Geelen, 2014). For example, in rose (*Rosa spp.*) short periods of heat stress result in partial restitution of male meiosis and the formation of unreduced dyads, but also triads and polyads (Pecrix et al., 2011).

Analysis of the effect of redox status on germline specification in maize revealed another link to abiotic stress. In anthers, germ cell formation is stimulated by a low oxygen environment or by a low abundance of reactive oxygen species (ROS), which accumulate under different kinds of stress (Kelliher and Walbot, 2012, 2014). This led to the conclusion that reduced oxygen concentration promotes the acquisition of meiotic fate in maize (Kelliher and Walbot, 2012). Contrasting the idea that meiotic fate is acquired under low ROS levels, a recent hypothesis postulates that the evolution and maintenance of meiosis depends on stress and elevated ROS levels (Hörandl and Hadacek, 2013, see Box 2).

In conclusion, although abiotic and oxidative stresses seems to play a role in the transition from somatic to reproductive fate and the regulation of (apo)meiosis, their potential role as a driving force promoting sexual or asexual reproduction remains unclear and warrants further investigation.

### Epigenetic regulatory pathways are important for germline specification and the control of sexual versus apomictic reproduction

Disturbance of the meiotic programme typically results in sterility or the diplospory-like formation of unreduced gametes. However, phenotypes resembling apospory or diplospory have also been observed in mutants perturbing epigenetic regulatory pathways, in particular those involving DNA methylation and small RNA-based gene regulation (Olmedo-Monfil et al., 2010; Garcia-Aguilar et al., 2010; Singh et al., 2011).

Epigenetic regulation is involved in a variety of developmental and cell fate decisions by controlling gene activity through DNA or chromatin modifications. For example, ARGONAUTE (AGO) proteins are involved in gene regulation mediated by small RNAs such as microRNAs (miRNA), small interfering RNAs (siRNA) and PIWI-associated RNAs (piRNA) (Meister, 2013). In *Arabidopsis*, 10 AGO proteins have been identified, and these can be grouped into three major clades: the AGO1, AGO5 and AGO10 clade; the AGO2, AGO3 and AGO7 clade; and the AGO4, AGO6, AGO8 and AGO9 clade (Mallory and Vaucheret, 2010). These different clades of AGO proteins engage in different small RNA pathways, with the AGO9 clade being active in the siRNA heterochromatin pathway that regulates the transcriptional silencing of transposons and repeats by mediating DNA methylation and heterochromatin formation (Mallory and Vaucheret, 2010).

**Table 2. Mutations that lead to the formation of unreduced female gametophytes by an apospory- or diplospory-like mechanism**

| Mutation | Species | Description | Type of apomeiosis | Reference(s) |
|---|---|---|---|---|
| *dyad*/*swi1* | *A. thaliana* | Mutation in core meiotic gene | Diplospory like | Ravi et al., 2008 |
| *MiMe-1* (*spo11-1*, *osd1* and *rec8*) | *A. thaliana* | Triple mutant of core meiotic genes | Diplospory like | d'Erfurth et al., 2009 |
| *MiMe-2* (*spo11-1*, *osd1* and *tam*) | *A. thaliana* | Triple mutant of core meiotic genes | Diplospory like | d'Erfurth et al., 2010 |
| *ago9* | *A. thaliana* | Mutation in gene involved in a small RNA pathway | Apospory like | Olmedo-Monfil et al., 2010 |
| *rdr6* | *A. thaliana* | Mutation in gene involved in a small RNA pathway | Apospory like | Olmedo-Monfil et al., 2010 |
| *sgs3* | *A. thaliana* | Mutation in gene involved in a small RNA pathway | Apospory like | Olmedo-Monfil et al., 2010 |
| *mem* | *A. thaliana* | Mutation in gene encoding a RNA-helicase | Apospory like | Schmidt et al., 2011 |
| *dmt102* | *Z. mays* | Mutation in gene involved in DNA methylation | Apospory like | Garcia-Aguilar et al., 2010 |
| *dmt103* | *Z. mays* | Mutation in gene involved in DNA methylation | Apospory like | Garcia-Aguilar et al., 2010 |
| *ago104* | *Z. mays* | Mutation in gene involved in a small RNA pathway | Diplospory like | Singh et al., 2011 |

*A. thaliana*, *Arabidopsis thaliana*; *Z. mays*, *Zea mays*.

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**Box 2. The evolution of apomixis and meiosis**

Evidence suggest that apomixis evolved from a deregulation of the sexual pathway several times independently (Koltunow, 1993; Vielle-Calzada et al., 1996; Leblanc et al., 1997; Grimanelli et al., 2001; Grossniklaus, 2001; Tucker et al., 2003; Koltunow and Grossniklaus, 2003; Sharbel et al., 2009, 2010). Deregulation of genetic and epigenetic regulatory pathways has been hypothesized to be a consequence of hybridization and polyploidization, which have been proposed as preconditions for apomixis to occur (Asker and Jerling, 1992; Grossniklaus, 2001; Spillane et al., 2001; de Storme and Geelen, 2013).

Interestingly, according to a recent hypothesis, meiosis as a precondition for sexual reproduction is thought to have evolved as a repair mechanism for DNA damage induced by oxidative stress and ROS, and it has been proposed that the redox chemistry between oxidized DNA and the meiotic protein SPO11 is required for the generation of double-strand breaks, which are required for meiotic recombination and the repair of damaged DNA (Hörandl and Hadacek, 2013). However, low levels of ROS promote the acquisition of meiotic fate in maize anthers (Kelliher and Walbot, 2012). In line with this hypothesis, the metabolism of polyamine and spermidine, which are quenchers of ROS activity, is enriched in the AIC in *B. gunnisoniana* (Schmidt et al., 2014). Similarly, increasing evidence suggests the importance of the redox state for the development of the anther and male germline (reviewed by Zhang and Yang, 2014). Nevertheless, the role of stress and reactive oxygen species on regulating sexual versus apomictic reproduction remains unknown.

Increasing evidence highlights the importance of *AGO* activity in plant germline development and gamete formation (Nonomura et al., 2007; Wüest et al., 2010; Olmedo-Monfil et al., 2010; Singh et al., 2011; Borges et al., 2011; Tucker et al., 2012). This is reminiscent of the role of AGO proteins in the animal germline; proteins of the animal-specific PIWI-clade protect the genomic integrity of the germline, in particular by repressing the activity of transposons in invertebrates, although their role in vertebrates is less clear (Clark and Lau, 2014). AGO/PIWI proteins, and potentially other proteins involved in small RNA pathways, interact with VASA or VASA-like RNA helicases that are preferentially expressed in the germline (Yajima and Wessel, 2011). Although neither the PIWI clade of AGOs nor VASA RNA helicases have been identified in plants, recent evidence suggests that similar regulatory mechanisms evolved in the plant reproductive lineage, likely by convergence (Wüest et al., 2010; Schmidt et al., 2011).

In *Arabidopsis*, a role for *AGO9* and *MNEME* (*MEM*), a RNA helicase that is preferentially expressed in the MMC, during germline specification has recently been described (Fig. 3; Table 2; Olmedo-Monfil et al., 2010; Schmidt et al., 2011). In plants heterozygous for *mem-1* or *mem-2*, more than one subepidermal enlarged cell instead of the single MMC develops in ~21% of ovules, whereas 37-48% of the ovules in *ago9* homozygotes show a similar phenotype, depending on the allele (Olmedo-Monfil et al., 2010; Schmidt et al., 2011). In *mem* and *ago9* mutants, these additional subepidermal enlarged cells directly give rise to a female gametophyte in the absence meiosis, closely resembling apospory (Olmedo-Monfil et al., 2010; Schmidt et al., 2011). Although the AGO9 protein has been detected only in the L1 layer of the developing ovule, and not in the MMC, *MEM* transcripts are highly enriched in the MMC but are also detected in the surrounding ovule tissue, albeit at much lower levels (Fig. 3; Olmedo-Monfil et al., 2010; Schmidt et al., 2011). Thus, MEM and AGO9 likely repress the acquisition of reproductive fate in the surrounding tissues in a non-cell-autonomous manner (Olmedo-Monfil et al., 2010; Schmidt et al., 2011). Interestingly, *AGO9* plays a role in repressing transposons in the germline, reminiscent of the role played by PIWI proteins in animals (Olmedo-Monfil et al., 2010). It remains to be determined whether MEM – like VASA in the animal germline – is involved in this process, potentially acting by aiding the unwinding of RNAs prior to their association with AGO proteins.

Similar phenotypes have also been reported for mutations that disrupt *RNA-DEPENDENT RNA POLYMERASE6* (*RDR6*) and *SUPPRESSOR OF GENE SILENCING3* (*SGS3*), which are known to be required for the biogenesis of *trans*-acting siRNAs (Table 2; Olmedo-Monfil et al., 2010). In addition, features of apospory have been reported for the maize *dmt102* and *dmt103* lines, which carry mutations in the homologues of the *Arabidopsis CHROMOMETHYLTRANSFERASE3* (*CMT3*) and *DOMAINS REARRANGED METHYLASE1* (*DRM1*) and *DRM2*, respectively (Table 2; Garcia-Aguilar et al., 2010). Currently, no evidence has been reported that demonstrates the formation of viable offspring from the unreduced, supernumerous gametophytes seen in the maize *dmt102* and *dmt103* mutants, or in the *Arabidopsis ago9* and *mem* mutants. By contrast, a mutation in maize *AGO104*, a homologue of *Arabidopsis AGO9*, leads to features of diplospory and to the formation of tripoid and tetraploid offspring following the fertilization of unreduced gametophytes (Table 2; Singh et al., 2011). The *ago104* phenotype is caused by a mutation that leads to defects in chromosome condensation during meiosis (affecting mega- and microsporogenesis) and, subsequently, to the formation

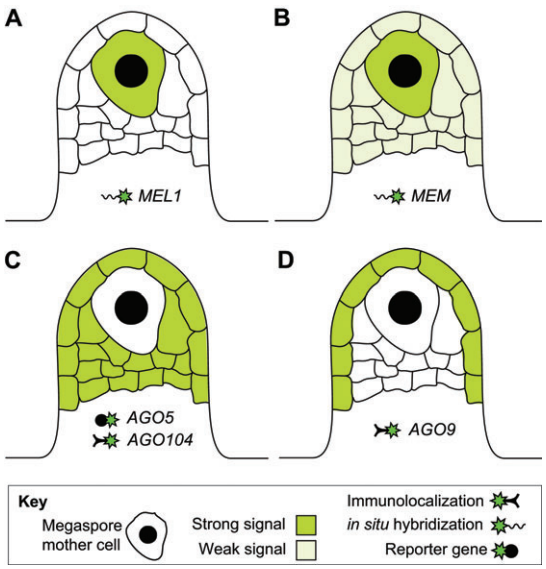


**Fig. 3. The expression patterns of proteins/genes whose perturbations mimic apomeiosis.** The expression patterns or abundance of protein are schematically shown for: (A) *MEL1* (Nonomura et al., 2007); (B) *MEM* (Schmidt et al., 2011); (C) *AGO5* (Tucker et al., 2012) and AGO104 (Singh et al., 2011); and (D) AGO9 (Olmedo-Monfil et al., 2010). During female germline formation, *MEL1* is expressed in the MMC, suggesting a cell-autonomous effect to cause failure of meiosis (Nonomura et al., 2007). However, a more complex regulation cannot be excluded, as in rare cases germline formation fails. AGO104 and AGO5 are both localized in the nucellus tissue, suggesting signalling from the sporophytic tissues of the nucellus to the developing germline (Singh et al., 2011; Tucker et al., 2012). Interestingly, AGO9 was described to be restricted to the L1 layer of the nucellus, suggesting a non-cell-autonomous mechanism (Olmedo-Monfil et al., 2010). By contrast, highest expression of *MEM* has been observed in the MMC, so that a non-cell-autonomous mechanism to repress germline fate in the surrounding cells is likely. However, as *MEM* is also expressed in the nucellus surrounding the MMC at low levels, other mechanisms cannot be excluded.

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of unreduced dyads (Singh et al., 2011). Interestingly, *AGO104* is expressed not in the MMC but in the surrounding somatic tissues, suggesting that the meiotic defect is mediated by a mobile signal (Fig. 3; Singh et al., 2011). Effects of AGO activity on meiosis have been described previously: in rice, mutations in the MMC-expressed gene *MEIOSIS ARRESTED AT LEPTOTENE1* (*MEL1*) (Fig. 3) lead to meiotic arrest and sterility (affecting mega- and microsporogenesis) (Nonomura et al., 2007). MEL1 is closely related only to *Arabidopsis* AGO5 and thus belongs to a different AGO clade than AGO104 (Nonomura et al., 2007). Together, the data suggest diverse and important functions for AGO proteins that are active in different small RNA-dependent regulatory pathways during germline specification and meiosis in different plant species.

Predominant expression of *AGO1*, *AGO2*, *AGO5*, *AGO8* and *AGO9* has also been observed in the *Arabidopsis* egg cell (Wüest et al., 2010), although the function of these AGO proteins in the egg cell remains to be elucidated. *AGO5* is also highly enriched in sperm (Borges et al., 2011), and a role for AGO5 in a putative miRNA complex in the male germline has been proposed (Borges et al., 2011). During megasporogenesis, *AGO5* can be detected in sporophytic ovule tissues, but not in the developing female germline, similar to *AGO9* (Fig. 3; Tucker et al., 2012). Furthermore, plants carrying the semi-dominant *ago5-4* allele do not initiate female gametophyte development, suggesting that this particular mutation inhibits a somatic small RNA pathway that promotes the initiation of gametogenesis (Tucker et al., 2012).

In addition to involving DNA methylation and small RNA pathways, epigenetic pathways regulate chromatin organization and histone modifications. It was recently reported that large-scale chromatin reprogramming establishes an epigenetic and transcriptional state in the *Arabidopsis* MMC that is distinct from that in the surrounding tissue (She et al., 2013). These changes likely contribute to the acquisition of germline fate and to the transition to the gametophytic phase, rather than being only a precondition for meiosis (She et al., 2013). This is supported by the finding of similar histone modifications and histone variant dynamics in the additional subepidermal enlarged cells in *ago9*, *sds3* and *rdr6* mutants (She et al., 2013).

In conclusion, epigenetic regulatory pathways play important roles during the acquisition of germline fate, during germline differentiation and for discriminating between a meiotic and a mitotic fate. It remains unknown whether the influence of stress on germline specification described above acts via changes in the activity of epigenetic pathways or through independent mechanisms.

## Polarity and cell fate determination during megagametogenesis

Whether generated by sexual reproduction or apomixis, a highly polarized structure harbouring functionally distinct cell types is established during megasporogenesis from a single FMS by only two or three mitotic divisions and cellularization. Recent studies in *Arabidopsis* have identified a number of factors that can influence this polarity and the subsequent development of the gametophyte (summarized in Table 3; reviewed by Sundaresan and Alandete-Saez, 2010; Sprunck and Gross-Hardt, 2011; Lituiev and Grossniklaus, 2014).

### Factors regulating FMS selection

To initiate megagametogenesis, typically only the chalazal-most spore in the tetrad survives and differentiates into the FMS, but the mechanism governing FMS selection and survival is unclear. The *Arabidopsis antikevorkian* mutant affects FMS selection but the corresponding gene remains to be cloned (Yang and Sundaresan, 2000). More recently, *ARABINOGALACTAN PROTEIN18* (*AGP18*) was found to be important for the survival and selection of the FMS (Table 3; Acosta-García and Vielle-Calzada, 2004; Demesa-Arévalo and Vielle-Calzada, 2013). Overexpression of *AGP18* in ovules results in the survival of more than one of the four megaspores (Demesa-Arévalo and Vielle-Calzada, 2013). The mechanism by which *AGP18* determines FMS selection remains unknown, although it has been hypothesized that AGP proteins, which are attached to the plasma membrane through a glycosylphosphotidylinositol (GPI) anchor, can act as components of signalling pathways (Youl et al., 1998; Borner et al., 2003; Ellis et al., 2010; Seifert and Roberts, 2007; Zhang et al., 2011).

Subsequent to megaspore selection, the FMS forms the mature gametophyte, which harbours four functionally distinct cell types, typically through three mitotic divisions. How cell fate acquisition is regulated and when cell fate is determined during this process is still largely unclear. It has been proposed that positional information might be involved in the determination of cell fate (Grossniklaus and Schneitz, 1998; Sundaresan and Alandete-Saez, 2010; Sprunck and Gross-Hardt, 2011; Lituiev and Grossniklaus, 2014). During the syncytial phase, nuclei migrate and occupy predefined positions in the female gametophyte. In mutants with supernumerary nuclei, the position of the nuclei along the micropylar-chalazal axis of the embryo sac affects their cell fate, indicating that they are influenced by positional information (Table 3; Gross-Hard et al., 2007; Pagnussat et al., 2007; Moll et al., 2008a; Moll et al., 2008b; Johnston et al., 2010).

### The role of auxin and cytokinin in establishing and maintaining polarity

It has been proposed that the plant hormone auxin plays a pivotal role in establishing and maintaining polarity by forming a gradient in the developing embryo sac (Pagnussat et al., 2009). The auxin gradient was thought to be mediated by auxin influx from sporophytic tissues at early stages and by localized biosynthesis at later stages of female gametophyte development. Abolishing the auxin gradient, by expressing the YUCCA1 (YUC1) auxin biosynthetic protein in the entire embryo sac or by modulating the auxin response by downregulating selected *AUXIN RESPONSE FACTOR* (*ARF* genes), led to the loss or, at low frequencies, the mis-expression of cell fate markers in the female gametophyte (Table 3; Pagnussat et al., 2009). However, theoretical models attempting to describe the auxin gradient in the female gametophyte showed that only very shallow auxin gradients can be established even when using the most favourable parameters (Lituiev et al., 2013). A sensitivity analysis demonstrated that the steepness of the obtained gradients is not sufficient to determine distinct cell fates (Lituiev et al., 2013). Furthermore, the reinvestigation of auxin signalling using various sensors failed to detect auxin in the female gametophyte but instead found auxin signalling to be restricted to the surrounding ovule tissues in a dynamic polar pattern (Ceccato et al., 2013; Lituiev et al., 2013). This polar auxin pattern in sporophytic tissues may non-cell-autonomously influence cell specification in the female gametophyte and may have been affected by manipulating the expression of *YUC1* and *ARF*s (Lituiev et al., 2013).

Auxin signalling is interrelated with the cytokinin pathway (Müller and Sheen, 2008; Bencivenga et al., 2012, Cheng et al., 2013) and, not surprisingly therefore, cytokinin signalling has also been shown to play a role in germline development. For example, cytokinin levels influence ovule patterning by affecting the

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**Table 3. Mutations involved in polarity and cell fate determination in the *Arabidopsis thaliana* female gametophyte**

| Mutation | Description | Phenotype | Reference |
|---|---|---|---|
| *cki1* | Mutation in a gene causing cytokinin-independent activation of the cytokinin signalling pathway | Arrest starting from FG4 | Pischke et al., 2002; Hejátko et al., 2003 |
| *agp18* | RNAi targeting *ARABINOGALACTAN PROTEIN18* transcripts | Arrest at FG1 | Acosta-García and Vielle-Calzada, 2004 |
| *rbr1* | Mutation in a core cell cycle regulator gene | Nuclear overproliferation | Ebel et al., 2004; Johnston et al., 2010 |
| *lis* | Mutation in a gene encoding a component of the RNA splicing machinery | Synergids and the central cell adopt an egg cell-like fate | Gross-Hardt et al., 2007; Völz et al., 2012 |
| *eostre* | Mutation leading to the misexpression of *BLH1* | One synergid cell differentiates into an additional egg cell | Pagnussat et al., 2007 |
| *clo/gfa1* | Mutation in a gene encoding a component of the RNA splicing machinery | Synergids and the central cell adopt an egg cell-like fate | Moll et al., 2008b |
| *ato* | Mutation in a gene encoding a component of the RNA splicing machinery | Synergids and the central cell adopt an egg cell-like fate | Moll et al., 2008b |
| *amiR-ARFa* | amiRNA targeting transcripts of the auxin signalling pathway | Synergid identity lost, partly adopting an egg cell-like fate | Pagnussat et al., 2009 |
| *ahk2-7, ahk3-3, cre1-12* | Triple mutant in genes encoding components of the cytokinin signalling pathway | Arrest at FG1 | Cheng et al., 2013 |
| *ahp1, ahp2-1, ahp3, ahp4, ahp5* | Multiple mutant in genes encoding components of the cytokinin signalling pathway | Arrest at FG7 | Cheng et al., 2013 |
| *hda7* | Mutation in a histone deacetylase gene | Arrest at FG4 | Cigliano et al., 2013 |
| *myb64, myb119* | Double mutant in MYB transcription factor genes | Arrest during FG5 transition | Rabiger and Drews, 2013 |

amiRNA, artificial microRNA; *BLH1*, *BELL1-LIKE HOMEODOMAIN1*; RNAi, RNA interference.
Female gametophyte stages (FG) are according to Christensen et al. (1997).

expression of *PIN-FORMED1* (*PIN1*), which encodes an auxin efflux carrier (Bencivenga et al., 2012; Luschnig and Vert, 2014). This is consistent with the finding that cytokinin regulates *PIN1* expression in roots (Dello Ioio et al., 2008; Ruzicka et al., 2009). In ovules, the regulatory pathway involves two transcription factors, SPOROCYTLESS/NOZZLE (SPL/NZZ) and the homeodomain protein BELL1 (BEL1). Mutations in the gene encoding SPL/NZZ, which is required for the initiation of megasporogenesis, lead to reduced expression of *PIN1*, while the effects of exogenous cytokinin are mediated by BEL1, which is important for ovule identity, and lead to an altered pattern of auxin signalling in the ovule (Table 3; Schiefthaler et al., 1999; Yang et al., 1999; Balasubramanian and Schneitz, 2000; Sieber et al., 2004; Brambilla et al., 2007; Bencivenga et al., 2012). In addition to *PIN1*, *PIN3* is expressed during ovule development (Ceccato et al., 2013). However, no effect on ovule or female germline development has been reported in *pin3* mutants and a potential functional interaction between cytokinin and *PIN3* has not been investigated (Ceccato et al., 2013). Thus, although an auxin gradient in the embryo sac could not be confirmed, auxin and cytokinin do play important roles in the sporophytic tissues of the ovule.

Consistent with the crosstalk between the auxin and cytokinin pathways, cytokinin is involved in communication between sporophytic ovule tissues and the developing female gametophyte (Table 3; Cheng et al., 2013). In *Arabidopsis*, different cytokinin receptors expressed in the chalazal ovule tissues act redundantly to regulate FMS specification (Table 3; Cheng et al., 2013). During megagametogenesis, the histidine protein kinase CYTOKININ-INDEPENDENT1 (CKI1) has important functions, and *cki1* mutants affect the mitotic divisions during gametophyte development (Table 3; Pischke et al., 2002; Hejátko et al., 2003; Cheng et al., 2013). Although related to the *Arabidopsis* histidine kinases (AHKs) AHK2, AHK3 and AHK4, which act as cytokinin receptors, CKI1 lacks a cytokinin-binding domain and activates the cytokinin signalling pathway in the absence of cytokinin (Kakimoto, 1996; Nakamura et al., 1999; Urao et al., 2000; Hwang and Sheen, 2001; Yamada et al., 2001; Mähönen et al., 2006). *Arabidopsis* double mutants affecting *MYB-DOMAIN PROTEIN64* (*MYB64*) and *MYB119*, which encode two closely related R2R3-MYB domain transcription factors, also display a *cki1*-like phenotype (Table 3; Rabiger and Drews, 2013). Double mutant *myb64 myb119* gametophytes undergo extra mitotic division cycles and usually fail to cellularize (Rabiger and Drews, 2013). In the few cellularized mutant embryo sacs, cell fate is not properly established and the polarity of the embryo sac is affected (Rabiger and Drews, 2013). Furthermore, while *MYB64* and *MYB119* act redundantly during female gametophyte development, *MYB119* but not *MYB64* is regulated by *CKI1* (Rabiger and Drews, 2013).

### Epigenetic and post-transcriptional regulation of gametophyte development

In addition to hormonal pathways and transcription factors, epigenetic regulators are involved in establishing polarity in the developing gametophyte. Recently, a role for HISTONE DEACETYLASE7 (HDA7) during megagametogenesis and embryo development has been demonstrated (Cigliano et al., 2013). In *hda7-2* mutants at the four-nucleate stage of megagametogenesis, the two nuclei located at the micropylar pole degenerate, suggesting that histone deacetylation is required for survival and possibly for fate determination of the micropylar nuclei (Table 3; Cigliano et al., 2013). Other important gene regulatory control mechanisms involve the storage of mRNAs in mRNA-protein complexes, mRNA processing and mRNA degradation (reviewed by Hafidh et al., 2011). Regulation of the asymmetric distribution and processing of mRNAs involving RNA-binding proteins is known to be a determinant of protein gradients, cell polarity, cell fate decisions and patterning during development (Hafidh et al., 2011). For example, this is well described in *Drosophila* embryo genesis but also relevant for polar pollen tube growth in plants (Hafidh et al., 2011). In agreement with the emerging roles of mRNA storage and processing, components of the RNA splicing machinery have been identified as being crucial for cell type specification and the restriction

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of gametic fate in the *Arabidopsis* embryo sac (Table 3; Gross-Hardt et al., 2007: Moll et al., 2008b; Völz et al., 2012). In *lachesis* (*lis*) mutants, the expression of a marker for egg cell identity extends to adjacent gametophytic cells, the synergids and the central cell (Table 3; Gross-Hardt et al., 2007). As the phenotype becomes stronger as time progresses, LIS may predominantly play a role in maintaining egg cell identity. *LIS* encodes a homologue of the yeast splicing factor PRP4 (Gross-Hardt et al., 2007). Similar to *LIS*, *CLOTHO*/*GAMETOPHYTIC FACTOR1* (*CLO*/*GFA1*) and *ATROPUS* (*ATO*) are also important for restricting gametic fate in the mature gametophyte (Table 3; Moll et al., 2008b). *CLO*/*GFA1* encodes a homologue of Snu114, an essential component of the spliceosome, while *ATO* encodes the *Arabidopsis* homologue of SF3a60, which plays a role in pre-spliceosome formation (Moll et al., 2008b). The activities of *LIS* and *CLO* are related, as *CLO* is important for the tissue specificity of *LIS* expression (Moll et al., 2008b). *LIS* is strongly enriched in female gametes, suggesting that it regulates the maintenance of cell fate by lateral inhibition of the adjacent accessory cells in the female gametophyte, the synergid and antipodal cells (Gross-Hardt et al., 2007; Moll et al., 2008b; Völz et al., 2012). In summary, the splicing machinery is important for the specification and maintenance of cellular identity in the female gametophyte. Whether this is mediated through specific effects of some of its components in the embryo sac or caused by a general deficiency in splicing – also affecting pre-mRNAs of cell specification factors – remains to be determined.

In conclusion, although many mutants that exhibit disrupted embryo sac polarity or cell type-specific expression have been identified over the past decade, we are still far from understanding these processes at the molecular level. Currently, we have a partial list of components involved in cell specification but we do not understand how they work together to pattern the female gametophyte. Importantly, many of the observed effects may be indirect, e.g. caused by the mis-positioning of nuclei in the embryo sac, or the identified factors act after the initial specification of cell fate, in the maintenance of cell identity or during cell differentiation. A clear candidate for a cell fate determinant – one that cell-autonomously specifies cell type identity – is still being sought after. Transcription factors of the RKD family can at least partially reprogramme sporophytic cells towards an egg cell fate when overexpressed (Koszegi et al., 2011) but, owing to genetic redundancy, functional analyses of these transcription factors proved difficult and their potential gametophytic phenotypes are unknown.

## Conclusions

The male and female plant germlines are ideal models for studying the role of polarity, cell specification processes and the transition from sporophytic to gametophytic fate, which is a key step in the plant life cycle. Apart from being scientifically fascinating, understanding the molecular mechanisms underlying the specification and development of plant reproductive lineages is relevant for targeted manipulations of reproduction for crop improvement and seed production, in particular to achieve the longstanding goal of engineering apomixis in crop plants. Important aspects will be to determine whether distinct or similar genetic and epigenetic modifications govern apomixis in different species, involving aposporous and diplosporous accessions, and to identify common features.

Recent studies have yielded important insights into various aspects of sexual and apomictic germline formation, providing a glimpse of the complex regulatory mechanisms required to control reproductive development in plants. In this Review, we have discussed studies that address the genetic basis underlying the transition from somatic to germline fate, the repression of additional reproductive lineages, and cell specification during megagametogenesis, which together ensure reproductive success. As highlighted above, many different pathways are involved, including hormonal pathways, epigenetic regulation via small RNAs, transcriptional regulation by transcription factors and post-transcriptional control mechanisms. However, we currently do not know how these pathways are interconnected to form regulatory networks. Building on recent investigations, an important aim of future research will be to compare whole transcriptome analyses and combine these with detailed studies of the molecular mechanisms at play in different species, while taking evolutionary aspects into consideration.

Finally, little is known about the influence of stress or changing environmental conditions on germline specification. Interestingly, heat stress as well as the abundance of ROS were reported to influence the meiotic versus apomeiotic fate decision of spore mother cells. However, contradicting hypotheses have been portrayed, highlighting a need for more investigations in this area. In the longer term, this may be important not only for a better understanding of the regulatory networks underlying reproductive development and their interactions with environmental factors, but also for the improvement of agricultural plants under changing climate conditions.

#### Acknowledgements

We thank our colleagues in the Grossniklaus laboratory for interesting discussions and three anonymous reviewers for their helpful comments, which helped us to improve the manuscript.

#### Competing interests

The authors declare no competing financial interests.

#### Funding

Work on gametophyte development, apomixis and epigenetic gene regulation in U.G.'s laboratory is supported by the University of Zürich, and by grants from the 'Staatssekretariat für Bildung und Forschung' in the framework of COST action FA0903 (to U.G. and A.S.), the Swiss National Science Foundation (to U.G.) and the European Research Council (to U.G.).

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