Control of male germ-cell development in flowering plants

Mohan B. Singh and Prem L. Bhalla*

Summary

Plant reproduction is vital for species survival, and is also central to the production of food for human consumption. Seeds result from the successful fertilization of male and female gametes, but our understanding of the development, differentiation of gamete lineages and fertilization processes in higher plants is limited. Germ cells in animals diverge from somatic cells early in embryo development, whereas plants have distinct vegetative and reproductive phases in which gametes are formed from somatic cells after the plant has made the transition to flowering and the formation of the reproductive organs. Recently, novel insights into the molecular mechanisms underlying male germ-line initiation and male gamete development in plants have been obtained. Transcriptional repression of male germ-line genes in non-male germ-line cells have been identified as a key mechanism for spatial and temporal control of male germ-line development. This review focuses on molecular events controlling male germ-line development especially, on the nature and regulation of gene expression programs operating in male gametes of flowering plants.

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Introduction

Most of the grains and seeds that form the world staple food supply are the result of successful functioning of male and female gametes during fertilization. Despite the importance of plant reproduction, our understanding of the development, differentiation and fertilization processes of gamete lineages in higher plants is limited. Most studies in recent decades have investigated the reproductive development of the anther and ovule[1–5] within the context of androecium and gynoecium, and can be regarded as early sexual organ differentiation stages of the flower, which, except for a few cells, is largely a sporophytic and thus somatic lineage. In contrast, this paper focuses on reviewing progress towards understanding the molecular basis of male germ-line initiation and male gamete development in flowering plants.

The germ-line cells in most animals diverge from somatic cells during early embryo development and remain as a distinct stem cell population throughout the life of the animal (Fig. 1A). In contrast, plants exhibit distinct vegetative and reproductive phases, and the male germ line in plants originates in flowers from the cells of a previous somatic lineage (Fig. 1B, Fig. 2). Cell-cycle decisions in plant anther tissues from mitosis to meiosis initiate a complicated cascade of development processes that leads to the formation of four haploid microspores from a single diploid microspore mother cell (Fig. 2). The male germ line is initiated from the haploid microspore via asymmetric division, following which the smaller generative cell, which defines the male germ-cell lineage becomes wholly encased within the much larger vegetative cell to form a unique ''cell-within-a-cell structure''. The generative cell subsequently divides to produce two sperm cells (Fig. 3). In some plants, such as *Arabidopsis* and rice, the division of generative cell to produce two sperm cells takes place during pollen maturation in the anther, whereas in other plants, such as tobacco and lily, this division occurs after pollination, inside the pollen tube (Fig. 3). During pollen germination, the wall of the vegetative cell extends to produce a pollen tube via tip elongation and, by this mechanism, the two sperm cells are ultimately delivered to the embryo sac.

Asymmetric division of the microspore is essential for establishing the male germ line, since experimental manipulations of division lead to the failure of generative cell formation.[6] Moreover, *Arabidopsis* pollen mutants (such as gem1 and gem2 and scp) that show defects in asymmetric division of the microspores show failure of male gamete transmission[7–10] indicating that the male germ-line initiation pathway that leads to the formation of generative and sperm cells is decisively dependent on asymmetric division of the haploid microspore. Use of colchicine as a microtubule

Plant Molecular Biology and Biotechnology Laboratory, Australian Research Council Centre of Excellence for Integrative Legume Research, Faculty of Land and Food Resources, The University of Melbourne, Australia.

*Correspondence to: Prem L. Bhalla, Plant Molecular Biology and Biotechnology Laboratory, Australian Research Council Centre of Excellence for Integrative Legume Research, Faculty of Land and Food Resources, The University of Melbourne, Parkville, Victoria 3010, Australia. E-mail: premlb@unimelb.edu.au

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Abbreviations: EST, expressed sequence tags; GC, generative cell; GFP, green florescent protein; GRSF, germ-line-restrictive silencing factor; GUS, β-glucuronidase; LGC1, lily generative-cell-specific 1; SC, sperm cells; VC, vegetative cell.

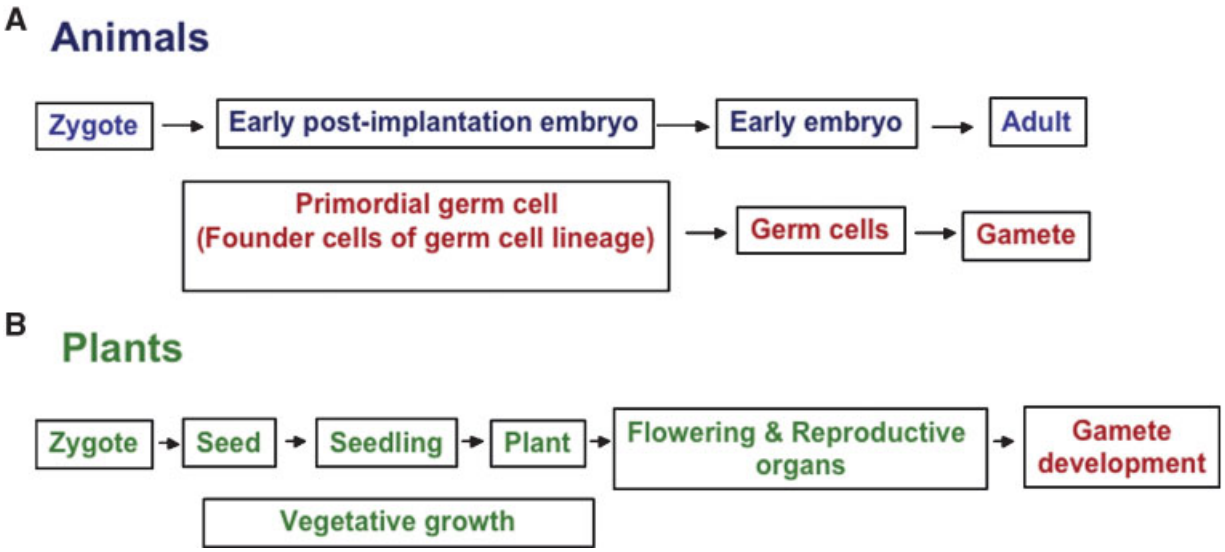

Figure 1. Outline of the landmark stages in the formation of germ lines in animals and plants. **A:** An early embryo formed from a zygote that results from the fusion of male and female gametes generates not only all the parts of animal body (somatic cells) but also demarcates germ cells for the next generation. Note pre-meiosis establishment of germ lines and the continuous production of functional male gametes is dependent on germ-line stem cells during the adult life of an animal. **B:** Seeds resulting from double fertilization in higher plants have well-developed embryos. Seed germination results in a fully developed plant at the vegetative stage. The transition to the flowering stage initiates the formation of the reproductive organs, followed by gametes. Origin of gametes from gametophyte is a post-meiotic event. Gamete development within a reproductive organ is not a continuous process.

disruptive drug[6] and identification of GEM1/MOR1 as a microtubule-associated protein also indicated that the cell cytoskeleton appears to play a key role in establishing the division plane for asymmetric cell division.[7,10,11] Despite these advances in our knowledge, the key intrinsic molecular determinants that control asymmetric polarity division and the founding of male germ line have not been identified. It appears that the key intrinsic steps that specify asymmetric division of the microspore and subsequent determination of the cell fate are determined by the haploid genome in a cell-autonomous manner, since microspores removed from their anther niche and cultured in artificial media are capable of sperm cell formation in vitro.[12]

In contrast to the significant understanding of the molecular genetic basis of male gamete development and fertilization in animals, most of the mechanisms underlying the molecular

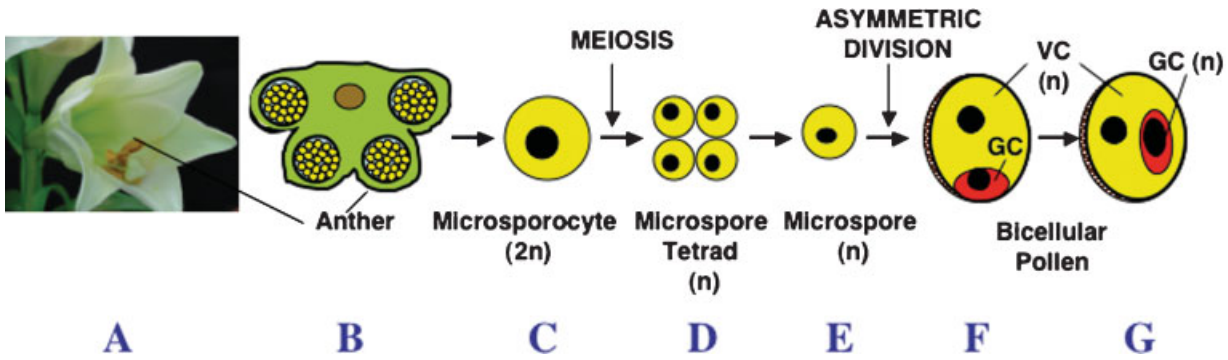

Figure 2. Male germ-line development in plants. **A:** Lily flower showing the male reproductive anther organ (yellow). **B:** Diagrammatic representation of early anther showing microsporocytes (2n) within the developing anther. **C:** Microsporocyte undergoing meiosis resulting in the formation of a tetrad of haploid microspores (n) (**D**). **E:** A microspore. **F:** Early bicellular pollen. Asymmetric division within a microspore results in the formation of a larger vegetative cell (VC) and a smaller generative cell (GC). **G:** Mature bicellular pollen.

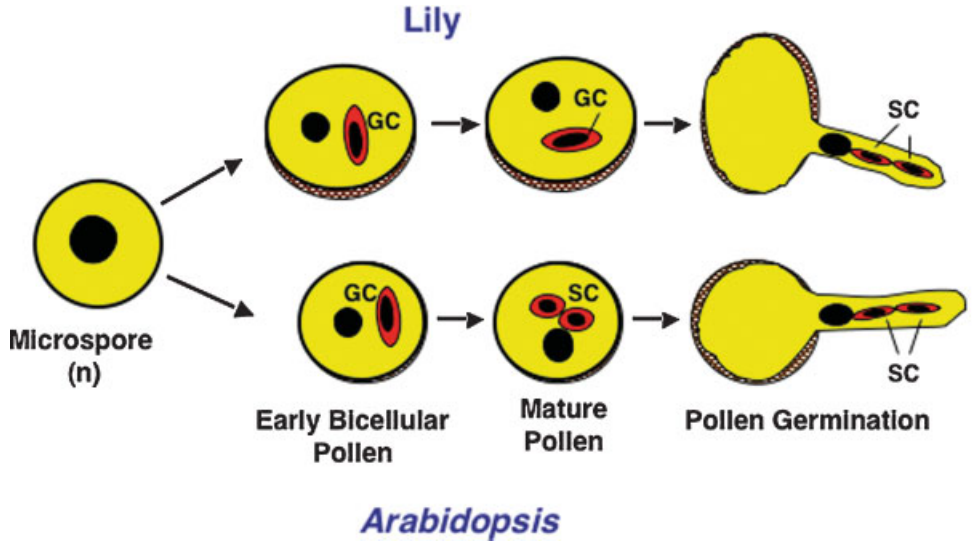

Figure 3. Formation of sperm cells. Bicellular pollen showing a smaller generative cell (GC) and a larger vegetative cell. Mature pollen in plants such as lily is released from the anther at this stage for pollination. In plants such as *Arabidopsis* and rice, the pollen undergoes further division whereby the generative cell divides mitotically to produce two sperm cells (SC). Tricellular pollen is released from the anther for pollination, whereas bicellular pollen undergoes a second mitotic division during pollen tube elongation while growing through female reproductive tissues.

basis of flowering-plant germ-cell specification, differentiation and gamete interactions remain unknown. However, with the availability of new molecular biology and genetic tools, there has been exciting progress in recent years towards the understanding of flowering plant gamete development. Therefore, this review will focus on molecular and genetic control of male germ-line development in plants and in particular on the nature and regulation of gene expression programs operating in the male gametes.

Transcriptome-based approaches to investigating male germ-line development

Until recently little information was available regarding the gene expression programs underpinning flowering plant male germ-line initiation and male gamete development. This lack of knowledge was mainly attributable to the inaccessibility of generative and sperm cells due to their encasement within the male gametophyte. Moreover, this important area remained neglected because of a long-held view in the literature that the condensed state of the chromatin and the very small amount of cytoplasm relative to the nucleus probably reflected the transcriptionally quiescent nature of generative and sperm cells. The development of novel protocols to isolate generative and sperm cells[13,14] eventually led to dawn of molecular era in plant gamete research. These gamete isolation protocols provided opportunities to address several outstanding questions such as whether these cells are transcriptionally active and have protein synthesis machinery that is independent of the outer vegetative cell.[15–17] The presence of translatable mRNA in generative and sperm cells was initially confirmed by metabolic labelling experiments.[18–20] This observation led to the construction of cDNA libraries from isolated generative and sperm cells and the identification of male gamete-specific genes in flowering plants.[21,22]

Currently, transcriptome data are available for *Arabidopsis* pollen[23–25] as well as EST data from maize sperm cell[22] and lily generative cell.[26] Transcriptional profiling of *Arabidopsis* pollen using an Affymetrix 8K chip showed that the transcriptome of pollen is clearly distinct from that of vegetative tissues.[23] Out of 7,792 annotated genes, 992 were shown to be pollen expressed and 40% of these pollen-expressed genes were found to be pollen specific. Subsequent use of ATH1 genome arrays identified 13,977 male gametophyte-expressed transcripts of which 9.7% (1355) were male gametophytic specific[24] while use of same ATH1 chip by different investigators identified 6,587 genes to be expressed in pollen.[25] While RNA prepared using total *Arabidopsis* pollen contains contributions from both vegetative and sperm cells, it is worth noting that due to very small size of the latter, the *Arabidopsis* pollen transcriptome is likely to highly biased towards reporting genes expressed in much larger vegetative cells. Hence, transcriptome data for isolated sperm cells would be desirable to gain insight into molecular events governing sperm cell development and function. Fluorescence-activated cell sorting (FACS) has been used to isolate maize (*Zea mays*) sperm cells.[22] Isolated maize sperm cells were used to

prepare cDNA library and 5,093 ESTs sequences have been obtained. Several transcripts encoding proteins similar to hypothetical *Arabidopsis* proteins were identified in the sperm cells of mature pollen. Analysis of 886 expressed sequence tags (ESTs) from lily generative cells revealed the presence of 637 non-redundant genes,[26] nearly 61% of which represented novel genes and hence targets for investigating male germ-line-specific functions.

The identification of several transcripts present specifically in the generative and sperm cells indicated that despite their condensed chromatin organization, these male germ cells have their own genetic program.[27–29] Sequencing of representative sets of cDNAs revealed classes of genes that are developmentally regulated in the male germ-line cells, including (a) genes that are shared with somatic cells but are upregulated in the male germ line, (b) germ-cell-specific gene variants and (c) genes that are exclusively expressed in male germ line cells.[26,30]

Genes that are shared with somatic cells but are upregulated in plant male germ-line cells include those involved in the DNA repair pathway.[31] A plant homologue of the human nucleotide excision repair gene ERCC1 was found to be upregulated several-fold in lily generative cells compared to pollen vegetative cells and plant somatic cells. In plants, the germ line is not set aside early in embryo development, with germ cells instead originating from cells of a previous somatic lineage. Thus, plant germ-line cells can carry several mutations that accumulate during somatic growth. It has been proposed that a stringent haploid selection process during gametophyte development filters out most of the deleterious mutations. However, the fully developed male pollen gametophyte is exposed to solar UV radiation and other environmental mutagens after being released from the anther. Upregulation of DNA repair genes in the male germ line most likely protects germ-line DNA from heritable mutations resulting from DNA damage. Other genes that are upregulated in generative cells include a cluster of genes related to the cell-cycle-progression pathway.[26,30]

Genes encoding ubiquitin-pathway-related proteins such as polyubiquitin, proteasome subunit, ubiquitin-conjugating enzyme, Skp1 and Ring box protein were found to be highly upregulated in the generative cells.[30] Ubiquitin-pathway-related genes have been found to be active in *Plumbago* species and maize sperm cells.[22,28] The ubiquitin system has also been shown to play an essential role in male gametogenesis in mice and humans.[32,33] The high level of expression of ubiquitin-pathway-related genes in generative cells suggests that the ubiquitin proteolysis system plays a critical role in the male gametogenesis of higher plants. In addition to the normal complement of histones present in plant somatic cells and pollen vegetative cell, the male germ-line cell possesses cell-specific variants of histones H3 and H2B.[26,27,29,30,34–37]

Comparison of lily generative cell ESTs with maize sperm cell ESTs indicated an overlap (168 out of 637 lily ESTs) in male gamete gene expression in generative and sperm cells in these plant species while comparison with *Arabidopsis* male gametophyte-specific transcripts[24] indicated that 129 lily generative cell ESTs showed significant similarity to Arabidopsis male gametophyte-specific genes. In addition, microarray studies showed unique gene expression profile of lily generative cells;[30] 83% (356 transcripts out of 430 genes) of the transcripts were enriched in generative cells. A high percentage of cell-specific transcripts in generative cell, a distinctive feature, is unique to these male gametic cells. Further, a significant overlap in the expressed genes among lily generative-cell ESTs, maize sperm-cell ESTs and *Arabidopsis* pollen-specific transcriptome is apparent throughout all functional role categories.[26] This comparative approach has been successful in maize to identify germ-line-specific promoters GEX1, GEX2 in *Arabidopsis*.[38] Further, these genome-wide and expressed genes data sets could be useful in identifying conserved male-gamete-specific genes and formulating hypothesis about their potential cellular functions.

Genes that are vital for male gamete development and for controlling gamete interactions are likely to include those that encode proteins that are exclusively expressed in germ-line cells. Several transcripts in lily generative cells that encode proteins that are similar to proteins classified as hypothetical in the *Arabidopsis* databases appear to be specific to the male germ line in mature pollen. The first such identified gene from a generative cell cDNA library was LGC1 (lily generative cell-specific 1; accession no. AF110779), which encodes a small protein of 128 amino acids with a calculated molecular mass of 13.8 kDa.[21] The presence of a hydrophobic domain exhibiting the characteristics of a GPI anchor suggests that this protein is located on the surface of the cell membrane of the male germ cell, and this has been confirmed by immunolocalization experiments, with expression analysis showing that LGC1 is expressed specifically in the generative and sperm cells of lily.[21,26,30] We recently found that LGC1 was represented by 11 out of 886 sequenced lily generative cell ESTs.[26]

The initial identification of male germ-line-specific genes in lily and subsequent investigation of their functions via disrupting the expression of their homologues in the model plant *Arabidopsis* has turned out be a highly successful strategy for determining the male gamete proteins that are critical to development and fertilization of higher plants. Mori et al.[39] used isolated lily generative cells to identify a higher plant homologue of GlsA, which is a chaperone-like protein essential for gonidia formation in *Volvox*.[40] Using degenerate PCR primers, the authors reported amplification of cDNA from lily generative cell RNA that was highly similar to *Volvox* counterparts. Immunolocalization analysis revealed the co-localization of this putative chaperone protein with

α-tubulin, suggesting that this plays an important role in the morphogenesis of the generative cell by stabilizing cytoskeletal structures.

Further differential display experiments performed in the same laboratory to compare the gene expression patterns of unicellular microspores, bicellular pollen and isolated generative cells led to the identification of a novel generative-cell-specific protein (GCS1) from lily generative cells.[41] GCS1 possesses a carboxy (C)-terminal transmembrane domain and is localized to the surface of generative and sperm cells, suggesting its role in gamete interactions. A GCS1 homologue is also present in *Arabidopsis*. The failure of gamete fusion leading to male sterility in *Arabidopsis* plants possessing a mutation in the GCS1 locus suggests that this gene-encoded function is essential for fertilization. It was further proposed that GCS1 is anchored on the surface of sperm cells via its C-terminal transmembrane domain.

The presence of LGC1 on the surface of lily male germ cells and the conservation of LGC1 homologues in *Arabidopsis* and rice suggest that this protein is a key player in the gamete interactions of flowering plants. Other lily genes reported to be represented in the lily generative cell EST library, particularly those showing homology in *Arabidopsis*, represent a unique starting point for investigating the repertoire of proteins involved in male gamete differentiation. Although these transcriptomic approaches are providing valuable insight into the portions of flowering-plant genomes that are expressed in male germ-line cells, they do not provide information on which of the mRNAs are actually translated and when this occurs. Whether there is a temporal disconnection between mRNA transcription and translation to proteins in male germ cells is unclear. Whether certain mRNAs are sequestered in ribonucleoprotein particles and stored prior to being translated also remains to be addressed. It should be noted that pollen generative cells that have already undergone dehydration as part of the maturation process contain abundant and diverse mRNAs, even when protein synthesis is not active. Since transcriptional profiling of generative and sperm cells has been based on cells isolated from mature desiccated pollen, it is suggested that these RNAs will be translated following rehydration of the pollen on the stigma surface following pollination. An integrative transcriptomic approach is likely to identify the most-relevant male-germ-cell-expressed proteins.

Genetic approaches for unravelling the male-germ-cell genes critical to male-germ-cell differentiation

Several genetic segregation screening studies of the genes expressed in haploid male gametophyte have been performed using *Arabidopsis* as a model system. The genetic lesions in most of the mutants identified via such screenings are in genes expressed in the larger cell of the pollen, whose knockout induces aberrations in pollen development that lead to male sterility.[8,42–47]. Since pollen has a haploid genome, any mutation in the genes that are essential for general cellular functions is likely to lead to the termination of development. For example, mutations in pollen-expressed genes that are involved in sucrose transport, membrane trafficking or cation transport showed a sterility phenotype.[48] Genetic compensation due to gene redundancy is also another shortcoming of the genetic approach, since gene redundancy is a normal phenomenon in plants and is considered to be responsible for the absence of phenotypes in the majority of single loss-of-function mutants.[49] These limitations question the ability to identify genes that are expressed only in the male germ cells of pollen. The screening for such mutants has to be limited to a subset in which pollen development and tube growth are normal, but where there is still no transmission of the male genome to the next generation. *Arabidopsis* mutants *duo1* and *duo2* are two such identified mutants, in which the pollen morphology appears normal but where blocked generative cell division results in the formation of bicellular pollen at anthesis.[50] It is worth mentioning here that these mutants were not identified by segregation screen, but in a direct morphological screen for germ-line defects. In duo2 mutant specifically generative cell mitosis at pro-metaphase was blocked.[52] *DUO*, which was subsequently identified by map-based cloning, encodes a novel R2R3-MYB transcription factor that is expressed specifically in the male germ line.[51] The DUO1 protein was reportedly localized to the nucleus of generative and sperm cells, with a proposed function of promoting generative cell by activating specific targets such as cyclin genes. The mitotic division of generative cell also fails in *Arabidopsis* mutant cdc2a,[52,53] in which only one sperm cell (rather than two) is produced. The viable mutant pollen can only fertilize one cell in the embryo sac. Intriguingly, the single gamete fertilizes only the egg cell. The serine/threonine protein kinase cdc2 is a key regulator of the cell cycle, acting through cyclin-dependent phosphorylation.

Another *Arabidopsis* mutant has been described in which the generative cell divides normally and sperm cells that are delivered to the embryo sac fail to fuse with either the egg cell or the central cell.[42,45,54] It was also confirmed that the absence of fertilization in HAP2 mutants was not due to a defect in sperm development or to migration of sperm within the pollen tube. HAP2 was found to be allelic to GCS1.[41] HAP2 encodes a 705-amino-acid membrane protein with a histidine-rich C terminus. This protein is not similar to genes of known function and has no obvious functional motifs. Database searches have revealed that HAP2 homologues are present in other flowering plants. HAP2 is specifically expressed in sperm cells, as confirmed by reporter gene analysis in transgenic *Arabidopsis* plants. Thus, these data suggest that unique molecules are involved in gamete function.

Chromatin remodelling and male germ-cell-specific histone variants

As soon as asymmetric cell division of a microspore leads to the formation of two unequal cells, it becomes apparent that the chromatin of the generative nucleus is much more condensed than that of the vegetative nucleus.[55] This condensed nature of the chromatin is also retained in sperm nuclei. This characteristic of higher plants is also present in animals,[56] with the condensation of male chromatin in animal sperm cells being mediated by the exchange of somatic histones with transition proteins, followed by protamines or sperm-specific histones that are, in turn, replaced by somatic histones provided by the egg cell following fertilization. Proteins similar to protamines have been reported in the motile sperm cells of lower plants,[57] but these proteins are not conserved in higher plants. A biochemical approach to comparing chromatin proteins from vegetative and generative cell nuclei in lily and testing the comparative mobility of these proteins on two-dimensional electrophoresis gels revealed that at least five nuclear basic proteins were either specific to or enriched in generative cell nuclei.[34] Two of these proteins were identified as variants of histones H3 and H2B, and were designated gH3 and gH2B, respectively. Immunocytochemical staining of these histone variants showed that they were not only present in generative cells but also in the two sperm cells formed by the division of the generative nucleus.[35] The first study to investigate the expressed genes represented in cDNA libraries constructed from isolated generative cells revealed an abundance of transcripts encoding histone variants.[58] At least five cell-specific histone H3 variants are expressed in lily male germ cells.[31] In histone variants gcH3 and leH3, the lysine at position 9 is substituted by methionine. Despite sharing conserved structural features with centromeric histone H3, the germ-line-specific variant form gH3 is distributed throughout the chromatin. In addition, the methylation pattern of lily histones associated with male germ-line cell appears to differ from that observed in somatic cells.

In *Arabidopsis*, one histone H3 gene, *At1g19890*, is expressed specifically in germ cells.[27] In contrast to lily germ-line histone variants, the amino acid sequence of this *Arabidopsis* variant is highly conserved in somatic histones. It is notable that histone genes are highly conserved in plants, and the only histones that show significant sequence variations are in those variants expressed in male germ-line cells. There exists the tantalizing possibility of a causal relationship between the expression of histone variants and the differential condensation and/or differential gene expression programming in male germ cells of higher plants. Further experiments involving either ectopic expression of germ-line variants in somatic cells or individual knockout mutants exhibiting disrupted function of germ-line-specific genes are required to address these outstanding questions.

Transcriptional regulation of male germ-line-specific gene expression

Molecular studies are beginning to elucidate the regulators of germ-line-specific gene expression, which is essential to understanding the gene circuits underpinning male germ-cell differentiation. Transcriptome analyses have revealed that a significant number of flowering-plant genes are transcribed exclusively in the male germ line.[27,30,54,58,59] What is the nature of transcriptional regulation programs that control the cell specificity of male germ-cell-specific genes? The identification of *LGC1* as the first-identified male germ-line cell-specific transcript expressed under the control of generative/sperm cell promoter provided a unique model system in which to investigate the underlying transcriptional regulatory mechanisms.

Use of the LGC1-GFP reporter gene construct in transient transformation experiments of lily pollen revealed the generative cell-specific expression of LGC1,[60] while stable transformation of a heterologous plant, tobacco with LGC1-GUS, showed the generative cell-specific expression of a reporter gene in the generative cells of transgenic tobacco, indicating that the transcriptional factors required to control the specificity of expression of LGC1 promoter are conserved in male germ-line cells. The strict generative cell specificity of LGC1 promoter was confirmed by obtaining transgenic plants carrying the LGC1 promoter fused with the DT/A cytotoxin gene.[60] Such plants showed specific ablation of pollen grains containing DT/A expression in the generative cells. Deletion analysis of the *LGC1* promoter showed the presence of a 43 bp nucleotide regulatory silencer element whose excision from the promoter led to a constitutive pattern of expression of truncated promoter in all the plant tissues tested. Gel retardation assays showed that nuclear extracts of lily petal cells contain a protein that specifically interacts with the *LGC1* silencer sequence.[60] The gene encoding this repressor protein was recently cloned by southwestern blotting of a lily petal cDNA expression library.[61] GRSF (Germline Restrictive Silencer Factor) is a novel 24-kDa DNA-binding repressor protein encoded by a gene expressed ubiquitously in plant tissues with the exception of generative cells. Immunolocalization showed that GRSF is present in the nuclei of uninucleate microspores and pollen vegetative cells but is absent in the generative cell nucleus. Chromatin immunoprecipitation assays showed that GRSF interacts with a specific domain in promoter region of *LGC1* and with the male germ-line-specific histone *gcH3*. Promoter mutagenesis experiments led to the identification of a conserved 8-bp motif in the *LGC1* and *gH3* promoters. This sequence motif is likely to be core-binding site for GRSF. These data show that the male germ-cell-specific gene expression of *LGC1* and other coordinately expressed genes might be controlled by GRSF by repressing their expression in other plant cells. The promoter region of the *Arabidopsis* male germ-line-specific

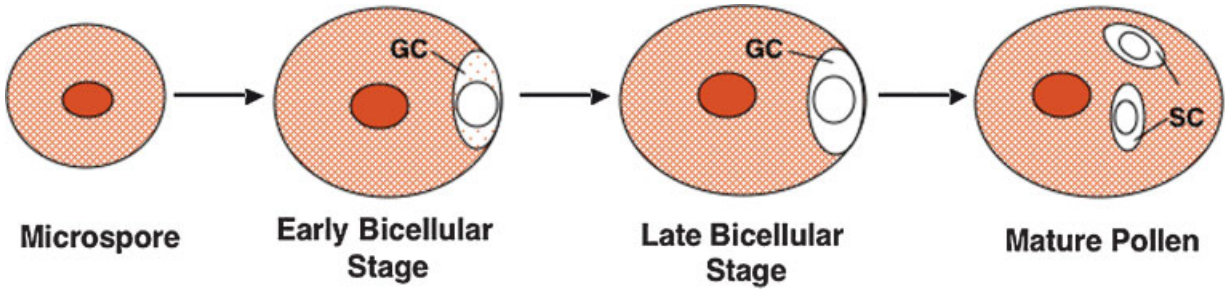

Figure 4. Proposed model for the asymmetric distribution of the repressor during pollen mitosis. A gene encoding a repressor protein, GRSF (shown in red), is active in the microspore, and this repressor protein acts as gametic cell restrictive factor. Following asymmetric division, GRSF is absent from the smaller generative cell, leading to activation of gamete-specific genes.

gene DUO1 is activated in both generative and sperm cells. It has been reported that the promoter *AtGEX2* (*At5g49150*) is active only in the sperm cells and in the progenitor generative cell of *Arabidopsis*.[38] The histone gene promoter *AtMGH3/At1g19890* is specifically activated in generative and sperm cells of *Arabidopsis*. Interestingly, all these *Arabidopsis* male germ-line-specific promoters contain a core GRSF-binding domain. It has been proposed that these *Arabidopsis* genes are direct targets of GRSF or a similar functionally conserved repressor that represses their expression in non-male germ-line cells. It has also been proposed that negative regulation is a general mechanism for controlling the expression of male germ-line-specific genes, and that release from GRSF-imposed repression is a determining event for initiating the male germ line of flowering plants (Fig. 4). Regulation of germ-cell-specific genes via the suppression of their transcription in somatic cells is a recurring theme in mammalian systems.[62–64] For example, histone variants that are expressed during mammalian spermatogenesis are subject to negative regulation in somatic cells.[62–64]

Significant numbers of genes appear to be expressed in the male germ line of flowering plants, and it is likely that some of these genes are regulated by a mechanism other than repressor-controlled negative regulation. This is likely to include the genes that are expressed in sperm cells but not in their progenitor generative cell. For example, *AtGEX1*[38] and *HAP2*[23] are not expressed in a generative cell but only in the sperm cells. The core GRSF-binding domain is not conserved in the promoter regions of these two genes. It appears that the genes that are activated in the generative cell immediately following its formation by asymmetric microspore division are under the control of the GRSF-type negative-regulation mechanism. It is thus apparent that despite recent advances, many of the regulatory mechanisms underlying male germ-cell differentiation remain to be uncovered. Future studies to define consensus *cis*-element motifs and/or shared transcriptional factors that control the male germ-line specificity of different gene clusters are likely to yield interesting data.

Conclusions and perspectives

Despite the availability of the complete genomic sequences of two model plants, *Arabidopsis* and rice, identification of the portions of genomes that are expressed in the male germ lines of flowering plants remains a challenge. Nevertheless,

Table 1. Summary of plant male gamete specific genes discussed in the text

Gene	Expression	Gene product & function	Localization	Plant studied	Reference
LGC1	GC & SC	128 aa glycoprotein with GPI anchor	Cell surface	Lily	(21)
GCS1	GC & SC	722 aa with transmembrane domain, essential for fertilization	Cell surface	Lily	(41)
HAP2	SC	705 aa cell surface protein, essential for fertilization	Cell surface	*Arabidopsis*	(54)
DUO1	GC	298 aa with MYB domain, required for sperm cell information	Nucleus	*Arabidopsis*	(51)
gH3	GC & SC	149 aa germ line Histone H3 variant	Nucleus	Lily	(35)
gH2.4	GC & SC	110 aa germ line Histone H2A variant	Nucleus	Lily	(58)
gcH3	GC & SC	111 aa germ line Histone H3 variant	Nucleus	Lily	(58)
AtMGH3	GC & SC	137 aa germ line Histone H3 variant	Nucleus	*Arabidopsis*	(27)

GC, generative cell; SC, sperm cell.

studying the development of flowering plant male germ lines has advanced to the molecular level (see Table 1). Germ-line cells are remarkably distinctive from other cells in that they have a haploid genome and are able to give rise to totipotent diploid zygotes. Investigations of the molecular processes that occur in the progression from somatic cells to germ-cell lineages offer unique opportunities for understanding complex developmental pathways that underpin general cellular processes. In particular, an understanding of the regulatory factors that induce haploid microspores to enter the asymmetric division program would greatly enhance our knowledge of cell fate decision making in plants. Understanding the molecular basis of cellular interactions among germ-line cells that are embedded deep within plant tissues remains a frontier challenge.

Acknowledgments

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