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Humboldt Review

Male gametophyte development in flowering plants: A story of quarantine and sacrifice

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ABSTRACT

Over 160 years ago, scientists made the first microscopic observations of angiosperm pollen. Unlike in animals, male meiosis in angiosperms produces a haploid microspore that undergoes one asymmetric division to form a vegetative cell and a generative cell. These two cells have distinct fates: the vegetative cell exits the cell cycle and elongates to form a tip-growing pollen tube; the generative cell divides once more in the pollen grain or within the growing pollen tube to form a pair of sperm cells. The concept that male germ cells are less active than the vegetative cell came from biochemical analyses and pollen structure anatomy early in the last century and is supported by the pollen transcriptome data of the last decade. However, the mechanism of how and when the transcriptional repression in male germ cells occurs is still not fully understood. In this review, we provide a brief account of the cytological and metabolic differentiation between the vegetative cell and male germ cells, with emphasis on the role of temporary callose walls, dynamic nuclear pore density, transcription repression, and histone variants. We further discuss the intercellular movement of small interfering RNA (siRNA) derived from transposable elements (TEs) and reexamine the function of TE expression in male germ cells.

1. Introduction

Male gametophyte (pollen) development of angiosperms (flowering plants) takes place in the anthers of a flower and can be divided into two phases — microsporogenesis and microgametogenesis. During microsporogenesis, each diploid pollen mother cell (PMC) undergoes meiotic divisions, giving rise to four haploid microspores in tetrad arrangement. Following the release of microspores from tetrads, pollen development enters microgametogenesis. The released microspores experience size enlargement and nuclear polarization, then undergo an asymmetrical division (pollen mitosis I, PMI), generating a larger vegetative cell and a smaller generative cell, engulfed in the vegetative cell. The generative cell completes a second division (pollen mitosis II, PMII) to produce two sperm cells. The vegetative cell acts as the companion cell of male germ cells (generative and sperm cells) to support their development and to transport sperm cells for double fertilization via pollen tubes. Common model plants such as Arabidopsis, rice, and maize shed tricellular pollen consisting of two sperm cells and one vegetative cell. However, in the natural world, about 70 % of flowering plants such as tomato, *Lilium,* and *Medicago* disperse pollen at the bicellular stage containing one generative cell and one vegetative cell (Brewbaker, 1967; Williams et al., 2014) (Fig. 1). The development of angiosperm male germ cells occurs in a relatively closed space, making it a unique system for developmental biology. Extensive research has been conducted to understand cell fate determination, cell differentiation, and cell cycle. Particularly in recent years, the combination of reproductive mutants, cell sorting, and high-throughput sequencing has been providing a more complete view of pollen development. Accumulating evidence suggests the reprogramming of gene expression involves histone variants, small RNA, and DNA methylation. Transposable elements (TE) reactivation in the vegetative cell has been proposed to generate siRNA that moves to the sperm cells to inhibit TE expression across generations. Many excellent reviews have summarized this progress from the perspectives of evolution (Hackenberg and Twell, 2019; Hisanaga et al., 2019), germ cell formation (Berger and Twell, 2011; Borg et al., 2009; Chang et al., 2011; Schmidt et al., 2015; Twell, 2011), cellular processes (Borg and Berger, 2015; Hafidh et al., 2016), transcriptome (Rutley and Twell, 2015; Schmidt et al., 2012), and small RNA (Borges et al., 2011; Wu and

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Zheng, 2019). Here, we focus on the functional differentiation between the vegetative cell and germ cells, and how metabolic and transcription repression occurs in the germ cells. We also discuss the current model of TE silencing in germ cells via vegetative-cell-derived TE-siRNAs.

2. Historical understanding of angiosperm pollen

2.1. The cell within a cell

A century and a half ago, the first microscopic observations found pollen grains of several angiosperm species to be binucleate (Reichenbach, 1852; Hartig, 1866). Strasburger first clarified the two nuclei's identities, that the smaller one was generative and could divide again in the pollen grain or pollen tube; the larger one was vegetative and remained undivided (Strasburger, 1884). It took several decades to confirm a distinct membrane that separated the male germ cells from the vegetative cytoplasm. The cytoplasm around the germ nuclei often contained mitochondria (Anderson, 1939) but stained differently from the vegetative cytoplasm under a light microscope (Maheshwari, 1949). Although no one suspected male germ cells were complete, whether a real cell wall or only double plasma membranes separated the generative cell from vegetative cytoplasm had been controversial for some time. With the development of the transmission electron microscope approaches, ultrastructural studies in the pollen grain of *Oenothera hookeri* (Diers, 1963), *Petunia* (Sassen, 1964), *Tradescantia paludosa* (Maruyama et al., 1965), and bluebell (Angold, 1968) finally confirmed the existence of a real cell wall around the generative cell.

The establishment of cell polarity before microspore division is evident in many species. For instance, in lily microspores, the nucleus moves from the central position to the long axis at the G1 phase of PMI (Tanaka et al., 1979). Migration of the microspore nucleus to the pole is often accompanied by an expansion of vacuoles, pushing most of the cytoplasm to one end of the cell (Mascarenhas, 1975). In *Tradescantia*, the microspore nuclear movement to the pole is microtubule independent, while the movement of the newly formed vegetative nucleus and elongation of the generative cell is microtubule dependent (Terasaka and Niitsu, 1990). PMI can be disrupted during in vitro culture of isolated microspores of many plant species, such as *Nicotiana tabacum*, *Tulipa gesneriana*, and *Brassica napus,* or by pharmaceutical treatment with low concentrations of colchicine (Lichter, 1982; Sunderland and Wicks, 1971; Tanaka and Ito, 1981). When microtubule dynamics are disrupted, the generative-cell-within-vegetative cell arrangement disappears.

2.2. The callose wall

It was Mangrin who introduced the term callose and first described callose walls in the microspore (Mangin, 1889). Callose is a β-1,3-glucan polysaccharide with β-1,6 branches. Compared with cellulose (polymer of β-1,4-glucan), callose is more rapidly synthesized and degraded by plant cells, which makes it suitable for regulating cell communication and cell differentiation (Ünal et al., 2013; Wu et al., 2018). For example, enhanced accumulation of callose at plasmodesmata can delay the intercellular trafficking of plant viruses and virus-encoded proteins by limiting the plasmodesmatal size (Bucher et al., 2001; Iglesias and Meins, 2000). Apart from serving as wall material to protect mature pollen grains, callose is transiently deposited and degraded during microsporogenesis and microgametogenesis. The appearance of a callose-containing wall is often a sign of cell fate transition.

At the prophase of meiosis I, PMCs are connected by abundant cytoplasmic strands, offering a gateway for molecular exchanges between PMCs (Gates, 1908; Heslopharrison, 1966). These channels

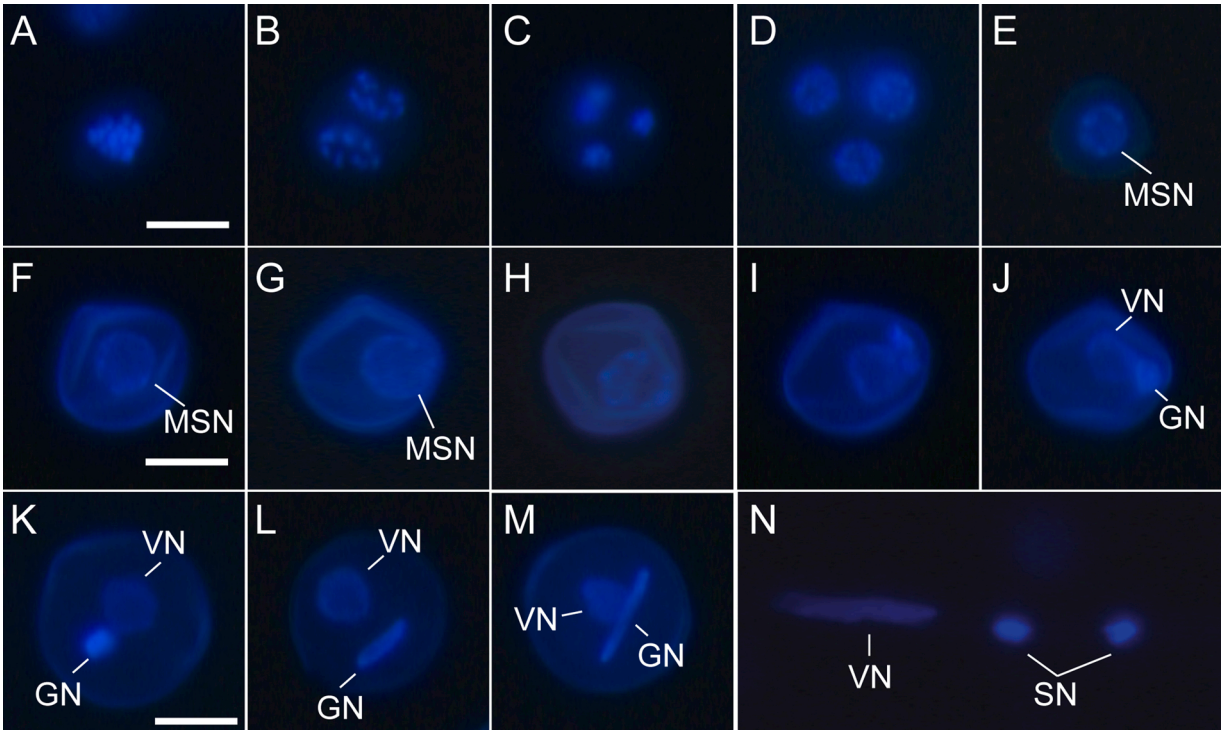

Fig. 1. Male gametophyte development in tomato. (A-D) A pollen mother cell undergoes meiotic divisions to produce four microspores in a tetrahedral arrangement (tetrad). (E) A haploid microspore just released from a tetrad. (F) The uninucleate microspore grows in size and accumulates more cell wall materials. (G-H) Migration of microspore nucleus to the cell periphery and initiation of asymmetrical mitosis (pollen mitosis I). (I-J) A newly formed generative nucleus is heavily stained and close to the cell wall; the vegetative nucleus is slightly stained and away from the cell wall. (K-M) Reposition of the generative and vegetative nucleus; Cell volume increases, and the generative nucleus elongates. Note that mature tomato pollen is bicellular, different from tricellular pollen, such as in Arabidopsis and rice. (N) Upon germination, the vegetative cell develops a pollen tube. The generative cell divides (pollen mitosis II) in the pollen tube to form two sperm cells. MSN, microspore nucleus; VN, vegetative nucleus; SN, sperm nucleus. Bars, 10 μm. The cell nuclei are visualized by DAPI (4′,6-diamidino-2-phenylindole) staining.

disappear before meiosis II, and the tetrads containing newly formed microspores are entirely isolated within the callose wall (Heslopharrison, 1966). Autoradiographic results demonstrated that isotopes could move freely into PMCs during the pre-meiotic and meiotic leptotene stages. However, the labeled trace was excluded from PMCs and microspores when the cytoplasmic strands were sealed, and cells were isolated within the callose walls (Heslopharrison and Mackenzie, 1967). Similarly, incorporating radioactive precursors into the developing *Gerbera jamesonii* pollen wall suggested that the callose wall was more like a molecular filter that allowed the transit of specific molecules (Southworth, 1971).

In microgametogenesis, the deposition of callose wall was proposed to enable the generative nucleus to exert greater control over the generative cell (Angold, 1968). Spindle microtubules were shown to be involved in the initial deposition of callose or callose precursors into the cell plate during PMI (Heslop-Harrison, 1968). In a survey including 82 angiosperm species, Górska-Brylass showed that the callose wall around generative cells was temporary and only lasted for about 10−20 h in *Chlorophytum elatum*. This temporary callose wall gradually disappeared as the generative cell moved away from the pollen wall (Górska-Brylass, 1967, 1970). In the late generative cell, the wall material mainly consisted of polysaccharides (Cresti et al., 1987). These studies indicate callose is one of the major components of the early generative cell wall and is gradually replaced by non-callose/cellulose polysaccharides in the late stage. A callose wall's isolation initiates two separate development paths for the generative and vegetative cells.

The proper deposition and dissolution of callose seem to be essential for pollen viability. In Arabidopsis mutant *limpet pollen (lip)*, the generative cell or sperm cells remain attached to the pollen wall, and inward migration is prevented, possibly due to a prolonged existence of the callose wall (Howden et al., 1998). Disruptions of callose metabolism in *GLUCAN SYNTHASE-LIKE 5 (GSL5)*, *CALLOSE SYNTHASE 5 (CALS5),* and C*ALLOSE DEFECTIVE MICROSPORE1 (CDM1)* result in no or a thinner callose wall around tetrads and severely reduced fertility (Dong et al., 2005; Lu et al., 2014; Shi et al., 2015), while the loss of function of *GLUCAN SYNTHASE-LIKE 8* and *10 (GSL8, GSL10)* and *POLLEN SPECIFIC PROTEIN 231 (PSP231)* lead to aberrant pollen mitosis due to irregular callose deposition (Li et al., 2020b; Toller et al., 2008). Intriguingly, several studies suggest that decreased callose content results in elevated plasmodesmata permeability and lesser constraint on phytohormone auxin mobility (Han et al., 2014). During somatic embryogenesis, callose deposition at plasmodesmata appears earlier than the establishment of embryo identity and is associated with auxin response changes (Godel-Jedrychowska et al., 2020). The auxin pathway is pivotal for stamen development and pollen maturation (Sundberg and Østergaard, 2009; Zheng et al., 2020), as dysfunction of auxin transporter PIN8, which is predominantly expressed in pollen, leads to misshaped pollen grains and reduced pollen viability (Dal Bosco et al., 2012; Ding et al., 2012). Major phytohormone levels and gene expression related to hormone signaling change dramatically during pollen development (Hirano et al., 2008; Liu et al., 2018). Therefore, it is possible that callose deposition around the cell promotes the efficient formation of a hormone gradient, which is important for cell fate transition.

2.3. The metabolic repression in male germ cells

In the 1930s to 1940s, multiple staining techniques indicated that the metabolism in the generative cell cytoplasm was low, contrary to that in the vegetative cytoplasm. The reduced metabolic potential in the generative cell cytoplasm was proposed to accord with its role of sperm cell production, not pollen tube growth (La Cour, 1949). In most plant species, the microspore produces two daughter cells with equal chromatin but unequal cell size. It allocates much more cytoplasm to the vegetative cell and less cytoplasm to the generative cell with fewer and smaller mitochondria, Golgi apparatus, and endoplasmic reticulum (ER), and almost no plastids and amyloplasts. In an extreme case, hardly any mitochondria and plastids were observed in the generative cell of *Cymbidium goeringii* (Yu and Russell, 1992). One noticeable exception is *Plumbago zeylanica*. Its generative cells and dimorphic sperm cells have a relatively large cytoplasm to accommodate numerous mitochondria, plastids, ER, Golgi apparatus, and vesicles (Russell and Cass, 1981; Russell and Strout, 2005). The unequal distribution of organelles in two daughter cells is attributed to the following reasons: 1) during PMI, the generative cell inherits less cytoplasm from the microspore and thus fewer organelles; 2) organelle degeneration occurs during the maturation of male germ cells (Schröder, 1985); 3) the perinuclear microtube system may serve to exclude organelles from the generative pole (Tanaka, 1997).

Biochemical analyses have suggested that male germ cells have lower transcriptional activity than do vegetative cells (Mascarenhas, 1975). In *Tradescantia paludosa*, vegetative nuclei synthesized about twice the amount of RNA as did generative nuclei (LaFountain and Mascarenhas, 1972). Transcription activity further decreased in sperm cells, as exemplified in the germinating *Hyoscyamus niger* pollen. There were only trace amounts to no uridine isotope incorporation (RNA synthesis indicator) in the generative and sperm nuclei, suggesting transcription in the male germ cells is dispensable for development (Reynolds and Raghavan, 1982). However, it should be pointed out that male germ cells are not always quiescent in gene expression. Upon pollen germination, reactivation of transcription and translation was observed in sperm cells of many plants such as maize and rye (Haskell and Rogers, 1985; Zhang et al., 1993).

Consistent with the biochemical analyses in the pre-omics era, RNA-seq studies suggest the pollen transcriptome decreases in size as pollen matures. In Arabidopsis, rice, and tobacco, pollen at early stages contains more transcripts than late pollen does, while transcript complexity further decreased in mature pollen (Bokvaj et al., 2015; Honys and Twell, 2004; Wei et al., 2010). The germ cell transcripts are mixed with and inevitably masked by abundant vegetative transcripts during transcriptome analysis. Therefore, to gain insight into the male germ cell transcriptome, cell isolation by osmotic shock, enzyme digestion, physical separation, or fluorescence-activated cell sorting (FACS) is required to enrich generative cells or sperm cells (Borges et al., 2012; Chaboud and Perez, 1992; Li et al., 2019; Lu et al., 2015; Uchiumi et al., 2006). Transcriptome data from isolated male germ cells indicate that gene expression from microspores to sperm cells is subject to global transcription repression. It appears that most transcripts in sperm cells were present in the microspores or generative cells and that only a small amount of transcript is sperm cell-specific (Anderson et al., 2013; Borges et al., 2008; Liu et al., 2018). Moreover, the protein level of RNA polymerase II and ribosomes is also negligible in sperm cells, suggesting few transcriptional and translational events take place in sperm cells (Liu et al., 2018). It is noteworthy that the 'expression' revealed by present transcriptomes is based only on the profiling of steady-state level of RNA but not on nascent transcripts that directly reflect transcriptional activity (Gardini, 2017). Similarly, protein synthesis activity within male germ cells or vegetative cells should not be inferred from the RNA-seq data because transcripts engaged with ribosomes are not measured.

Further evidence implies that low metabolic activity in male germ cells is associated with the downregulation of nuclear pore complexes (NPC). NPC are protein assemblies embedded in the nuclear envelope and serve as vital gateways for transporting large macromolecules between the nucleus and cytoplasm (Kim et al., 2018; Knockenhauer and Schwartz, 2016). Lower NPC density or deficiencies of nucleoporin are associated with perturbed nuclear import or export capacity (Jevtic et al., 2019; Tamura and Hara-Nishimura, 2014). The difference in NPC density between male germ cells and vegetative nuclei was observed in several species such as *Brassica napus*, *Medicago sativa*, *Tradescantia paludosa*, and *Zea mays*. A general rule of thumb is that in mature pollen, vegetative nuclei have about two- to four-fold of the NPC density that

generative/sperm nuclei do (LaFountain and LaFountain, 1973; Shi et al., 1991; Southworth, 1992; Southworth et al., 1988; Straatman et al., 2000). Compared to NPC on vegetative nuclei, NPC on generative nuclei are larger and irregular in distribution. Sperm nuclei usually have the lowest NPC number. In maize, for instance, the sperm nucleus lacks nuclear pores (Southworth et al., 1988), while in *Plumbago* the sperm nuclei contain numerous NPC in the nuclear envelope (Russell and Cass, 1981). Although the NPC poorly select against molecules smaller than 5 nm or 40 kDa in size (Knockenhauer and Schwartz, 2016), the green fluorescent protein (GFP) produced in the vegetative cytoplasm does not freely pass into sperm cells and vice versa (Engel et al., 2005), suggesting low permeability in sperm cell NPC.

NPC density increases in actively proliferating cells but decreases in terminally differentiated cells. Thus, NPC dynamics were proposed to be associated with nuclear transcriptional activity (Maul et al., 1980). NPC can interact directly with the chromatin to regulate gene expression via a transport-independent mechanism (Ibarra and Hetzer, 2015). The 'gene gating' hypothesis assumes that the association of chromatin regions with NPC could facilitate active gene expression (Blobel, 1985; Ibarra and Hetzer, 2015), while tethering of chromosomal regions to the inner nuclear membrane can result in transcriptional repression (Reddy et al., 2008). A recent study shows that NPC density controls heterochromatin reorganization during oncogene-induced senescence in mammalian nuclei, possibly through balancing the forces attracting heterochromatin to the nuclear lamina and repelling it away from the NPC (Boumendil et al., 2019). These findings suggest NPC number correlates with cell metabolic activity, but the causality between transcription repression and NPC reduction in germ cell development remains an open question.

2.4. Genes involved in pollen compartmentation

Genetic analyses demonstrate that the compartmentation process to ensure the generative cell receives little cytoplasm is crucial for germ cell fate determination. Studies in *Arabidopsis thaliana* mutants have shown that the microspore division and subsequent mitosis of the generative cell are determinants for male germ cell fate. In contrast to three-celled pollen in wild-type Arabidopsis, a portion of *sidecar pollen (scp)* contained two vegetative cells and two sperm cells. The extra vegetative cell was produced due to a defective LOB/AS2 domain protein required for correct timing and orientation of PMI (Chen and McCormick, 1996; Oh et al., 2010). Mutation in retinoblastoma (Rb) protein caused hyperproliferation of vegetative cells and male germ cells. At the bicellular stage of *rbr* pollen, a cell wall was symmetrically placed between two vegetative nuclei instead of between the generative and vegetative nucleus (Chen et al., 2009). In *gemini pollen1 (gem1)* mutants, the coupling of karyokinesis and cytokinesis at PMI was disrupted, resulting in binucleate cells and an additional internal wall (Park et al., 1998; Twell et al., 2002).

The lack of confinement by a cell wall also hinders germ cell development. In *two-in-one(tio)* mutants, the callose wall around the generative nucleus was not fully formed and degraded prematurely. The generative nucleus remained uncondensed and stopped further division (Oh et al., 2005). In double mutants of phragmoplast-associated *Kinesins-12A/B*, the generative cell wall was absent, and defective pollen grains containing two nuclei had severely reduced fertility (Lee et al., 2007). In *solo pollen*, mutants produced uninucleate pollen due to disruption in nuclear division and cytokinesis (Twell et al., 1998). The misplaced or extra cell wall is rarely seen in single-celled mutants, suggesting the nuclear division is a prerequisite for germ cell wall formation, not vice versa.

Expression analysis of vegetative cell fate marker *LAT52* suggests most abnormal nuclei in the cytokinesis-defective pollen adopt vegetative cell fate as a default program (Eady et al., 1995; Twell et al., 1998). Accordingly, two models have been proposed to explain the cell fate determination of daughter cells of PMI. Both models take into account microspore polarity and assume the expression of the *LAT52* marker reflects vegetative cell fate. In the active-repression model, unknown repressors are localized to the generative cell pole to block the transcription of vegetative-cell-specific genes. In the passive-repression model, a hypothetical factor is excluded from the generative cell pole, preventing the transcription of vegetative-cell-specific genes (Eady et al., 1995; Twell et al., 1998). Interestingly, pollen without germ cells showed normal germination, pollen tube growth, and guidance to the embryo sac (Glockle et al., 2018; Zhang et al., 2017). These findings suggest that the vegetative cell is indispensable for male germ cell differentiation, whereas the male germ cells' presence is unnecessary for vegetative cell function.

3. The role of histone variants in regulating pollen gene expression

The generative and sperm cells have tightly packed chromatin while vegetative cells contain dispersed chromatin. The morphological differences in chromosome condensation begin to appear at telophase or as early as mid-anaphase of PMI (Terasaka and Tanaka, 1974). In many animals as well as lower plants such as *Chara corallina* and *Marchantia polymorpha*, small arginine-rich proteins (50~110 amino acids) named protamine are synthesized to displace somatic histones during the late stages of spermatid maturation. These sperm-specific protamines bind to DNA and pack the genome more closely into a highly inactive state (Balhorn, 2007; Reynolds and Wolfe, 1984). Homologs of protamines are absent in higher plants; however, multiple histone proteins (variants) related to chromatin condensation show dynamic changes during pollen mother cell meiosis or pollen mitosis. In lily and tulip, histone H1-like meiotic histone was synthesized and persisted through microsporogenesis and pollen maturation (Sasaki et al., 1990; Sheridan and Stern, 1967). The meiotic histone does not displace normal H1 but is enriched in the centromeric region of meiotic chromosomes (Riggs and Hasenkampf, 1991; Suzuki et al., 1997).

Histone H1 (linker histone) is located at the entry-exit sites of core DNA on nucleosomes, where it compacts chromatins by reducing linker arm flexibility (Bednar et al., 2017; Woodcock et al., 2006). In animals, H1-containing nucleosomes are thought to reduce RNA polymerase II accessibility and gene expression (Fyodorov et al., 2018) and are often absent from a set of active promoters in *Drosophila* and human cells (Braunschweig et al., 2009; Krishnakumar et al., 2008). H1 dynamics affect chromatin condensation of vegetative cells and male germ cells. Several lines of evidence suggest that the vegetative chromatin has a low level of H1. In Arabidopsis microspores, H1 depletion occurs concurrently with the nuclear movement to the cell periphery, and H1 is lost in the vegetative nucleus but preserved in male germ cell nuclei (He et al., 2019; Hsieh et al., 2016). Similarly, histone H1 decreases gradually only in the vegetative nucleus but not in the generative nucleus of bicellular pollen (Tanaka et al., 1998).

Selective loss of H1 contributes to active transcription in the vegetative cell. Developmental depletion of H1 during microgametogenesis in Arabidopsis facilitates heterochromatin relaxation, whereas H1 overexpression results in intense heterochromatic foci in late microspores and partially aborted pollen (He et al., 2019; Rutowicz et al., 2019). Moreover, by combination with DNA methylation, H1 was found to play roles in silencing transposable elements and aberrant intragenic transcripts in Arabidopsis (Choi et al., 2020), and is suggested to repress some repetitive elements by promoting histone H3K9 methylation and chromatin compaction in mouse embryonic stem cells (Healton et al., 2020). These indicate that H1 is important for maintaining transcriptional homeostasis in male cells and their companion cell.

Other histone variants that specifically accumulate in male germ cells are also suggested to be involved in the regulation of chromatin compaction. Among all H2B variants in Arabidopsis, H2B.1, H2B.2, and H2B.8 expression were restricted solely to male germ cells and were not detected in the vegetative cell. In contrast, H2B.10 expression was

detected in microspores then remained largely restricted to the vegetative cell during pollen maturation (Jiang et al., 2020). In lily, gH2A, gH2B, and gH3 were abundantly present only in generative and sperm nuclei but not found in the vegetative nucleus (Ueda et al., 2000; Ueda and Tanaka, 1995). H3.3-like gene *YAH3* transcripts were present in microspores and generative cells. In contrast, *MPH3* transcripts were accumulated explicitly in the vegetative cell and preferentially incorporated into highly active chromatin of the vegetative nucleus (Sano and Tanaka, 2005).

The centromeric histone 3 CenH3 (HTR12) accumulates at heterochromatin around centromeres (Lermontova et al., 2006; Talbert et al., 2002) to ensure kinetochore establishment and chromosome integrity during mitosis (Keceli et al., 2020; Sanei et al., 2011). After PMI, CenH3 and AtMGH3 (*HTR10*) were still expressed in generative and sperm nuclei but depleted in the vegetative nuclei (Ingouff et al., 2007; Okada et al., 2005). In contrast, H3.3(*HTR8*) and H3.14 (*HTR14*) were observed only in vegetative nuclei (Ingouff et al., 2010). It has been suggested that H3.3 can prevent H1 recruitment or compete for H1 occupancy at the transcription start site (Braunschweig et al., 2009; Wollmann et al., 2017). It is probable that specific enrichment of H3.3 and other histone variants coordinately influences the accessibility of transcriptional machinery, hereby regulating differential gene expression in vegetative and germ cells.

4. Transposable element expression in pollen

4.1. Reactivation of transposable elements in vegetative cell

Transposable elements (TEs) or transposons are DNA segments capable of self-mobilization and duplication in the genome via the 'copy-and-paste' or 'cut-and-paste' mechanism. The concept of TEs was first introduced in 1950 during a study of unexpected variegation in maize kernels (McClintock, 1950). TEs can be categorized as Class I (retrotransposons, such as LINEs, SINEs, LTRs) and Class II (DNA transposons, such as TIRs, Helitrons) elements (Wicker et al., 2007), both of which make up a large proportion of the plant genome, and the percentage of TE-DNA in the genome has been shown to positively correlate with the genome size (Tenaillon et al., 2010; Zhou et al., 2020). The majority of plant TEs are inactive ancestral TE copies that have lost their ability to transpose (Chuong et al., 2017; Lisch, 2012), and recent transposition incidents are rare (Quadrana et al., 2016). On the other hand, TEs exhibit strong spatio-temporal expression in certain gametophytic cells. For instance, LTR retrotransposons in maize move mainly in male germ cells but rarely in female germ cells (Dooner et al., 2019). In Arabidopsis somatic cells, TE expression was barely detected, but a significant proportion (32.5 %) of TEs was expressed in male meiocytes (Chen et al., 2010). In addition, a developmental relaxation of transposable element silencing during plant sexual reproduction has also been observed in the vegetative cell, central cell, and fertilized endosperm (Martinez and Slotkin, 2012). These findings imply that TE activation is a natural process during pollen development and may play an essential role in plant reproduction.

TEs are an important source for generating long noncoding RNAs (lncRNAs) and small interfering RNAs (siRNAs) in plants. TE-lncRNAs diversity positively correlates with TE percentage in the genome. About 23 %, 50 %, and 65 % lncRNAs were associated with TEs in *Arabidopsis thaliana*, *Oryza sativa,* and *Zea mays*, respectively (Cho, 2018; Lv et al., 2019; Wang et al., 2017). TE-derived siRNAs have been shown to mediate genome dosage response in Arabidopsis endosperm (Borges et al., 2018). TE-derived *siRNA854* is synthesized in pollen and transferred to the central cell via sperm cells. Depletion of siRNA854 in the endosperm caused triploid seed abortion in Arabidopsis (Wang et al., 2018).

Suppression of TE activity is controlled by a multiple-layered mechanism of DNA methylation and histone modifications. TEs are often highly methylated in all cytosine contexts (CG, CHG, and CHH), while at protein-coding genes methylation occurs mainly in the CG context (Bewick et al., 2016; Schmitz et al., 2013; Stuart et al., 2016). Mobile TE families are generally subjected to higher methylation than non-mobile TE families (Quadrana et al., 2016), suggesting tighter surveillance on TE transposition than on transcription. The edges of TEs have the highest mCHH level, which may delimit the TE and neighboring chromatin (Li et al., 2015; Panda and Slotkin, 2020; Zemach et al., 2013). In plants, de novo CHH methylation of TEs is carried out by the RNA-directed DNA methylation (RdDM) machinery, which requires the guide of 24-nt or 21−22-nt sRNA and plant-specific RNA polymerase IV and V (Erdmann and Picard, 2020; Zhang et al., 2018).

During the decondensation of heterochromatin in the Arabidopsis vegetative nucleus, diverse TE families show reduced DNA methylation, reactivation of expression, and transposition only in the vegetative cell but not in sperm cells (Slotkin et al., 2009). TE reactivation is accompanied by downregulation of 24-nt siRNA and remodeler *DECREASE IN DNA METHYLATION 1 (DDM1)* (Slotkin et al., 2009; Zemach et al., 2013) as well as upregulation of DNA glycosylases such as *DEMETER (DME)* and *REPRESSOR OF SILENCING 1a (ROS1a)* (Kim et al., 2019; Park et al., 2017). Many TE transcripts can be targets of miRNA, and give rise to 21-nt 'epigenetically activated' siRNA (easiRNAs) (Creasey et al., 2014; Slotkin et al., 2009).

4.2. Transposable element silencing in male germ cells by mobile siRNA

siRNA is an essential component in the posttranscriptional silencing of transposons and foreign nucleic acid fragments. The movement of siRNA through plasmodesmata and the phloem is common in plants (Palauqui et al., 1997; Voinnet and Baulcombe, 1997). In Arabidopsis, most mobile siRNAs are associated with transposons and methylated regions (Molnar et al., 2011, 2010). During the selective DNA demethylation and TE reactivation in the vegetative cell, mature siRNAs but not their primary transcripts are detected in sperm cells. A theory has thus been proposed that TE-siRNAs produced from the vegetative cell can move to sperm cells, via an unknown pathway, to reinforce gene silencing in the germ genome or influence imprinted gene expression after fertilization (Martinez et al., 2016, 2018; Slotkin et al., 2009). In this process, the vegetative cell makes sacrifices to reactivate TE expression, and the TE-derived siRNAs are delivered to male germ cells to enhance TE silencing via the RdDM pathway (Fig. 2).

The model of intercellular transfer of siRNA relies on the following two postulates: Firstly, siRNAs need to move from the vegetative cell into the germ cell cytoplasm. In *Plumbago* and Arabidopsis, one of the sperm cells has a long cytoplasmic projection stretching to the periphery of the vegetative nucleus (McCue et al., 2011; Ndinyanka Fabrice et al., 2017; Russell and Cass, 1981). In *Medicago*, the vegetative cell's nuclear envelope facing the generative cell has a higher NPC density (Shi et al., 1991). The polarized distribution of NPC and cytoplasmic connection may facilitate siRNA transit from vegetative nuclei to male germ cells. As the plasmodesmata-like structure is absent between the vegetative nuclei and male germ cells (Cresti et al., 1987), the cross-membrane transportation of siRNAs may occur through unknown siRNA import receptors localized in the plasma membrane of the germ cells. Secondly, siRNA are retrogradely imported to the gametic nuclei. Although the central dogma indicates a unidirectional movement of RNA from the nucleus to the cytoplasm, research in animals shows that mature transfer RNAs (tRNAs) can move from the cytoplasm to the nucleus as part of the quality control mechanism (Kramer and Hopper, 2013; Shaheen and Hopper, 2005). Whether siRNA has a retrograde import pathway remains unresolved.

In addition to the model mentioned above, other explanations are that male germ cells may inherit some siRNAs from microspores. Alternatively, siRNAs may be transported from the newly formed vegetative nucleus to the generative nucleus before the generative cell develops its cell membrane and nuclear envelope (Fig. 2). This alternative model assumes generative nuclei could engulf sufficient siRNA

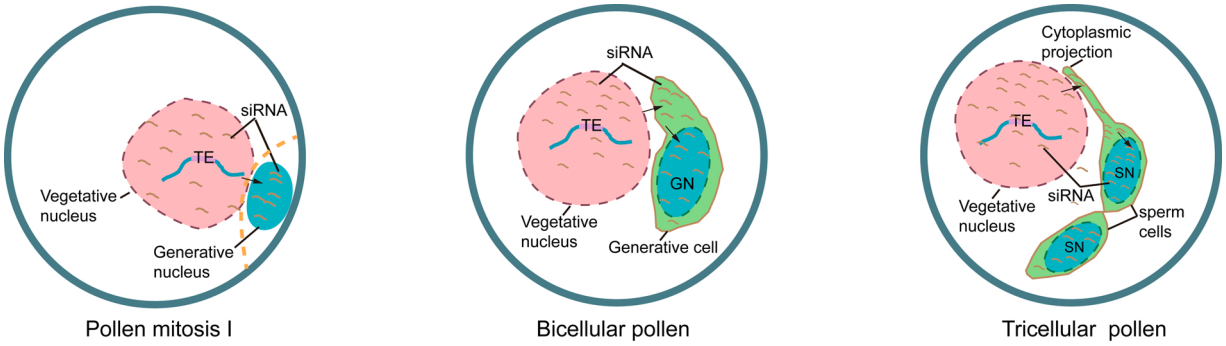

Fig. 2. Schematic illustration of three models for siRNA movement from the vegetative nucleus to the germ nucleus. 1) During the late stages of pollen mitosis I, the vegetative nucleus undergoes TE reactivation and TE-siRNA moves freely to the generative nucleus before the formation of its nuclear envelope and cell wall. 2) At the bicellular stage, TE-siRNA moves from the vegetative cytoplasm to the generative cytoplasm via unknown siRNA transporters localized in the generative cell's plasma membrane (step 1). Retrograde import of siRNA from the cytoplasm to the nucleus of the generative cell (step 2). 3) At the tricellular stage, TE-siRNA moves from the vegetative cytoplasm to the sperm cell's cytoplasm via cytoplasmic projection and unknown siRNA transporters localized in the sperm cell's plasma membrane (step 1). Retrograde import of siRNA from the cytoplasm to the sperm nuclei (step 2).

for TE repression, and the reinforcement of TE silencing by siRNA guided RdDM occurs in the early stage of the generative cell. In support of this model, a recent study of the male germ cell methylome shows that generative nuclei have nearly identical 5-mC DNA methylomes with sperm nuclei, suggesting siRNA guided DNA methylation is already established at the bicellular pollen stage or earlier (Lu et al., 2020).

Several transgenic experiments have tested the gene silencing effect in the male germ cells by vegetative nuclei-derived small RNA. These experiments use vegetative cell-specific promoters such as *LAT52*, *VCK1*, and *KRP6* to drive the production of artificial siRNA or miRNA originating from a partial GFP sequence. A functional GFP fused with an siRNA/miRNA target is expressed by germ cell-specific promoters such as *AtMGH3*. The reduction of GFP fluorescence in sperm cells signifies successful gene silencing due to siRNA/miRNA movement into sperm cells. Although these promoters' specificity at the protein level has been confirmed on many occasions, the specificity at the transcription level still needs to be examined. Indeed, H2B-GFP driven by *LAT52* is observed both in the vegetative and generative cells (Grant-Downton et al., 2013). Basal expression of *LAT52::GUS* was present in the endosperm (McCormick, 1993). Surprisingly, transcriptome analysis of male reproductive lineage cells from tomato suggests native *LAT52* gene transcripts (Solyc10g007270.2) are also present in the generative cell and sperm cells in medium abundance (Liu et al., 2018). These findings suggest that *LAT52* may be transcribed but not translated in male germ cells. The basal expression or inheritance of 'vegetative specific' transcripts in the male germ cells might inadvertently produce siRNA that reduces reporter GFP expression.

4.3. Transposable element expression in male germ cells — relaxed or reinforced?

In general, active TE transcription and transposition are detrimental to the germ cell genome. The spontaneous mutation rate induced by TEs is extremely low on the timescale of dozens of generations (Ossowski et al., 2010; Weng et al., 2019). In the transcriptome of wild-type Columbia Arabidopsis inflorescence tissue, only 0.59 % reads were shown to be TE-initiated transcripts. However, in DNA methylation establishment or maintenance mutants, elevated TE transcription and mutation rates were observed (Panda and Slotkin, 2020; Quadrana et al., 2019).

Many pieces of evidence support that TE suppression in male germ cells is reinforced by mobile siRNA originating from the surrounding companion cell (Ibarra et al., 2012; Lewis et al., 2018; Martinez et al., 2016; Slotkin et al., 2009). That is, the TE methylation level in male germ cells is enhanced by the siRNA guided RdDM pathway which mainly functions in non-CG methylation. However, compared to those in vegetative cells, TEs in male germ cells are hypermethylated in the CG context, but hypomethylated in the non-CG contexts, which include many CHG and CHH methylation sites. Several reports show that the non-CG methylation level is significantly reduced in the generative cell and sperm cells, rather than elevated (Table 1). Prominently, mCHH peaks are present at both boundaries of TEs in the vegetative cell but are absent in generative cells and sperm cells (Fig. 3) (Lu et al., 2020). The mCHH peaks at TE edges are usually thought to preserve the silencing of TEs via canonical 24-nt siRNA guided RdDM (Li et al., 2015; Zemach et al., 2013), and depletion of 24-nt siRNA from mCHH heterochromatin boundaries have been observed in sperm cells (Li et al., 2020a). Therefore, the downregulation of non-CG methylation in TE suggests a relaxation of TE repression in male germ cells.

In contrast to vegetative cell-specific reactivation of TEs in Arabidopsis, there is an active expression of TEs in microspores and sperm cells in maize. About 2000 of TEs are upregulated in sperm cells. This TE expression is family-independent and correlates with high-level expression of neighboring protein-coding genes, suggesting potential functional roles of TEs in sperm cells or fertilization (Warman et al., 2020). TE activity may be low in Arabidopsis sperm cells, but this may not be the same in plant species with larger genomes such as tomato and maize.

From an evolutionary perspective, TE activity is a rich source of mutations. TE insertion/deletion causes structure variation, which profoundly impacts genome size differences and genetic diversity during plant adaptation and evolution (Bennetzen et al., 2005; Lisch, 2012; Wendel et al., 2016). TE expression or transposition in somatic cells provides more plasticity on epigenetic regulation of adjacent gene expression but contributes little to genetic variation in progenies. In contrast, gene mutations induced by TE activity in male germ cells are more likely to be inherited by the next generation. Even though most mutations may affect male germ cells' viability, there are still sufficient viable germ cells to pass genetic information, because plants produce a vast number of sperm cells to ensure successful fertilization of relatively few egg cells. Damaging mutations will lower the sperm cells' ability to compete for fertilization and are very likely to be purged in the double fertilization process, while neutral and beneficial mutations are likely to survive (Quadrana et al., 2016; Stuart et al., 2016). To some extent, a controllable TE expression and transposition in the germ cell is more beneficial than severe TE suppression in the plant, especially for the propagation in a changeable environment.

5. Conclusion

The sperm cell lineage development is generally a self-enclosing process. The germ cell inherits less cytoplasm and fewer organelles

Table 1
Mean methylation level (%) for each cell type in pollen.

Methylation context	Species	Microspore	Vegetative cell	Generative cell	Sperm cell
CG	Arabidopsis	28.8	29.3	_	31.4
	Rice	_	36.3	_	47.9
	Tomato	85.4	78.7	86.3	86.9
CHG	Arabidopsis	12.5	15.0	_	12.3
	Rice	_	20.5	_	19.7
	Tomato	55.4	54.5	48.9	49.3
CHH	Arabidopsis	1.8	5.4	_	1.6
	Rice	_	7.8	_	3.4
	Tomato	3.4	8.8	6.6	6.7

Methylation data are adopted from (Calarco et al., 2012; Kim et al., 2019; Lu et al., 2020).

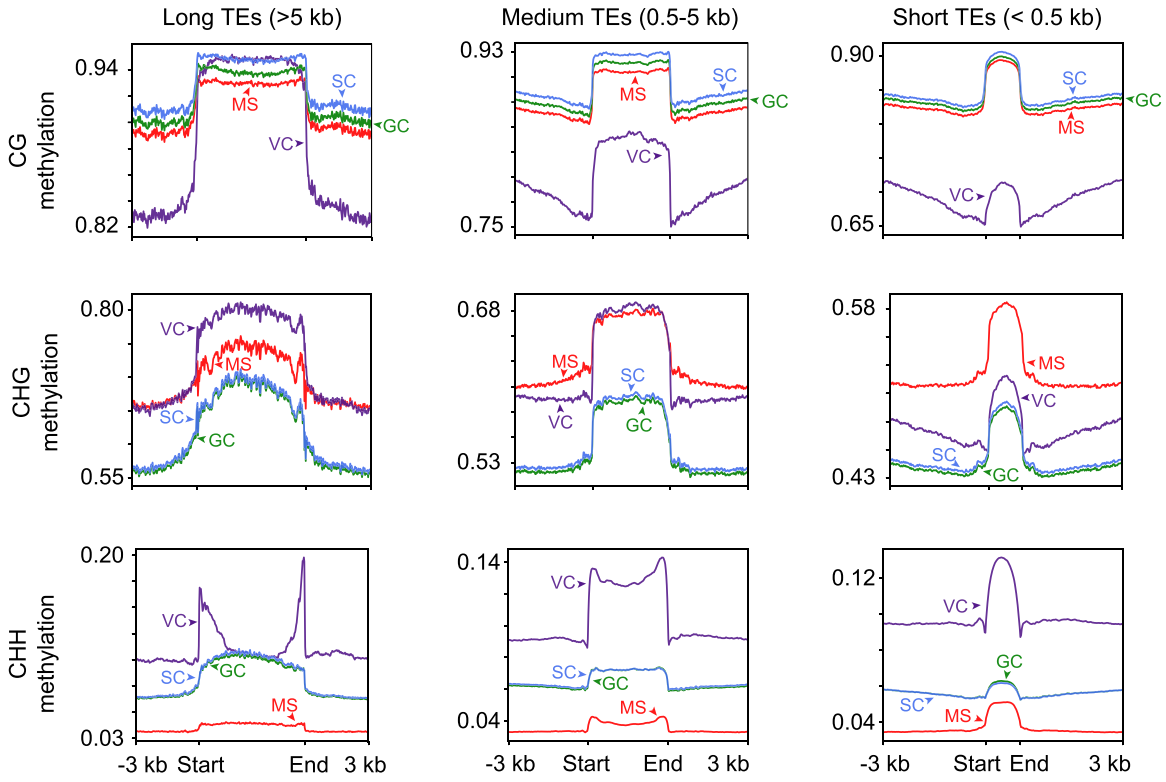

Fig. 3. DNA methylation level of transposable elements (TEs) in tomato pollen. TEs are grouped into 'long' (> 5 kb), 'medium' (0.5-5 kb), and 'short' (<0.5 kb) according to their length. Microspore, MS; vegetative cell, VC; generative cell, GC; sperm cell, SC.

from the microspore, reducing both gateways for intercellular and intracellular communication, lowering metabolic activity and gene transcription. The brief occurrences of callose walls may change the distribution of signal molecules such as auxin within the developing germ cell, and the question of how a 'closed-system' affects pollen gene expression remains mostly unanswered. To gain further insight into gene networks involved in sperm cell production, further transcriptomics studies should try to understand the functions of newly synthesized transcripts and inherited transcripts. It is very clear that epigenetic pathways play critical roles in the regulation of pollen gene expression. It is worth considering that the suppression of TEs may not be intensified in male germ cells. At least in some model plants with a larger genome size and higher proportion of TEs, TE methylation and expression seem to be relaxed in male germ cells as compared to the vegetative cell. Evidence shows leaky expression of 'vegetative cell-specific' genes in male germ cells. Therefore, how and when the siRNA move from the vegetative cell to male germ cells still needs to be explored.

Declaration of Competing Interest

The authors report no declarations of interest.

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References

Anderson, L.E., 1939. Cytoplasmic inclusions in the male gametes of Lilium. Am. J. Bot. 26, 761–766.
Anderson, S.N., Johnson, C.S., Jones, D.S., Conrad, L.J., Gou, X., Russell, S.D., Sundaresan, V., 2013. Transcriptomes of isolated *Oryza sativa* gametes characterized by deep sequencing: evidence for distinct sex-dependent chromatin and epigenetic states before fertilization. Plant J. 76, 729–741.
Angold, R.E., 1968. The formation of the generative cell in the pollen grain of *Endymion non-scriptus* (L). J. Cell. Sci. 3, 573–577.
Balhorn, R., 2007. The protamine family of sperm nuclear proteins. Genome Biol. 8, 227.

Bednar, J., Garcia-Saez, I., Boopathi, R., Cutter, A.R., Papai, G., Reymer, A., Syed, S.H., Lone, I.N., Tonchev, O., Crucifix, C., Menoni, H., Papin, C., Skoufias, D.A., Kurumizaka, H., Lavery, R., Hamiche, A., Hayes, J.J., Schultz, P., Angelov, D., Petosa, C., Dimitrov, S., 2017. Structure and dynamics of a 197 bp nucleosome in complex with linker histone H1. Mol. Cell 66 (384-397), e388.

Bennetzen, J.L., Ma, J., Devos, K.M., 2005. Mechanisms of recent genome size variation in flowering plants. Ann. Bot. 95, 127–132.

Berger, F., Twell, D., 2011. Germline specification and function in plants. Annu. Rev. Plant Biol. 62, 461–484.

Bewick, A.J., Ji, L., Niederhuth, C.E., Willing, E.M., Hofmeister, B.T., Shi, X., Wang, L., Lu, Z., Rohr, N.A., Hartwig, B., Kiefer, C., Deal, R.B., Schmutz, J., Grimwood, J., Stroud, H., Jacobsen, S.E., Schneeberger, K., Zhang, X., Schmitz, R.J., 2016. On the origin and evolutionary consequences of gene body DNA methylation. Proc. Natl. Acad. Sci. U. S. A. 113, 9111–9116.

Blobel, G., 1985. Gene gating - a hypothesis. Proc. Natl. Acad. Sci. U. S. A. 82, 8527–8529.

Bokvaj, P., Hafidh, S., Honys, D., 2015. Transcriptome profiling of male gametophyte development in *Nicotiana tabacum*. Genom. Data 3, 106–111.

Borg, M., Berger, F., 2015. Chromatin remodelling during male gametophyte development. Plant J. 83, 177–188.

Borg, M., Brownfield, L., Twell, D., 2009. Male gametophyte development: a molecular perspective. J. Exp. Bot. 60, 1465–1478.

Borges, F., Gomes, G., Gardner, R., Moreno, N., McCormick, S., Feijo, J.A., Becker, J.D., 2008. Comparative transcriptomics of Arabidopsis sperm cells. Plant Physiol. 148, 1168–1181.

Borges, F., Pereira, P.A., Slotkin, R.K., Martienssen, R.A., Becker, J.D., 2011. MicroRNA activity in the Arabidopsis male germline. J. Exp. Bot. 62, 1611–1620.

Borges, F., Gardner, R., Lopes, T., Calarco, J.P., Boavida, L.C., Slotkin, R.K., Martienssen, R.A., Becker, J.D., 2012. FACS-based purification of Arabidopsis microspores, sperm cells and vegetative nuclei. Plant Methods 8, 44.

Borges, F., Parent, J.S., van Ex, F., Wolff, P., Martinez, G., Kohler, C., Martienssen, R.A., 2018. Transposon-derived small RNAs triggered by miR845 mediate genome dosage response in Arabidopsis. Nat. Genet. 50, 186–192.

Boumendil, C., Hari, P., Olsen, K.C.F., Acosta, J.C., Bickmore, W.A., 2019. Nuclear pore density controls heterochromatin reorganization during senescence. Genes Dev. 33, 144–149.

Braunschweig, U., Hogan, G.J., Pagie, L., van Steensel, B., 2009. Histone H1 binding is inhibited by histone variant H3.3. EMBO J. 28, 3635–3645.

Brewbaker, J.L., 1967. The distribution and phylogenetic significance of binucleate and trinucleate pollen grains in the angiosperms. Am. J. Bot. 54, 1069–1083.

Bucher, G.L., Tarina, C., Heinlein, M., Di Serio, F., Meins Jr., F., Iglesias, V.A., 2001. Local expression of enzymatically active class I β-1,3-glucanase enhances symptoms of TMV infection in tobacco. Plant J. 28, 361–369.

Calarco, J.P., Borges, F., Donoghue, M.T., Van Ex, F., Jullien, P.E., Lopes, T., Gardner, R., Berger, F., Feijo, J.A., Becker, J.D., Martienssen, R.A., 2012. Reprogramming of DNA methylation in pollen guides epigenetic inheritance via small RNA. Cell 151, 194–205.

Chaboud, A., Perez, R., 1992. Generative cells and male gametes - isolation, physiology, and biochemistry. Int. Rev. Cytol. 140, 205–232.

Chang, F., Wang, Y., Wang, S., Ma, H., 2011. Molecular control of microsporogenesis in Arabidopsis. Curr. Opin. Plant Biol. 14, 66–73.

Chen, Y.C., McCormick, S., 1996. *Sidecar pollen*, an *Arabidopsis thaliana* male gametophytic mutant with aberrant cell divisions during pollen development. Development 122, 3243–3253.

Chen, Z., Hafidh, S., Poh, S.H., Twell, D., Berger, F., 2009. Proliferation and cell fate establishment during Arabidopsis male gametogenesis depends on the Retinoblastoma protein. Proc. Natl. Acad. Sci. U. S. A. 106, 7257–7262.

Chen, C., Farmer, A.D., Langley, R.J., Mudge, J., Crow, J.A., May, G.D., Huntley, J., Smith, A.G., Retzel, E.F., 2010. Meiosis-specific gene discovery in plants: RNA-Seq applied to isolated Arabidopsis male meiocytes. BMC Plant Biol. 10, 280.

Cho, J., 2018. Transposon-derived non-coding RNAs and their function in plants. Front. Plant Sci. 9, 600.

Choi, J., Lyons, D.B., Kim, M.Y., Moore, J.D., Zilberman, D., 2020. DNA Methylation and histone H1 jointly repress transposable elements and aberrant intragenic transcripts. Mol. Cell 77 (310-323), e317.

Chuong, E.B., Elde, N.C., Feschotte, C., 2017. Regulatory activities of transposable elements: from conflicts to benefits. Nat. Rev. Genet. 18, 71–86.

Creasey, K.M., Zhai, J., Borges, F., Van Ex, F., Regulski, M., Meyers, B.C., Martienssen, R. A., 2014. miRNAs trigger widespread epigenetically activated siRNAs from transposons in Arabidopsis. Nature 508, 411–415.

Cresti, M., Lancelle, S.A., Hepler, P.K., 1987. Structure of the generative cell wall complex after freeze substitution in pollen tubes of *Nicotiana* and *Impatiens*. J. Cell. Sci. 88, 373–378.

Dal Bosco, C., Dovzhenko, A., Liu, X., Woerner, N., Rensch, T., Eismann, M., Eimer, S., Hegermann, J., Paponov, I.A., Ruperti, B., Heberle-Bors, E., Touraev, A., Cohen, J.D., Palme, K., 2012. The endoplasmic reticulum localized PIN8 is a pollen-specific auxin carrier involved in intracellular auxin homeostasis. Plant J. 71, 860–870.

Diers, L., 1963. Elektronenmikroskopische Beobachtungen an der generativen Zelle von Oenothera hookeri Torr. et Gray. Zeitschrift für Naturforschung B 18, 562.

Ding, Z., Wang, B., Moreno, I., Duplakova, N., Simon, S., Carraro, N., Reemmer, J., Pencik, A., Chen, X., Tejos, R., Skupa, P., Pollmann, S., Mravec, J., Petrasek, J., Zazimalova, E., Honys, D., Rolcik, J., Murphy, A., Orellana, A., Geisler, M., Friml, J., 2012. ER-localized auxin transporter PIN8 regulates auxin homeostasis and male gametophyte development in Arabidopsis. Nat. Commun. 3, 941.

Dong, X.Y., Hong, Z.L., Sivaramakrishnan, M., Mahfouz, M., Verma, D.P.S., 2005. Callose synthase (CalS5) is required for exine formation during microgametogenesis and for pollen viability in Arabidopsis. Plant J. 42, 315–328.

Dooner, H.K., Wang, Q., Huang, J.T., Li, Y., He, L., Xiong, W., Du, C., 2019. Spontaneous mutations in maize pollen are frequent in some lines and arise mainly from retrotranspositions and deletions. Proc. Natl. Acad. Sci. U. S. A. 116, 10734–10743.

Eady, C., Lindsey, K., Twell, D., 1995. The significance of microspore division and division symmetry for vegetative cell-specific transcription and generative cell differentiation. Plant Cell 7, 65–74.

Engel, M.L., Holmes-Davis, R., McCormick, S., 2005. Green sperm. Identification of male gamete promoters in Arabidopsis. Plant Physiol. 138, 2124–2133.

Erdmann, R.M., Picard, C.L., 2020. RNA-directed DNA methylation. PLoS Genet. 16, e1009034.

Fyodorov, D.V., Zhou, B.R., Skoultchi, A.I., Bai, Y., 2018. Emerging roles of linker histones in regulating chromatin structure and function. Nat. Rev. Mol. Cell Biol. 19, 192–206.

Gardini, A., 2017. Global run-on sequencing (GRO-Seq). Methods Mol. Biol. 1468, 111–120.

Gates, R.R., 1908. A study of reduction in *Oenothera rubrinervis* - Contributions from the Hull Botanical Laboratory III. Bot. Gaz. 46, 1–34.

Glockle, B., Urban, W.J., Nagahara, S., Andersen, E.D., Higashiyama, T., Grini, P.E., Schnittger, A., 2018. Pollen differentiation as well as pollen tube guidance and discharge are independent of the presence of gametes. Development 145 dev152645.

Godel-Jedrychowska, K., Kulinska-Lukaszek, K., Horstman, A., Soriano, M., Li, M., Malota, K., Boutilier, K., Kurczynska, E.U., 2020. Symplasmic isolation marks cell fate changes during somatic embryogenesis. J. Exp. Bot. 71, 2612–2628.

Górska-Brylass, A., 1967. Temporary callose wall in the generative cell of pollen grains. Naturwissenschaften 54, 230–231.

Górska-Brylass, A., 1970. The "callose stage" of the generative cells in pollen grains. Grana 10, 21–30.

Grant-Downton, R., Kourmpetli, S., Hafidh, S., Khatab, H., Le Trionnaire, G., Dickinson, H., Twell, D., 2013. Artificial microRNAs reveal cell-specific differences in small RNA activity in pollen. Curr. Biol. 23, R599–R601.

Hackenberg, D., Twell, D., 2019. The evolution and patterning of male gametophyte development. Curr. Top. Dev. Biol. 131, 257–298.

Hafidh, S., Fila, J., Honys, D., 2016. Male gametophyte development and function in angiosperms: a general concept. Plant Reprod. 29, 31–51.

Han, X., Hyun, Tae K., Zhang, M., Kumar, R., Koh, E.-j., Kang, B.-H., Lucas, William J., Kim, J.-Y., 2014. Auxin-callose-mediated plasmodesmal gating is essential for tropic auxin gradient formation and signaling. Dev. Cell 28, 132–146.

Hartig, T.H., 1866. der Füllkern, der diaphragmatische und der intercellulare Zellkern, 1. Bot. Unters. Physik. Labor. Landw. Lehranst, Berlin, p. 294.

Haskell, D.W., Rogers, O.M., 1985. RNA-synthesis by vegetative and sperm nuclei of trinucleate pollen. Cytologia 50, 805–809.

He, S., Vickers, M., Zhang, J., Feng, X., 2019. Natural depletion of histone H1 in sex cells causes DNA demethylation, heterochromatin decondensation and transposon activation. elife 8, e42530.

Healton, S.E., Pinto, H.D., Mishra, L.N., Hamilton, G.A., Wheat, J.C., Swist-Rosowska, K., Shukeir, N., Dou, Y., Steidl, U., Jenuwein, T., Gamble, M.J., Skoultchi, A.I., 2020. H1 linker histones silence repetitive elements by promoting both histone H3K9 methylation and chromatin compaction. Proc. Natl. Acad. Sci. U. S. A 117, 14251–14258.

Heslopharrison, J., 1966. Cytoplasmic connexions between angiosperm meiocytes. Ann. Bot. 30, 221–222.

Heslopharrison, J., Mackenzie, A., 1967. Autoradiography of soluble [2-^{14}C]thymidine derivatives during meiosis and microsporogenesis in *Lilium* anthers. J. Cell. Sci. 2, 387–400.

Heslop-Harrison, J., 1968. Synchronous pollen mitosis and the formation of the generative cell in massulate orchids. J. Cell. Sci. 3, 457.

Hirano, K., Aya, K., Hobo, T., Sakakibara, H., Kojima, M., Shim, R.A., Hasegawa, Y., Ueguchi-Tanaka, M., Matsuoka, M., 2008. Comprehensive transcriptome analysis of phytohormone biosynthesis and signaling genes in microspore/pollen and tapetum of rice. Plant Cell Physiol. 49, 1429–1450.

Hisanaga, T., Yamaoka, S., Kawashima, T., Higo, A., Nakajima, K., Araki, T., Kohchi, T., Berger, F., 2019. Building new insights in plant gametogenesis from an evolutionary perspective. Nat. Plants 5, 663–669.

Honys, D., Twell, D., 2004. Transcriptome analysis of haploid male gametophyte development in Arabidopsis. Genome Biol. 5, R85.

Howden, R., Park, S.K., Moore, J.M., Orme, J., Grossniklaus, U., Twell, D., 1998. Selection of T-DNA-tagged male and female gametophytic mutants by segregation distortion in Arabidopsis. Genetics 149, 621–631.

Hsieh, P.H., He, S., Buttress, T., Gao, H., Couchman, M., Fischer, R.L., Zilberman, D., Feng, X., 2016. Arabidopsis male sexual lineage exhibits more robust maintenance of CG methylation than somatic tissues. Proc. Natl. Acad. Sci. U. S. A. 113, 15132–15137.

Ibarra, A., Hetzer, M.W., 2015. Nuclear pore proteins and the control of genome functions. Genes Dev. 29, 337–349.

Ibarra, C.A., Feng, X., Schoft, V.K., Hsieh, T.F., Uzawa, R., Rodrigues, J.A., Zemach, A., Chumak, N., Machlicova, A., Nishimura, T., Rojas, D., Fischer, R.L., Tamaru, H., Zilberman, D., 2012. Active DNA demethylation in plant companion cells reinforces transposon methylation in gametes. Science 337, 1360–1364.

Iglesias, V.A., Meins Jr, F., 2000. Movement of plant viruses is delayed in a β-1,3-glucanase-deficient mutant showing a reduced plasmodesmatal size exclusion limit and enhanced callose deposition. Plant J. 21, 157–166.

Ingouff, M., Hamamura, Y., Gourgues, M., Higashiyama, T., Berger, F., 2007. Distinct dynamics of histone 3 variants between the two fertilization products in plants. Curr. Biol. 17, 1032–1037.
Ingouff, M., Rademacher, S., Holec, S., Šoljić, L., Xin, N., Readshaw, A., Foo, S.H., Lahouze, B., Sprunck, S., Berger, F., 2010. Zygotic resetting of the histone 3 variant repertoire participates in epigenetic reprogramming in Arabidopsis. Curr. Biol. 20, 2137–2143.
Jevtic, P., Schibler, A.C., Wesley, C.C., Pegoraro, G., Misteli, T., Levy, D.L., 2019. The nucleoporin ELYS regulates nuclear size by controlling NPC number and nuclear import capacity. EMBO Rep. 20.
Jiang, D., Borg, M., Lorkovic, Z.J., Montgomery, S.A., Osakabe, A., Yelagandula, R., Axelsson, E., Berger, F., 2020. The evolution and functional divergence of the histone H2B family in plants. PLoS Genet. 16, e1008964.
Keceli, B.N., Jin, C., Van Damme, D., Geelen, D., 2020. Conservation of centromeric histone 3 interaction partners in plants. J. Exp. Bot. 71, 5237–5246.
Kim, S.J., Fernandez-Martinez, J., Nudelman, I., Shi, Y., Zhang, W., Raveh, B., Herricks, T., Slaughter, B.D., Hogan, J.A., Upla, P., Chemmama, I.E., Pellarin, R., Echeverria, I., Shivaraju, M., Chaudhury, A.S., Wang, J., Williams, R., Unruh, J.R., Greenberg, C.H., Jacobs, E.Y., Yu, Z., de la Cruz, M.J., Mironska, R., Stokes, D.L., Aitchison, J.D., Jarrold, M.F., Gerton, J.L., Ludtke, S.J., Akey, C.W., Chait, B.T., Sali, A., Rout, M.P., 2018. Integrative structure and functional anatomy of a nuclear pore complex. Nature 555, 475–482.
Kim, M.Y., Ono, A., Scholten, S., Kinoshita, T., Zilberman, D., Okamoto, T., Fischer, R.L., 2019. DNA demethylation by ROS1a in rice vegetative cells promotes methylation in sperm. Proc. Natl. Acad. Sci. U. S. A. 116, 9652–9657.
Knockenhauer, K.E., Schwartz, T.U., 2016. The nuclear pore complex as a flexible and dynamic gate. Cell 164, 1162–1171.
Kramer, E.B., Hopper, A.K., 2013. Retrograde transfer RNA nuclear import provides a new level of tRNA quality control in *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. U. S. A. 110, 21042–21047.
Krishnakumar, R., Gamble, M.J., Frizzell, K.M., Berrocal, J.G., Kininis, M., Kraus, W.L., 2008. Reciprocal binding of PARP-1 and histone H1 at promoters specifies transcriptional outcomes. Science 319, 819–821.
La Cour, L.F., 1949. Nuclear differentiation in the pollen grain. Heredity (Edinb) 3, 319–337, 312 pl.
LaFountain, J.R., LaFountain, K.L., 1973. Comparison of density of nuclear pores on vegetative and generative nuclei in pollen of *Tradescantia*. Exp. Cell Res. 78, 472–476.
LaFountain, K.L., Mascarenhas, J.P., 1972. Isolation of vegetative and generative nuclei from pollen tubes. Exp. Cell Res. 73, 233–236.
Lee, Y.R., Li, Y., Liu, B., 2007. Two Arabidopsis phragmoplast-associated kinesins play a critical role in cytokinesis during male gametogenesis. Plant Cell 19, 2595–2605.
Lermontova, I., Schubert, V., Fuchs, J., Klatte, S., Macas, J., Schubert, I., 2006. Loading of Arabidopsis centromeric histone CENH3 occurs mainly during G2 and requires the presence of the histone fold domain. Plant Cell 18, 2443–2451.
Lewis, S.H., Quarles, K.A., Yang, Y., Tanguy, M., Frezal, L., Smith, S.A., Sharma, P.P., Cordaux, R., Gilbert, C., Giraud, I., Collins, D.H., Zamore, P.D., Miska, E.A., Sarkies, P., Jiggins, F.M., 2018. Pan-arthropod analysis reveals somatic piRNAs as an ancestral defence against transposable elements. Nat. Ecol. Evol. 2, 174–181.
Li, Q., Gent, J.I., Zynda, G., Song, J., Makarevitch, I., Hirsch, C.D., Hirsch, C.N., Dawe, R. K., Madzima, T.F., McGinnis, K.M., Lisch, D., Schmitz, R.J., Vaughn, M.W., Springer, N.M., 2015. RNA-directed DNA methylation enforces boundaries between heterochromatin and euchromatin in the maize genome. Proc. Natl. Acad. Sci. U. S. A. 112, 14728–14733.
Li, C., Xu, H., Russell, S.D., Sundaresan, V., 2019. Step-by-step protocols for rice gamete isolation. Plant Reprod. 32, 5–13.
Li, C., Xu, H., Fu, F.F., Russell, S.D., Sundaresan, V., Gent, J.I., 2020a. Genome- wide redistribution of 24-nt siRNAs in rice gametes. Genome Res. 30, 173–184.
Li, Y., Li, L., Wang, Y., Wang, Y.C., Wang, N.N., Lu, R., Wu, Y.W., Li, X.B., 2020b. Pollen-specific protein PSP231 activates callose synthesis to govern male gametogenesis and pollen germination. Plant Physiol. 184, 1024–1041.
Lichter, R., 1982. Induction of haploid plants from isolated pollen of *Brassica napus*. Zeitschrift für Pflanzenphysiologie 105, 427–434.
Lisch, D., 2012. How important are transposons for plant evolution? Nat. Rev. Genet. 14, 49–61.
Liu, L., Lu, Y., Wei, L., Yu, H., Cao, Y., Li, Y., Yang, N., Song, Y., Liang, C., Wang, T., 2018. Transcriptomics analyses reveal the molecular roadmap and long non-coding RNA landscape of sperm cell lineage development. Plant J. 96, 421–437.
Lu, P.L., Chai, M.F., Yang, J.E., Ning, G., Wang, G.L., Ma, H., 2014. The Arabidopsis *CALLOSE DEFECTIVE MICROSPORE1* gene Is required for male fertility through regulating callose metabolism during microsporogenesis. Plant Physiol. 164, 1893–1904.
Lu, Y., Wei, L., Wang, T., 2015. Methods to isolate a large amount of generative cells, sperm cells and vegetative nuclei from tomato pollen for "omics" analysis. Front. Plant Sci. 6, 391.
Lu, Y., Song, Y., Liu, L., Wang, T., 2020. DNA methylation dynamics of sperm cell lineage development in tomato. Plant J. https://doi.org/10.1111/tpj.15098.
Lv, Y., Hu, F., Zhou, Y., Wu, F., Gaut, B.S., 2019. Maize transposable elements contribute to long non-coding RNAs that are regulatory hubs for abiotic stress response. BMC Genomics 20, 864.
Maheshwari, P., 1949. The male gametophyte of angiosperms. Bot. Rev. 15, 1–75.
Mangin, L., 1889. Observations sur la membrane du grain de pollen mûr. Bull. Soc. bot. France 36, 274–284.
Martinez, G., Slotkin, R.K., 2012. Developmental relaxation of transposable element silencing in plants: functional or byproduct? Curr. Opin. Plant Biol. 15, 496–502.
Martinez, G., Panda, K., Kohler, C., Slotkin, R.K., 2016. Silencing in sperm cells is directed by RNA movement from the surrounding nurse cell. Nat. Plants 2, 16030.
Martinez, G., Wolff, P., Wang, Z., Moreno-Romero, J., Santos-Gonzalez, J., Conze, L.L., DeFraia, C., Slotkin, R.K., Kohler, C., 2018. Paternal easiRNAs regulate parental genome dosage in Arabidopsis. Nat. Genet. 50, 193–198.
Maruyama, K., Gay, H., Kaufmann, B.P., 1965. The nature of the wall between generative and vegetative nuclei in the pollen grain of *Tradescantia paludosa*. Am. J. Bot. 52, 605–610.
Mascarenhas, J.P., 1975. The biochemistry of angiosperm pollen development. Bot. Rev. 41, 259–314.
Maul, G.G., Deaven, L.L., Freed, J.J., Campbell, G.L.M., Beçak, W., 1980. Investigation of the determinants of nuclear pore number. Cytogenet. Cell Genet. 26, 175–190.
McClintock, B., 1950. The origin and behavior of mutable loci in maize. Proc Natl. Acad. Sci. U. S. A. 36, 344–355.
McCormick, S., 1993. Male gametophyte development. Plant Cell 5, 1265–1275.
McCue, A.D., Cresti, M., Feijo, J.A., Slotkin, R.K., 2011. Cytoplasmic connection of sperm cells to the pollen vegetative cell nucleus: potential roles of the male germ unit revisited. J. Exp. Bot. 62, 1621–1631.
Molnar, A., Melnyk, C.W., Bassett, A., Hardcastle, T.J., Dunn, R., Baulcombe, D.C., 2010. Small silencing RNAs in plants are mobile and direct epigenetic modification in recipient cells. Science 328, 872–875.
Molnar, A., Melnyk, C., Baulcombe, D.C., 2011. Silencing signals in plants: a long journey for small RNAs. Genome Biol. 12, 215.
Ndinyanka Fabrice, T., Kaech, A., Barmettler, G., Eichenberger, C., Knox, J.P., Grossniklaus, U., Ringli, C., 2017. Efficient preparation of Arabidopsis pollen tubes for ultrastructural analysis using chemical and cryo-fixation. BMC Plant Biol. 17.
Oh, S.A., Johnson, A., Smertenko, A., Rahman, D., Park, S.K., Hussey, P.J., Twell, D., 2005. A divergent cellular role for the FUSED kinase family in the plant-specific cytokinetic phragmoplast. Curr. Biol. 15, 2107–2111.
Oh, S.A., Park, K.S., Twell, D., Park, S.K., 2010. The *SIDECAR POLLEN* gene encodes a microspore-specific LOB/AS2 domain protein required for the correct timing and orientation of asymmetric cell division. Plant J. 64, 839–850.
Okada, T., Endo, M., Singh, M.B., Bhalla, P.L., 2005. Analysis of the histone H3 gene family in Arabidopsis and identification of the male-gamete-specific variant AtMGH3. Plant J. 44, 557–568.
Ossowski, S., Schneeberger, K., Lucas-Lledo, J.I., Warthmann, N., Clark, R.M., Shaw, R. G., Weigel, D., Lynch, M., 2010. The rate and molecular spectrum of spontaneous mutations in *Arabidopsis thaliana*. Science 327, 92–94.
Palauqui, J.C., Elmayan, T., Pollien, J.M., Vaucheret, H., 1997. Systemic acquired silencing: transgene-specific post-transcriptional silencing is transmitted by grafting from silenced stocks to non-silenced scions. EMBO J. 16, 4738–4745.
Panda, K., Slotkin, R.K., 2020. Long-read cDNA sequencing enables a "gene-like" transcript annotation of transposable elements. Plant Cell 32, 2687–2698.
Park, S.K., Howden, R., Twell, D., 1998. The *Arabidopsis thaliana* gametophytic mutation *gemini pollen1* disrupts microspore polarity, division asymmetry and pollen cell fate. Development 125, 3789–3799.
Park, J.S., Frost, J.M., Park, K., Ohr, H., Park, G.T., Kim, S., Eom, H., Lee, I., Brooks, J.S., Fischer, R.L., Choi, Y., 2017. Control of DEMETER DNA demethylase gene transcription in male and female gamete companion cells in *Arabidopsis thaliana*. Proc. Natl. Acad. Sci. U. S. A. 114, 2078–2083.
Quadrana, L., Bortolini Silveira, A., Mayhew, G.F., LeBlanc, C., Martienssen, R.A., Jeddeloh, J.A., Colot, V., 2016. The *Arabidopsis thaliana* mobilome and its impact at the species level. eLife 5, e15716.
Quadrana, L., Etcheverry, M., Gilly, A., Caillieux, E., Madoui, M.A., Guy, J., Bortolini Silveira, A., Engelen, S., Baillet, V., Wincker, P., Aury, J.M., Colot, V., 2019. Transposition favors the generation of large effect mutations that may facilitate rapid adaption. Nat. Commun. 10, 3421.
Reddy, K.L., Zullo, J.M., Bertolino, E., Singh, H., 2008. Transcriptional repression mediated by repositioning of genes to the nuclear lamina. Nature 452, 243–247.
Reichenbach, H.G., 1852. De pollinis Orchidearum genesi ac structura et de Orchideis in artem ac systema regigendis.
Reynolds, T.L., Raghavan, V., 1982. An autoradiographic study of RNA synthesis during maturation and germination of pollen grains of *Hyoscyamus niger*. Protoplasma 111, 177–188.
Reynolds, W.F., Wolfe, S.L., 1984. Protamines in plant sperm. Exp. Cell Res. 152, 443–448.
Riggs, C.D., Hasenkampf, C.A., 1991. Antibodies directed against a meiosis-specific, chromatin-associated protein identify conserved meiotic epitopes. Chromosoma 101, 92–98.
Russell, S.D., Cass, D.D., 1981. Ultrastructure of the sperms of *Plumbago zeylanica*. Protoplasma 107, 85–107.
Russell, S.D., Strout, G.W., 2005. Microgametogenesis in *Plumbago zeylanica* (Plumbaginaceae). 2. Quantitative cell and organelle dynamics of the male reproductive cell lineage. Sex. Plant Reprod. 18, 113–130.
Rutley, N., Twell, D., 2015. A decade of pollen transcriptomics. Plant Reprod. 28, 73–89.
Rutowicz, K., Lirski, M., Mermaz, B., Teano, G., Schubert, J., Mestiri, I., Kroten, M.A., Fabrice, T.N., Fritz, S., Grob, S., Ringli, C., Cherkezyan, L., Barneche, F., Jerzmanowski, A., Baroux, C., 2019. Linker histones are fine-scale chromatin architects modulating developmental decisions in Arabidopsis. Genome Biol. 20, 157.
Sanei, M., Pickering, R., Kumke, K., Nasuda, S., Houben, A., 2011. Loss of centromeric histone H3 (CENH3) from centromeres precedes uniparental chromosome elimination in interspecific barley hybrids. Proc. Natl. Acad. Sci. U. S. A. 108, E498–505.
Sano, Y., Tanaka, I., 2005. A histone H3.3-like gene specifically expressed in the vegetative cell of developing lily pollen. Plant Cell Physiol. 46, 1299–1308.

Sasaki, Y., Yasuda, H., Ohba, Y., Harada, H., 1990. Isolation and characterization of a novel nuclear-protein from pollen mother cells of lily. Plant Physiol. 94, 1467–1471.
Sassen, M.M.A., 1964. Fine structure of petunia pollen grain and pollen tube. Acta Bot. Neerl. 13, 175–181.
Schmidt, A., Schmid, M.W., Grossniklaus, U., 2012. Analysis of plant germline development by high-throughput RNA profiling: technical advances and new insights. Plant J. 70, 18–29.
Schmidt, A., Schmid, M.W., Grossniklaus, U., 2015. Plant germline formation: common concepts and developmental flexibility in sexual and asexual reproduction. Development 142, 229–241.
Schmitz, R.J., Schultz, M.D., Urich, M.A., Nery, J.R., Pelizzola, M., Libiger, O., Alix, A., McCosh, R.B., Chen, H., Schork, N.J., Ecker, J.R., 2013. Patterns of population epigenomic diversity. Nature 495, 193–198.
Schröder, M.B., 1985. Ultrastructural studies on plastids of generative and vegetative cells in Liliaceae 3. Plastid distribution during the pollen development in *Gasteria verrucosa* (Mill.) Duval. Protoplasma 124, 123–129.
Shaheen, H.H., Hopper, A.K., 2005. Retrograde movement of tRNAs from the cytoplasm to the nucleus in *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. U. S. A. 102, 11290–11295.
Sheridan, W.F., Stern, H., 1967. Histones of meiosis. Exp. Cell Res. 45, 323–335.
Shi, L., Mogensen, H.L., Zhu, T., Smith, S.E., 1991. Dynamics of nuclear pore density and distribution patterns within developing pollen: implications for a functional relationship between the vegetative nucleus and the generative cell. J. Cell. Sci. 99, 115–120.
Shi, X., Sun, X.H., Zhang, Z.G., Feng, D., Zhang, Q., Han, L.D., Wu, J.X., Lu, T.G., 2015. GLUCAN SYNTHASE-LIKE 5 (GSL5) plays an essential role in male fertility by regulating callose metabolism during microsporogenesis in rice. Plant Cell Physiol. 56, 497–509.
Slotkin, R.K., Vaughn, M., Borges, F., Tanurdzic, M., Becker, J.D., Feijo, J.A., Martienssen, R.A., 2009. Epigenetic reprogramming and small RNA silencing of transposable elements in pollen. Cell 136, 461–472.
Southworth, D., 1971. In: Heslop-Harrison, J. (Ed.), Incorporation of Radioactive Precursors into Developing Pollen Walls. Pollen. Butterworth-Heinemann, pp. 115–120.
Southworth, D., 1992. Freeze fracture of male reproductive cells. In: Russell, S.D., Dumas, C. (Eds.), Int. Rev. Cytol. Academic Press, pp. 187–204.
Southworth, D., Platt-Aloia, K.A., Thomson, W.W., 1988. Freeze fracture of sperm and vegetative cells in *Zea mays* pollen. J. Ultrastruct. Mol. Struct. Res. 101, 165–172.
Straatman, K.R., Nijsse, J., Kieft, H., van Aelst, A.C., Schel, J.H.N., 2000. Nuclear pore dynamics during pollen development and androgenesis in *Brassica napus*. Sex. Plant Reprod. 13, 43–51.
Strasburger, Eduard, 1884. Neue Untersuchungen Über den Befruchtungsvorgang bei den Phanerogamen: Als Grundlage für eine Theorie der Zeugung. Jena, G. Fischer.
Stuart, T., Eichten, S.R., Cahn, J., Karpievitch, Y.V., Borevitz, J.O., Lister, R., 2016. Population scale mapping of transposable element diversity reveals links to gene regulation and epigenomic variation. elife 5, e20777.
Sundberg, E., Østergaard, L., 2009. Distinct and dynamic auxin activities during reproductive development. Cold Spring Harb. Perspect. Biol. 1, a001628.
Sunderland, N., Wicks, F.M., 1971. Embryoid formation in pollen grains of *Nicotiana tabacum*. J. Exp. Bot. 22, 213–226.
Suzuki, T., Ide, N., Tanaka, I., 1997. Immunocytochemical visualization of the centromeres during male and female meiosis in *Lilium longiflorum*. Chromosoma 106, 435–445.
Talbert, P.B., Masuelli, R., Tyagi, A.P., Comai, L., Henikoff, S., 2002. Centromeric localization and adaptive evolution of an Arabidopsis histone H3 variant. Plant Cell 14, 1053–1066.
Tamura, K., Hara-Nishimura, I., 2014. Functional insights of nucleocytoplasmic transport in plants. Front. Plant Sci. 5, 118.
Tanaka, I., 1997. Differentiation of generative and vegetative cells in angiosperm pollen. Sex. Plant Reprod. 10, 1–7.
Tanaka, I., Ito, M., 1981. Control of division patterns in explanted microspores of *Tulipa gesneriana*. Protoplasma 108, 329–340.
Tanaka, I., Taguchi, T., Ito, M., 1979. Studies on microspore development in liliaceous plants. Bot. Mag. 92, 291–298.
Tanaka, I., Ono, K., Fukuda, T., 1998. The developmental fate of angiosperm pollen is associated with a preferential decrease in the level of histone H1 in the vegetative nucleus. Planta 206, 561–569.
Tenaillon, M.I., Hollister, J.D., Gaut, B.S., 2010. A triptych of the evolution of plant transposable elements. Trends Plant Sci. 15, 471–478.
Terasaka, O., Niitsu, T., 1990. Unequal cell division and chromatin diffrentiation in pollen grain cells. Bot. Mag. 103, 133–142.
Terasaka, O., Tanaka, R., 1974. Cytological studies on nuclear differentiation in microspore division of some angiosperms. Bot. Mag. Tokyo 87, 209–217.
Toller, A., Brownfield, L., Neu, C., Twell, D., Schulze-Lefert, P., 2008. Dual function of Arabidopsis glucan synthase-like genes *GSL8* and *GSL10* in male gametophyte development and plant growth. Plant J. 54, 911–923.
Twell, D., 2011. Male gametogenesis and germline specification in flowering plants. Sex. Plant Reprod. 24, 149–160.
Twell, D., Park, S.K., Lalanne, E., 1998. Asymmetric division and cell-fate determination in developing pollen. Trends Plant Sci. 3, 305–310.
Twell, D., Park, S.K., Hawkins, T.J., Schubert, D., Schmidt, R., Smertenko, A., Hussey, P. J., 2002. MOR1/GEM1 has an essential role in the plant-specific cytokinetic phragmoplast. Nat. Cell Biol. 4, 711–714.
Uchiumi, T., Komatsu, S., Koshiba, T., Okamoto, T., 2006. Isolation of gametes and central cells from *Oryza sativa* L. Sex. Plant Reprod. 19, 37–45.
Ueda, K., Tanaka, I., 1995. The appearance of male gamete-specific histones gH2B and gH3 during pollen development in *Lilium longiflorum*. Dev. Biol. 169, 210–217.
Ueda, K., Kinoshita, Y., Xu, Z.J., Ide, N., Ono, M., Akahori, Y., Tanaka, I., Inoue, M., 2000. Unusual core histones specifically expressed in male gametic cells of *Lilium longiflorum*. Chromosoma 108, 491–500.
Ünal, M., Vardar, F., Ayturk, Ö., 2013. Callose in plant sexual reproduction. Curr. Prog. Biol. Res., pp. 319–343.
Voinnet, O., Baulcombe, D.C., 1997. Systemic signalling in gene silencing. Nature 389, 553.
Wang, D., Qu, Z., Yang, L., Zhang, Q., Liu, Z.H., Do, T., Adelson, D.L., Wang, Z.Y., Searle, I., Zhu, J.K., 2017. Transposable elements (TEs) contribute to stress-related long intergenic noncoding RNAs in plants. Plant J. 90, 133–146.
Wang, G., Jiang, H., Del Toro de Leon, G., Martinez, G., Kohler, C., 2018. Sequestration of a transposon-derived sirna by a target mimic imprinted gene induces postzygotic reproductive isolation in Arabidopsis. Dev. Cell 46 (696-705), e694.
Warman, C., Panda, K., Vejlupkova, Z., Hokin, S., Unger-Wallace, E., Cole, R.A., Chettoor, A.M., Jiang, D., Vollbrecht, E., Evans, M.M.S., Slotkin, R.K., Fowler, J.E., 2020. High expression in maize pollen correlates with genetic contributions to pollen fitness as well as with coordinated transcription from neighboring transposable elements. PLoS Genet. 16, e1008462.
Wei, L.Q., Xu, W.Y., Deng, Z.Y., Su, Z., Xue, Y., Wang, T., 2010. Genome-scale analysis and comparison of gene expression profiles in developing and germinated pollen in *Oryza sativa*. BMC Genomics 11, 338.
Wendel, J.F., Jackson, S.A., Meyers, B.C., Wing, R.A., 2016. Evolution of plant genome architecture. Genome Biol. 17, 37.
Weng, M.L., Becker, C., Hildebrandt, J., Neumann, M., Rutter, M.T., Shaw, R.G., Weigel, D., Fenster, C.B., 2019. Fine-grained analysis of spontaneous mutation spectrum and frequency in *Arabidopsis thaliana*. Genetics 211, 703–714.
Wicker, T., Sabot, F., Hua-Van, A., Bennetzen, J.L., Capy, P., Chalhoub, B., Flavell, A., Leroy, P., Morgante, M., Panaud, O., Paux, E., SanMiguel, P., Schulman, A.H., 2007. A unified classification system for eukaryotic transposable elements. Nat. Rev. Genet. 8, 973–982.
Williams, J.H., Taylor, M.L., O'Meara, B.C., 2014. Repeated evolution of tricellular (and bicellular) pollen. Am. J. Bot. 101, 559–571.
Wollmann, H., Stroud, H., Yelagandula, R., Tarutani, Y., Jiang, D., Jing, L., Jamge, B., Takeuchi, H., Holec, S., Nie, X., Kakutani, T., Jacobsen, S.E., Berger, F., 2017. The histone H3 variant H3.3 regulates gene body DNA methylation in *Arabidopsis thaliana*. Genome Biol. 18, 94.
Woodcock, C.L., Skoultchi, A.I., Fan, Y., 2006. Role of linker histone in chromatin structure and function: H1 stoichiometry and nucleosome repeat length. Chromosome Res. 14, 17–25.
Wu, S.W., Kumar, R., Iswanto, A.B.B., Kim, J.Y., 2018. Callose balancing at plasmodesmata. J. Exp. Bot. 69, 5325–5339.
Wu, W., Zheng, B., 2019. Intercellular delivery of small RNAs in plant gametes. New Phytol. 224, 86–90.
Yu, H.S., Russell, S.D., 1992. Male cytoplasmic diminution and male germ unit in young and mature pollen of *Cymbidium goeringii* : a 3-dimensional and quantitative study. Sex. Plant Reprod. 5, 169–181.
Zemach, A., Kim, M.Y., Hsieh, P.H., Coleman-Derr, D., Eshed-Williams, L., Thao, K., Harmer, S.L., Zilberman, D., 2013. The Arabidopsis nucleosome remodeler DDM1 allows DNA methyltransferases to access H1-containing heterochromatin. Cell 153, 193–205.
Zhang, G., Gifford, D.J., Cass, D.D., 1993. RNA and protein synthesis in sperm cells isolated from *Zea mays* L. pollen. Sex. Plant Reprod. 6, 239–243.
Zhang, J., Huang, Q., Zhong, S., Bleckmann, A., Huang, J., Guo, X., Lin, Q., Gu, H., Dong, J., Dresselhaus, T., Qu, L.J., 2017. Sperm cells are passive cargo of the pollen tube in plant fertilization. Nat. Plants 3, 17079.
Zhang, H., Lang, Z., Zhu, J.K., 2018. Dynamics and function of DNA methylation in plants. Nat. Rev. Mol. Cell Biol. 19, 489–506.
Zheng, Y., Wang, D., Ye, S., Chen, W., Li, G., Xu, Z., Bai, S., Zhao, F., 2020. Auxin guides germ cell specification in Arabidopsis anthers. bioRxiv preprint. https://doi.org/10.1101/2020.10.13.337634.
Zhou, W., Liang, G., Molloy, P.L., Jones, P.A., 2020. DNA methylation enables transposable element-driven genome expansion. Proc. Natl. Acad. Sci. U. S. A. 117, 19359–19366.