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Male Gametophyte Development and Function

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ABSTRACT

Male gametophyte development in higher plants is a complex process that requires the coordinated participation of various cell and tissue types and their associated specific gene expression patterns. The male gametophytic life cycle can be divided into a developmental phase leading to the formation of mature pollen grains, and a functional or progamic phase, beginning with the impact of the grains on the stigma surface and ending at double fertilisation. Pollen ontogeny is also an excellent model in which to dissect the cellular networks that control cell growth, polarity, cellular differentiation and cell signaling. Here we present an overview of important cellular processes in male gametophyte development and recent advances in genetics and genomic approaches that are advancing the field significantly. Genetic approaches have identified a growing number of gametophytic mutants affecting discrete steps during the developmental or progamic phases that are now beginning to uncover some of the key molecular processes involved. With recent technological advances pollen transcriptomic studies now provide the first genome-wide view of male reproductive cell development in *Arabidopsis* These studies reveal at least two successive global gene expression programs and the identity of a large number of male gametophyte-specific genes and putative transcriptional regulators. Transcriptome analysis has revealed a striking overrepresentation of cell wall metabolism, cytoskeleton and signaling genes in preparation for the progamic phase. This quantum leap in gene-centered knowledge highlights the functional specialization of this pathway and offers many new opportunities for the dissection of cellular processes that control male reproductive success.

1. INTRODUCTION – MALE GAMETOPHYTE

Male gametophyte development in higher plants is a complex process that requires the coordinated participation of various cell and tissue types and their associated specific gene expression patterns. Both gametophytic (pollen) and sporophytic (anther, tapetum) tissues participate in this process. The male gametophytic life cycle can be divided into two distinct phases: (1) a developmental phase, which takes places within the anther locules, and leads to formation of mature pollen grains, and (2), the functional or progamic phase, which begins with the impact of the grains on the stigma surface, continues with pollen tube growth and ends at double fertilisation. Developmentally, it is a simple and highly reduced system compared with the sporophyte and therefore provides unique opportunities to study the developmental regulation of gene expression and functional interactions between different cell types.

With regard to microsporogenesis, a large body of evidence shows that gametophytic gene expression is both complex and extensive. Moreover, a large fraction of such genes is also expressed in the sporophyte. This phenomenon of “haplo-diploid transcription” has important implications with respect of the efficiency of gametophytic selection and its role in the evolutionary success of flowering plants. Competition between male gametophytes for fertilization of a limited number of egg cells is common, and pronounced differences in pollen tube growth rate reflect genetic differences between individual microgametophytes (Mulcahy 1979, Mulcahy *et al.* 1996). Gametophytic selection provides a barrier against poorly functioning haploid genomes, serving to reduce the influence of random events and to promote the rigorous selection of superior haploid genotypes. These conditions argue for the rapid evolution of gametophytic genes that encode specialized functions that improve the fitness of the male gametophyte. On the contrary, gametophytic selection can influence sporophytic fitness only if there is significant overlap between genes expressed in both generations (Mulcahy 1979, Mulcahy *et al.* 1996). Recent transcriptomic studies of developing male gametophytes conclusively demonstrate that the extent of haplo-diploid overlap is greater than 95% (Honys and Twell 2004, Twell *et al.* 2006) thereby providing enormous potential for the gametophytic selection of genes affecting sporophytic traits.

Abbreviations: PMI, pollen mitosis I; **PMII**, pollen mitosis II

2. POLLEN DEVELOPMENT

Pollen development begins in the young anther locules and consists of two major phases -microsporogenesis and microgametogenesis. The primary sporogenous layer gives rise to the diploid microsporocytes or meiocytes. Meiotic division produces tetrads of four haploid microspores enclosed within a unique callosic (β-1,3-glucan) cell wall. Callose is degraded by the activity of an enzyme complex (callase) secreted by the tapetum leading to the separation of tetrads into individual microspores (**Fig. 1**). This demonstrates tight co-operation of sporophytic and gametophytic tissues. Separation of tetrads into individual microspores is under sporophytic control (Preuss *et al.* 1994) and in *quartet3* mutants microspores remain attached in a tetrad as a result of a mutation in a polygalacturonase, normally secreted from the tapetal cell layer (Rhee *et al.* 2003). Microspore growth and development proceed through a progressive cycle of vacuole biogenesis, fusion and fission events (Owen and Makaroff 1995, Yamamoto *et al.* 2003). The morphogenetic role of the vacuole in microspore expansion is associated with extreme polarization of the microspore nucleus against the microspore wall. Polarization of the microspore nucleus may provide a signal for entry into the highly asymmetric cell division at pollen mitosis I (PMI).

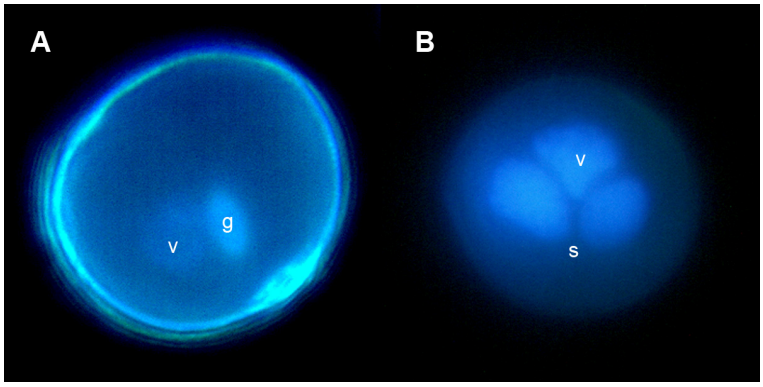

Fig. 1 Bicellular and tricellular pollen. Pollen of bicellular - hop (**A**), and tricellular - elderberry (**B**), stained with the DNA stain DAPI. Nuclear DNA within the vegetative (V), generative (G) or sperm-cells (S) are indicated.

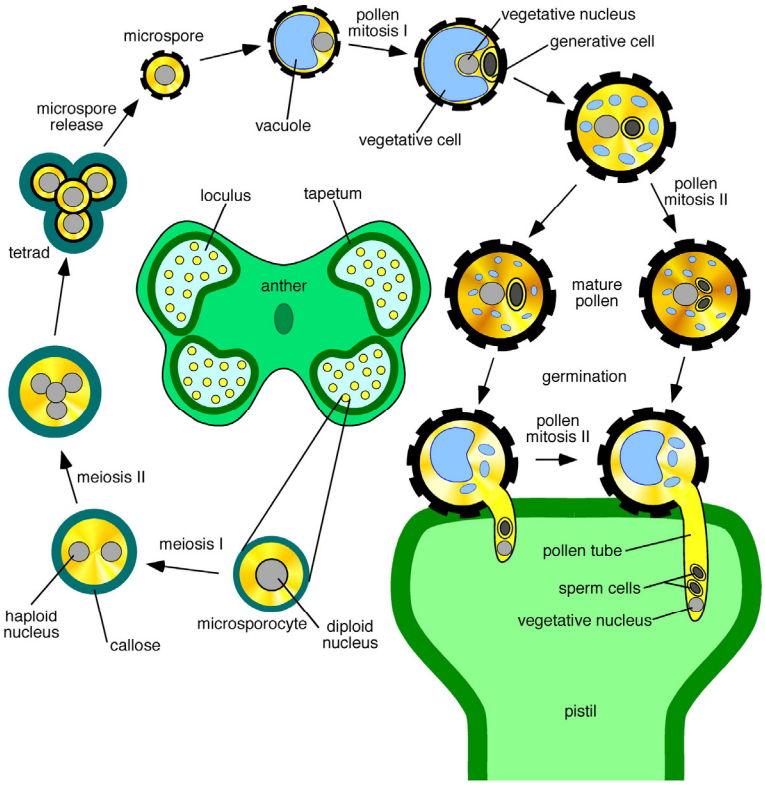

Fig. 2 Schematic diagram illustrating pollen development.

Pollen mitosis I gives rise to two daughter cells with completely different structures and cell fates (Twell *et al.* 1998). The large vegetative cell has dispersed nuclear chromatin and constitutes the bulk of the pollen cytoplasm. In contrast, the smaller generative cell has condensed nuclear chromatin and contains relatively few organelles and stored metabolites. Whereas the vegetative cell exits the cell cycle at G1 phase, the generative cell remains division-competent and completes pollen mitosis II (PMII) to form the two sperm cells. Asymmetric cytokinesis following PMI possesses two special features in that: (1) no preprophase band of microtubules marks the future division plane, and (2) a unique curved cell plate is formed to enclose the generative nucleus. Two general models have been proposed to account for differential cell fate arising from asymmetric division at PMI (Eady *et al.* 1995). Both assume that vegetative cell gene expression is the default pathway resulting from the accumulation of gametophytic factors, and provide alternative mechanisms to explain how vegetative cell-specific genes are repressed in the generative cell. In essence gametophytic determinants may be simply excluded from the generative cell pole, or polarized hypothetical generative cell repressors may block vegetative cell-specific gene expression. It is possible that a combination of both mechanisms operate to specify and effectively seal generative cell fate through asymmetric cell division.

After pollen mitosis I, the generative cell migrates inward resulting in a 'cell within a cell' structure, enabling gamete transport within the pollen tube. Generative cell migration follows degradation of the callose wall that separates the vegetative and generative cells. This presumably involves targeted secretion of β(1-3)-glucanases. Subsequently, the generative cell forms an elongated, or spindle-like shape that is maintained by a cortical cage of bundled microtubules. The generative cell undergoes further mitotic division at pollen mitosis II (PMII) to produce the two sperm cells. In tricellular pollen this division occurs within the anther, whereas in bicellular pollen it occurs within the growing pollen tube. Although the majority of flowering plants produce bicellular pollen, many important food crop plants such as rice, wheat and maize produce short-lived, tricellular pollen grains (**Figs. 1, 2**). Pollen mitosis II takes place within a membrane-bound compartment of the vegetative cell cytoplasm and a physical association is established between the gametes and the vegetative nucleus known as the male germ unit (MGU). The MGU exists in both bicellular and tricellular pollen systems and is thought to be important for the coordinated delivery of the gametes and sperm cell fusion events (Dumas *et al.* 1998). In *Arabidopsis* the MGU is first assembled in tricellular pollen (Lalanne and Twell 2002). During pollen maturation the vegetative cell accumulates carbohydrate and/or lipid reserves required for the demands of plasma membrane and pollen tube wall synthesis (Pacini 1996). The substantial accumulation of RNA and proteins during the final phases of pollen maturation (reviewed in Twell 2002) is functionally required for rapid pollen tube growth after pollination. Pollen grains are usually strongly dehydrated when finally released from the anthers. During this maturation phase, the accumulation of sugars and proline or glycine-betaine are thought to act as osmoprotectants to protect vital membranes and proteins (Schwacke *et al.* 1999).

2.1. Cell wall

The specialized activities and biological role of the pollen grain are reflected in the unique composition of the pollen wall. The pollen wall and its coatings isolate and protect the male gametophyte and its precious cargo, and mediate the complex communication with the stigma surface (Scott *et al.* 2004). The pollen wall consists of inner intine and outer exine layers. Its synthesis begins in the microspores newly formed within the tetrad, when the pectocellulosic intine and the primexine are laid down. The primexine serves as a matrix for deposition of sporopollenin precursors and their polymerisation. Sporopollenin is one of the most resistant biopolymers known and is a complex containing fatty acids and phenylpropanoids. Although the pathways involved in sporopollenin formation remain unknown its synthesis involves tight cooperation between microspore cytoplasm and tapetal cells. The exine is not evenly distributed over the pollen grain surface and regions with reduced thickness or that lack sporopollenin can form apertures that are used as sites for pollen tube emergence. The number and size of apertures and exine patterning are also under strict sporophytic control.

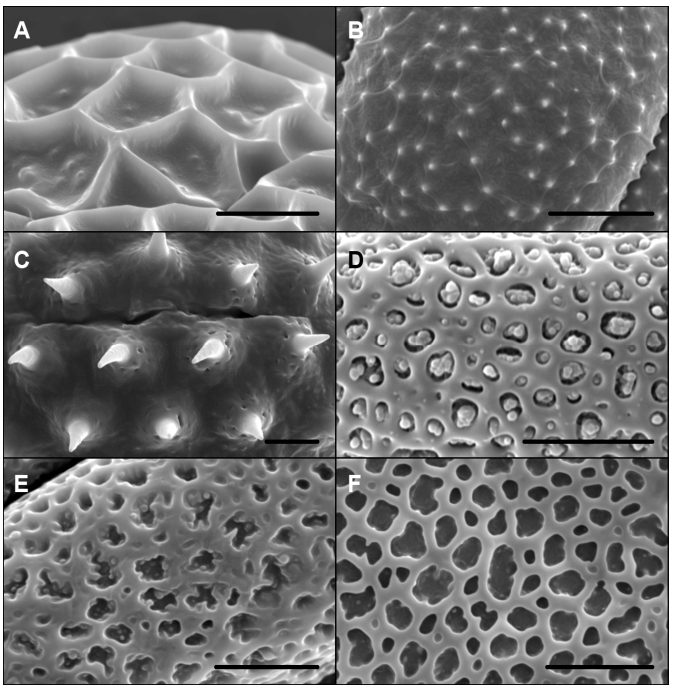

Fig. 3 Pollen wall patterning. Scanning electron micrographs of passion flower (**A**), poppy (**B**), sunflower (**C**), papaya (**D**), *Euconis* (**E**), and *Clivia* (**F**) pollen. Bar length = 5 μm.

The formation of pollen coatings is completed at later stages of microgametogenesis during which remnants of degenerating tapetal cells are deposited onto the pollen grain surface. The pollen coat is involved in pollen-pistil signalling, self incompatibility, pollen hydration, adhesivity, colour and odour. The yellow or purple colours of mature pollen grain results from the presence of both carotenoid and phenylpropanoid compounds. These features as well as the elaborate patterning of sporopollenin are highly variable among different plant species. In animal-pollinated species pollen is often decorated with elaborate structures that facilitate vector adhesion, whereas in wind pollinated species pollen lacks such sculpturing (**Fig. 3**).

2.2. Microspore embryogenesis

Microspores normally develop into pollen grains, but following certain stress treatments microspores can also undergo development that leads to the formation of a haploid embryo. Haploid plants are not fertile because of meiotic disturbances, but fertile doubled haploid plants can be regenerated by colchicine-treatment (Wang *et al.* 2000). This capability, together with the large number of microspores produced by a single plant, are of particular value for plant breeding and transformation through the production and selection of large numbers of doubled haploid lines and the rapid production of homozygous elite transformants.

Androgenesis was first described in *Datura innoxia in vitro* cultured anthers by Guha and Maheshwari (1964). Since then numerous of examples of successful androgenesis in different species have been reported. Androgenesis has been successfully induced by stress treatment of whole plants, flower buds, anthers or isolated microspores (reviewed in Touraev *et al.* 1997). The range of inductive stress conditions is very wide and is dependent on plant species as well as on the developmental stage of microspores and pollen. Among the most efficient are starvation or osmotic stress caused by mannitol treatment (*Hordeum vulgare*), heat stress (*Brassica napus* and *Capsicum annuum*) and heat stress accompanied by starvation (*Triticum vulgare* and *Nicotiana tabacum*). Androgenesis can be also induced by nitrogen starvation (*N. tabacum*), cold stress (*Zea mays*), colchicine treatment of microspores (*Brassica napus*, *Triticum vulgare*, *Zea mays*) and exposure to auxin (*T. vulgare*, *Hordeum vulgare*), chemicals or gamma irradiation (*B. napus*, *T. vulgare*; reviewed in Maraschin *et al.* 2005).

Androgenesis can be divided into three partially overlapping phases: induction, dedifferentiation and pattern formation. The induction-responsive period lies within a developmental window surrounding the asymmetric division of microspores (Maraschin *et al.* 2005). This window generally closes when young bicellular pollen grains start to accumulate starch (Binarová *et al.* 1997). The timing also correlates well with the major switch between two global male gametophytic developmental programs in *A. thaliana* (Honys and Twell 2004) that are described in more detail below. Significant progress has been made in identifying the first androgenesis-related regulatory gene, *BABY BOOM* (*BBM*) from *B. napus*. BBM is a member of the AP2/EREBP family and is preferentially expressed upon androgenesis and zygotic embryogenesis. Moreover, ectopic overexpression of BBM in *Brassica* and *Arabidopsis* was sufficient to induce spontaneous formation of somatic embryos on leaves of young seedlings (Boutilier *et al.* 2002).

3. PROGAMIC PHASE

3.1. Pollen germination

Pollination is initiated when a pollen grain lands on the surface of a compatible stigma. Rehydration, the first step of pollen activation, involves regulated uptake of water through the germination apertures. Pollen hydration is initially accompanied by a massive efflux of amino acids, especially of proline, esterases, catalases, nucleases, cellulase and pectinase. Aquaporins are considered to be involved in water uptake when changes occur in membrane architecture and subcellular rearrangement (Ikeda 1997, Becker 2004). The most evident processes during rehydration involve the formation of large numbers of vesicles, lipid droplets and vacuoles. Simultaneously, the endoplamasmic reticulum becomes encrusted with ribosomes and the respiratory activity of mitochondria increases rapidly. Bicellular pollen grains have structurally simple

mitochondria in which numerous cristae appear during activation, whereas tricellular pollen grains have highly organized mitochondria that adapt to the activation phase without further development. Other features of activation include polysome formation, RNA and protein synthetic activity, changes in the cytoskeleton and higher dictyosome activity (Raghavan 1997). In the activated pollen grains, actin microfilaments are already disposed toward the germination site (Heslop-Harrison and Heslop-Harrison 1992). Their essential role in the outgrowth of the pollen tube has been established with the use of cytochalasin B that causes microfilament fragmentation and arrest of pollen germination. Much less is known about microtubules and their role in pollen germination. Microtubules are not organized at potential germination sites, but some short and branched microtubules converge around the base of the emerging pollen tube as a collar. Microtubules are generally associated with the generative cell and prior to germination their polarized arrangement is similar to that of actin filaments. On the contrary disruption of microtubules with pharmacological inhibitors has little effect on pollen germination (Raghavan 1997).

Pollen grains contain a stored complement of mRNAs that encode proteins required for germination and early pollen tube growth. Because of this, the germination, early pollen tube growth and migration of vegetative cell nucleus in a medium containing the transcription inhibitor actinomycin D occur normally (Lafleur and Mascarenhas 1978). In contrast, cycloheximide, an inhibitor of translation, has immediate and fatal impact on germination and pollen tube growth (Čapková *et al.* 1980, Tsao and Linskens 1986), demonstrating a requirement for protein synthesis in pollen tube growth. Interestingly some species are not affected by protein synthesis inhibitors, suggesting that a full complement of proteins required for germination is stored in the pollen grains of these species.

3.2. Pollen tube growth

Once certain critical changes have occurred in the pollen grain during the hydration and activation phases, a pollen tube appears. During this process the cell becomes significantly polarized with the migration of organelles, in particular Golgi and large numbers of vesicles, towards the germination pore and growing apex (Malhó and Pais 1992). Compared to binucleate pollen types, which contain polysomes, germinating trinucleate pollen grains form very few polysomes and have low protein synthetic activity. The rapid germination of trinucleate pollen grains indicates that the synthesis of necessary proteins is completed during pollen maturation.

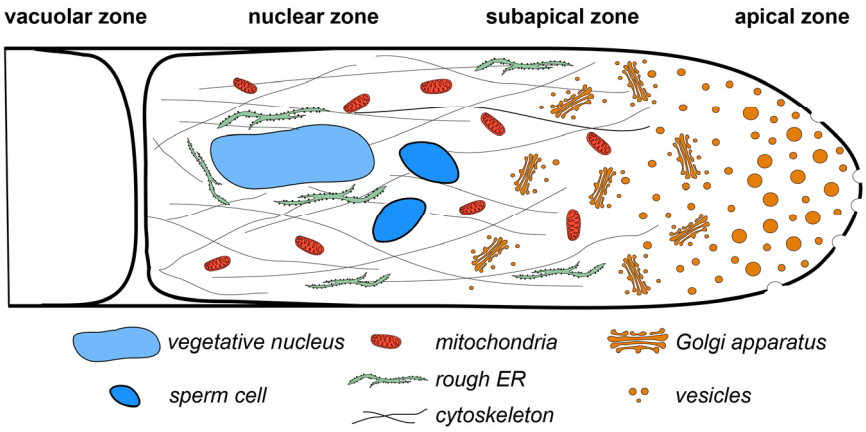

Fig. 4 Schematic diagram of pollen tube apical region.

Pollen, in addition to root hairs, fungal hypha or moss protonema provides excellent material for studying the mechanisms of cell growth by tip extension. Pollen also provides a model for analysis of metabolic pathways and the dynamics of cell wall deposition and maturation in an individual cell type. Within this cell-tubular structure cytoplasmic streaming occurs in a specific pattern named 'reverse fountain' (Justus 2004, Cardenas 2005). Growing pollen tubes (**Fig. 4**) present a formidable example of tip growing cells that can achieve growth rates of 1 cm/h in maize (Bedinger 1992). The protoplast is present only in the distal part of the tube and becomes separated from the proximal region by the periodic deposition of callose plugs, keeping the protoplast volume nearly constant. Apical growth of pollen tubes is associated with the presence of large numbers of vesicles in the tip region concerned with the transport of cell wall materials to the extending tip. Secretory vesicles track along the actin cytoskeleton toward the apical region (Cai *et al.* 1997, Yokuta *et al.* 1999). Pollen tube growth is oscillatory and is correlated with an oscillatory influx of the cations Ca^{2+}, H^+, and K^+ and the anion Cl^-. Periodic elongation of short actin bundles into the apical dome occurs between the exocytosis of synthetic materials delivered by the highly active vesicle-trafficking system.

Cytoplasmic movement occurs within a cylindrical cell that is continuously growing by extension at the apex and involves four more or less distinct activities: (a) circulation along the length of the tube of organelles, lipid bodies and other cytoplasmic inclusions, (b) continuous insertion of numerous polysacharide-containing precursor particles into the forming wall at the growing tip, (c) forward passage of the vegetative nucleus which is at some distance behind the apex and (c) migration along the tube of the generative cell or the gametes produced from it (Heslop-Harrison 1988).

In an ultrastructural view of the organization of the pollen tube four different zones are recognized - (a) the apical zone, enriched with vesicles; (b) the subapical zone, populated by organelles and especially active dictyosomes; (c) the nuclear zone, containing the vegetative nucleus and gametic cells, and (d) a vacuolar zone with large vacuoles separated by callose plugs (Cresti *et al.* 1977). Pollen tube zonation and intercellular movements are directly generated in association with cytoskeletal elements (Pierson *et al.* 1985, Lancelle *et al.* 1987). It is now clear that the actomyosin system has a primary role in cytoplasmic streaming, and not only the vegetative nucleus and generative cells, but also vesicles are connected with myosin and kinesin (Raghavan 1997). Microtubules occur mainly in the peripheral cytoplasm in axial groups and in the cage enclosing the generative and sperm cell nuclei. Microfilaments abound in the perinuclear zone, some of them extending into the cortical areas of the pollen tube (Suresh *et al.* 1988); besides, they are associated with elements of endoplasmic reticulum, cylindrical vacuoles, dictyosomal vesicles and the vegetative nucleus. The importance of microfilaments on vesicular trafficking and various intracellular processes became clear after experiments employing cytoskeletal inhibitors such as cytochalasin D disrupting of both the cortical and microfilaments and the cortical microtubules, whereas colchicine disrupted cortical microtubules only (Picton and Steer 1981).

The composition and structure of the pollen tube wall are complex. In addition to cellulose, pectic substances, hemicelluloses, and particularly callose coating the inner side are also constituents of the pollen tube wall. Pectin methylesterases, which are responsible for cross-linking unesterified pectins, are hypothesized to control the rate of production of cell wall binding sites for Ca^{2+} when incoming Ca^{2+} also releases H^+. The resulting acidification could deactivate methylesterase activity, and assuming that exocytosis continues, unesterified methylpectin would

be pushed into the tip zone and would stretch the plasma membrane here, leading to tip extension.

Knowledge of pollen tube biology is largely based on *in vitro* cultivated pollen tubes. Pollen tubes interact intimately with the tissue of the stigma and the extracellular matrix and transmitting tissue plays a significant role in pollen-pistil recognition (Palanivelu and Preuss 2000, Higashiyama *et al.* 2003, Lord 2003, Swanson *et al.* 2004). Compatible interactions are guaranteed by multiple-step pathways based on various factors e.g. cysteine-rich proteins (Mollet *et al.* 2000), glycoproteins (Bosch and Derksen 2003), pectins (Mollet *et al.* 2000), flavonoids (Mo *et al.* 1992), calcium gradients (Zhao and Yang 2003) and gamma amino-butyric acid (Palanivelu 2003). Moreover, our understanding of pollen tube growth, guidance and fertilization has been significantly advanced by the development of techniques for *in vitro* pollination and fertilization (Janson 1993, Zenkteler 1993, Higashiyama 2001, Vervaeke 2002, Willemse and van Lammeren 2002).

4. GAMETOPHYTIC MUTANTS AFFECTING POLLEN DEVELOPMENT

Mutational analysis is a powerful approach to identify structural and regulatory components that participate in a particular developmental pathway. Mutants affecting pollen development also provide excellent material to analyze processes of cell fate specification and cellular function. Currently we know about forty-five such gametophytic mutants for which 13 corresponding genes have been identified (**Table 1**). Seven of these were identified through forward genetics, by positional cloning (*GEM1, DUO1, TIO*), or by isolating flanking sequence tags (*UNG3, AGM, HAP5, HAP12).* The remainder were functionally identified using reverse genetics approaches (*AGP1, AtPTEN, ADL1C, AGM, AHA3, GPT1, VHA-A*). Proteins with a variety of cellular roles have been defined including those with roles in cell division (GEM1, DUO1, TIO), nutrition and metabolism (AHA3, GPT1), pH regulation and ion transport (VHA-A, HAP5), and other groups with roles in transcriptional regulation (DUO1, HAP12*)* or post-translational (AtPTEN*,* TIO) intracellular signaling events (**Table 1**).

Positional cloning has led to the identification of three classes of mutants affecting gametophytic cell divisions. *gemini pollen1* (*gem1*) affects male and female transmission and displays a range of microspore division phenotypes including equal, unequal and partial divisions (Park *et al.* 1998). Neither daughter cell completes a further division and cell fate analysis in *gem1* supports the role of cell size or nuclear/cytoplasmic ratio as a factor in determination of cell fate (Park *et al.* 1998, Park and Twell 2001). *GEM1* was the first male gametophytic gene to be positionally cloned, revealing its identity to the MOR1 (Whittington *et al.* 2001). MOR1/GEM1 is homologous to the MAP215 family of microtubule-associated proteins that stimulate plus-end microtubule growth. MOR1/GEM1 is associated with interphase, spindle and phragmoplast microtubule arrays and plays a vital role in microspore polarity as well as cytokinesis (Twell *et al.* 2002). In the *two-in-one* (*tio*) mutant microspores complete nuclear division, but fail to complete cytokinesis resulting in binucleate pollen grains. In contrast to *gem1, tio* shows normal nuclear polarity before pollen mitosis I, but displays cytokinesis-specific defects including the formation of incomplete callosic cell plates that fail to expand. TIO, localises to the phragmoplast midline and was recently identified as the plant homologue of the Ser/Thr protein kinase FUSED (Oh *et al.* 2005), which is a key component of the hedgehog signalling pathway in fruitflies and humans (Lum and Beachy 2004). The *duo pollen* (*duo*) mutants complete normal asymmetric division at PMI, but fail to enter or complete generative cell division (Durbarry *et al.* 2005). Heterozygous *duo* mutants produce ~50% bicellular pollen containing a vegetative cell nucleus and a single 'generative-like' cell with condensed nuclear chromatin. DUO1 was recently identified as a novel R2R3 MYB protein specifically expressed in generative and sperm cells (Rotman *et al.* 2005). DUO1 represents the first germ line specific regulator to be identified and DUO1 homologues in maize, rice and tobacco all possess a supernumerary lysine signature in the R3 MYB domain that defines the DUO1 family (Rotman *et al.* 2005). Mutant generative cells in *duo1* pollen fail to enter PMII and enter a partial endocycle, suggesting that DUO1 may be a specific regulator of genes required for G2 to M-phase transition (Durbarry *et al.* 2005).

Forward genetic screens using marker segregation ratio distortion have also been successful in identifying genes involved in microgametogenesis. These screens have mostly employed T-DNA or transposon insertion populations that harbor dominant antibiotic or herbicide resistance markers. For example, if an insertion inactivates an essential male gametophytic gene, then the ratio of resistant to sensitive progeny will deviate significantly below the expected 3:1 ratio toward 1:1. Such screens are inclusive in that mutants affecting both pollen develomental and progamic phases are recovered as well as those affecting female gametogenesis. Insertional segregation ratio distortion screens have the advantage of straightforward identification of the mutated sequences and a number of 'tagged' mutants have now been identified that affect microgametogenesis (Bonhomme *et al.* 1998, Christensen *et al.* 1998, Howden *et al.* 1998, Procissi *et al.* 2001, Johnson *et al.* 2004, Lalanne *et al.* 2004a 2004b). A number of these cause aborted pollen phenotypes that could arise from various mechanisms (**Table 1**). For example *abnormal gametophytes* (*agm*) encodes putative transmembrane protein; *vha-A* a subunit of the vacuolar H^+ ATPase, tagged sequences in *hap5* encode a putative AP2 domain transcription factor and in *hap12* a cation-chloride transporter. The *ung3* mutant results in early bicellular pollen arrest (Lalanne *et al.* 2004b) sequences in *ung3* encode a gibberellin-2-oxidase suggesting a role for GA in pollen development, as well as its recently discovered role in pollen tube growth (Singh *et al.* 2002).

In an increasing number of cases gametophytic roles have been discovered by characterising loss of function phenotypes caused by knockout or knockdown mutants in genes of interest. This approach has led to the identification of four genes, *AtPTEN, ADL1C*, *AHA3* and *GPT1,* with important roles during microgametogenesis (**Table 1**). In some cases progress was directed by knowledge of pollen-enhanced gene expression. For example, the essential role of a pollen-specific dual-specificity protein tyrosine phosphatase homolog (*AtPTEN1*) was revealed by *LAT52* promoter driven RNA interference (Gupta *et al.* 2002). This resulted in pollen cell death after pollen mitosis II, consistent with its peak expression in tricellular pollen. For the dynamin-like *ADL1C,* gene expression profiles and promoter-GUS analysis hinted at a role for *ADL1C* in pollen. T-DNA insertions in *ADL1C* caused plasma membrane and cell wall defects that could suggest a role for ADL1C in plasma membrane maintenance (Kang *et al.* 2003). The authors speculate that the ability of AtPTEN1 to convert phosphatidylinositol triphosphate (PIP3) to phosphatidylinositol bisphosphate (PIP2) may be required for activation of ADL1C.

The requirement of two ATPases for pollen development was also demonstrated by isolating T-DNA insertion alleles. Knockout of a single member of the 11 plasma membrane proton H^+-ATPases, AHA3, leads to pollen abortion that suggests a role in secondary ion transport and microspore nutrition (Robertson *et al.* 2004). A T-DNA insertion in the A subunit of the vacuolar H^+ ATPase, VHA-A, leads to mutant pollen with curved and swollen Golgi cisternae suggesting a role in Golgi organization (Dettmer *et al.* 2005). The essential role of a plastidic glucose 6-

Table 1 Gametophytic mutants that act during pollen development or the progamic phase.

Gene ID	Gene	Mutant	Mutant Phenotype	Protein identity	Protein function	Reference
At1g14830	ADL1C	*Arabidopsis dynamin-like 1C*	Pollen abortion during maturation	Dymanin-Like Protein	Pollen plasmamembrane maintenance, cell wall synthesis.	Kang *et al.* 2003
At5g44860	AGM	*Abnormal gametophytes*	Pollen degenerates at late microspore stage	Putative transmembrane protein	Microspore development and/or division?	Sorensen *et al.* 2004
At1g24520	AGP1	*Arabinogalactan protein1[AtBCP1]*	Pollen aborts and collapsed at bicellualar stage	Arabinogalactan protein	Tapetum, microspore and bicellular pollen viability.	Xu *et al.* 1995
At5g57350	AHA3	*Arabidopsis H+-ATPase 3*	Collapsed and aborted mature pollen	Plasma membrane H+-ATPase	Nutrient transport, microspore/pollen maturation.	Robertson *et al.* 2004
At3g60460	*DUO1*	*duo pollen1*	Bicellular pollen: generative cell fails to enter & skips mitosis.	R2R3 MYB transcription factor (MYB125)	Regulator of gene expression required for mitotic entry.	Durbarry *et al.* 2005, Rotman *et al.* 2005
At2g35630	*GEM1*	*gemini pollen1*	Twin-celled and binucleate pollen: abnormal divisions at pollen mitosis I	MOR1/GEM1: Homologous to chTOGp/XMAP215 family of microtubule associated proteins	Microspore polarity and cytokinesis through microtubule organisation.	Park *et al.* 1998 Park and Twell 2001, Twell *et al.* 2002
At5g54800	*GPT1*	*glucose-6-phosphate translocator1*	Aborted pollen. Reduced lipid bodies, vesicles and vacuoles. Defects at tricellular stage.	glucose-6-phosphate translocator	Glc6P import for plastidic starch & fatty acid biosynthesis and carbon for OPPP.	Niewiadomski *et al.* 2005
At1g30450	*HAP5*	*hapless5*	Aborted mature pollen	Cation-chloride cotransporter	Ion homeostasis during development.	Johnson *et al.* 2004
At4g36900	*HAP12*	*hapless12*	Aborted mature pollen	Contains AP2 domain (RAP2.10)	Regulator of pollen gene expression	Johnson *et al.* 2004
At5g39400	*PTEN*	*Arabidopsis phosphatase and tensin homologue*	Pollen death after pollen mitosis II	Phosphatase and tensin homologue [Tyrosine/PIP3 phosphatase]	Pollen maturation.	Gupta *et al.* 2002
At1g50230	*TIO*	*two-in-one*	Microspores initiate, but fail to complete cytokinesis at pollen mitosis I	TIO: Homologous to FUSED-kinase family	Signalling role in phragmoplast expansion.	Oh *et al.* 2005
At2g34550	*UNG4*	*ungud4*	Pollen aborts at bicellular stage	GA-2-oxidase	Regulation of giberrellin.	Lalanne *et al.* 2004
At1g78900	*VHA-A*	*Vacuolar-ATPase V_1 subunit A*	Pollen aborted at bicellualar stage and later: Swollen ER cisternae.	Vacuolar-ATPase V1 subunit A	Golgi organization, pH homeostasis, secondary active transport.	Dettmer *et al.* 2005
At4g32500	*AKT5*	*spik*	Impaired pollen tube growth	Shaker family inward K^+ channel	Ion transport	Mouline *et al.* 2002
At3g04080+ At5g18280	*AtAPY1 AtAPY2*	*apyrase 1+2 double mutant*	Nongerminating pollen, no pollen tube	Apyrase	Mediation of signalling, nucleotide metabolism	Steinebrunner *et al.* 2003
At2g35650	*AtCSL A7*	*cellulose synthase-like 7*	Slower pollen tube growth, defects in embryo development	Processive β-glycosyltransferase	Synthesis of cell wall polysaccharides	Goubet *et al.* 2003
At1g04950	*AtTAF6*	*attaf6*	Short pollen tube growth	TBP-associated factor 6	Basal transcription factor	Lago *et al.* 2005
At1g02140	*HAP1*	*hapless1*	Disrupted guidance of pollen tube growth, failure to leave the septum	*Mago nashi* (*Drosophila melanogaster*, e=3x10^{-61}; Boswell *et al.* 1991)	mRNA metabolism/localisation	Johnson *et al.* 2004
At4g11720	*HAP2*	*hapless2*	Chaotic pollen tube growth in the ovary	Expressed likely transmembrane protein	Unknown	Johnson *et al.* 2004
At1g66570/At 1g66580	*HAP3*	*hapless3*	Short pollen tube growth, failure to exit style	Sucrose transporter SUC1 / 60S ribosomal protein L40	Saccharide metabolism / protein synthesis	Johnson *et al.* 2004
At3g52590	*HAP4*	*hapless4*	Chaotic pollen tube growth in the ovary	Ubiquitin extension protein 1 (UBQ1) / 60S ribosomal protein L40	Protein synthesis/ubiquinitation	Johnson *et al.* 2004
At4g21150	*HAP6*	*hapless6*	Short pollen tube growth, failure to exit style	Ribophorin II (RPN2) family protein	Membrane trafficking	Johnson *et al.* 2004
At5g56250	*HAP8*	*hapless8*	Short pollen tube growth, failure to exit style	Expressed protein	Unknown	Johnson *et al.* 2004
At5g47020/At 5g47030	*HAP11*	*hapless11*	Normal pollen tube growth, failure to enter the micropyle	Unknown protein / Mitochondrial ATP synthase δ chain	Unknown / energy metabolism	Johnson *et al.* 2004
At1g60780	*HAP13*	*hapless13*	Short pollen tube growth, failure to exit style	Clathrin adaptor complexes medium subunit family protein	Membrane trafficking	Johnson *et al.* 2004
At1g20200	*HAP15*	*hapless15*	Short pollen tube growth, failure to exit style	26S proteasome regulatory subunit protein S3	Protein degradation	Johnson *et al.* 2004
At5g49680	*KIP*	*kinky pollen*	Kinky-shaped pollen tubes	SABRE-like protein	Component of the secretory pathway	Procissi *et al.* 2003
At2g43040	*NPG1*	*no pollen germination 1*	Nongerminating pollen, no pollen tube	Calmodulin-binding protein	Binding to calmodulin in calcium-dependent manner	Golovkin and Reddy 2003
At1g71270	*POK*	*poky pollen tube*	Short pollen tubes	Vps52/Sac2 family protein	Polar tube growth, component of vesicle-transport machinery	Lobstein *et al.* 2004
Atg310380	*SEC8*	*exocyst complex component*	Nongerminating pollen, no pollen tube	Subunit of the exocyst complex	Polarised exocytosis of secretory vesicles	Cole *et al.* 2005
At2g34980	*SETH1*	*seth1*	Strongly reduced germination, abnormal callose deposition	Phosphatidylinositol-glycan synthase subunit C	Component of the GPI-anchor biosynthetic pathway	Lalanne *et al.* 2004b
At3g45100	*SETH2*	*seth2*	Strongly reduced germination, abnormal callose deposition	GPI-GnT catalytic subunit PIG-A	Component of the GPI-anchor biosynthetic pathway	Lalanne *et al.* 2004a 2004b
At3g54690	*SETH3*	*seth3*	Reduced pollen germination & tube growth	Arabinose-5-phosphate isomerase	Synthesis of rare rhamnogalactouronans	Lalanne *et al.* 2004b, unpublished

Table 1 (cont.)

Gene ID	Gene	Mutant	Mutant Phenotype	Protein identity	Protein function	Reference
At4g34940	*SETH4*	*seth4*	Strongly reduced pollen germination	ARM repeat containing protein	Unknown	Lalanne *et al*. 2004b, unpublished
At4g00800	*SETH5*	*seth5*	Strongly reduced pollen germination & tube growth	Unknown transmembrane protein	Unknown	Lalanne *et al*. 2004b, unpublished
At2g47860	*SETH6*	*seth6*	Strongly reduced germination and tube growth	RPT2/NPH3-like protein	Scaffold protein within signalling pathway	Lalanne *et al*. 2004b
At2g41930	*SETH7*	*seth7*	Strongly reduced germination and tube growth	Ser/Thr protein kinase	Initiation of pollen germination	Lalanne *et al*. 2004b
At5g13650	*SETH8*	*seth8*	Reduced competitive ability, slower pollen tube growth	GTP-binding typA-related protein	Unknown	Lalanne *et al*. 2004b
At5g12250	*SETH9*	*seth9*	Reduced competitive ability, slower pollen tube growth	Alcohol dehydrogenase-like protein	Alcohol metabolism	Lalanne *et al*. 2004b
At2g03070	*SETH10*	*seth10*	Reduced competitive ability, slower pollen tube growth	Putative protein	Unknown	Lalanne *et al*. 2004b
At4g04710	*UNG6*	*ungud6*	Reduced male transmission	Calcium-dependent protein kinase	Calcium-dependent signalling	Lalanne *et al*. 2004b
At2g34680	*UNG9*	*ungud9*	Reduced male transmission	Auxin-induced protein (AIR9), similar to leucine-rich repeat family protein	Cell wall metabolism/signalling	Lalanne *et al*. 2004b
At3g54090	*UNG10*	*ungud10*	Reduced male transmission	Fructokinase-like protein	Sugar metabolism	Lalanne *et al*. 2004b
At2g47040	*VGD1*	*vanguard1*	Retarded pollen tube growth	Pectin methylesterase-homologous protein	Cell wall metabolism	Jiang *et al*. 2005

Only gametophytic mutants that show detectable phenotypes are shown. Mutated genes (or sites of insertion of T-DNA or transposable elements) responsible for phenotypes are shown.

phosphate/phosphate translocator (GPT1) in pollen was recently demonstrated by isolating knockout mutants in one of the two homologous GPT genes (Niewiadomski *et al.* 2005). *gpt1* mutant pollen shows reduced formation of lipid bodies and vacuoles essential for cell viability. The suggested role for GPT1 in pollen is to support Glc6P import into plastids as a source of carbon for starch and fatty acid biosynthesis, or as a starter for the oxidative pentose phosphate pathway.

Although significant progress has been slowed because of the limitations of positional cloning and the effort required for large-scale segregation distortion screening, renewed effort in morphological screening and cloning genes responsible for already known and novel mutant classes will continue to yield valuable discoveries. Insertional mutagenesis however will continue to dominate as the primary method for the discovery of genes with essential male gametophytic functions. With such resources in hand the total number of insertions that need to be screened to achieve saturation is more realistic and similar to the total number of annotated genes in *Arabidopsis*. Moreover, many genes with redundant roles will not be revealed until double and multiple mutant combinations can be assembled.

5. GAMETOPHYTIC MUTANTS AFFECTING THE PROGAMIC PHASE

The progamic phase is delimited by pollination and fertilization. This process involves pollen hydration, germination, pollen tube growth, guidance through the transmitting tissue, penetration of the micropyle and finally fusion of male and female gametes. Although post-pollination events have been extensively described at the cellular level, the identification and characterization of mutants affecting male progamic phase functions remains a challenge because of two major constraints. First, female sporophytic tissues constitute a physical barrier for morphological observation of growing pollen tubes; second, mutant pollen grains strictly affecting progamic development are by definition morphologically indistinguishable from their wild-type counterparts. Visual screens for reduced fertility have identified gametophytic mutations mainly affecting the female control of the late progamic phase events (Wilhelmi and Preuss 1996, Moore *et al.* 1997, Christensen *et al.* 1998, Shimizu and Okada 2000, Huck *et al.* 2003, Rotman *et al.* 2003). To solve the problem efficiently, insertional mutagenesis approaches based on screening for distorted segregation ratios have proven to be effective.

Forward genetics using Ds transposon insertion lines has led to identification of 20 *seth* and *ungud* mutations affecting male, or male and female gametophyte functions respectively. *SETH1* and *SETH2* genes encode proteins involved in the first step of the glycosylphosphatidylinositol (GPI) biosynthetic pathway and their mutations specifically block male transmission. Mutant pollen is similar in size and appearance to wild-type, but failed germination or tube growth is associated with abnormal callose deposition that may result from aberrant GPI-anchored protein localisation (Lalanne *et al.* 2003). *SETH3* gene encodes a potential component involved in synthesis of rare rhamnogalactouronans, the *seth3* mutant shows normal pollen phenotype but pollen transmnisison is completely blocked. *seth4 – seth7* mutations are strongly reduced in pollen germination and pollen tube growth, whereas *seth8 – seth10* mutations result in slower pollen tube growth and reduced competitive ability. Other male defective mutants with reduced male transmission are *ungud6, ungud9* and *ungud10* with no obvious defect in pollen size, appearance or viability. Their genes encode proteins involved in signalling, cell wall, saccharide and alcohol metabolism (Lalanne *et al.* 2004). Recently, a *Ds*-induced mutation, *vanguard1* was described that shows a severe retardation in pollen tube growth through the transmitting tract. *VANGUARD1* encodes a pollen-specific pectin methylesterase that may function via modification of the pollen tube wall pectin extensibility and interaction with transmitting tissues (Jiang *et al.* 2005).

A number of T-DNA-tagged male gametophytic mutants have been isolated using a modified segregation-distortion approach that enables precise analysis of pollen mutant phenotype. T-DNA carrying BASTA resistance and β-glucuronidase (GUS) under the control of the pollen-specific LAT52 promoter was transformed into the *quartet1* background (Johnson *et al.* 2004). This allows phenotypic analysis of marked mutant pollen tubes with GUS following an initial screen for segregation distortion. Nine *hapless* genes were identified affecting the progamic phase. These fall into a few phenotypic classes: a) *hap3*, *hap6, hap8, hap13* and *hap15*, have short pollen tubes that fail to exit the style; b) *hap1* pollen tubes fail to exit the septum; c) *hap11* fails to enter the micropyle and d) *hap2* and *hap4* show chaotic pollen tube growth in the ovary. Genes

identified at insertion sites encode proteins involved in sugar transport, membrane trafficking, mRNA localisation, protein degradation and energy metabolism (Johnson *et al.* 2004).

Reverse genetics approaches have led to the identification of 9 genes that have important roles during the progamic phase. *Cellulose synthase-like 7* (*AtCSLA7*) is a member of the CSLA subfamily of putative processive β-glycosyltransferases. The gene is ubiquitously expressed, is important for pollen tube growth and essential for embryogenesis, suggesting a requirement for a specific β-linked polysaccharide in plant development. The defect in pollen tube growth in *Atcsla7* could result from changes in cell wall properties, including its extensibility and stability (Goubet *et al.* 2003). A T-DNA insertion in *spik* revealed the importance of K^+ uptake in pollen tube growth. SPIK is a member of the Shaker K^+ channel gene family and the first ion channel active in the pollen membrane to be identified at the molecular level. The sensitivity of SPIK to external pH could allow regulation of K^+ influx by the apoplastic pH, itself under the control of H^+ transport activity of the germinating pollen grain or neighbouring cells (Mouline *et al.* 2002). *apyrase1* and *apyrase2* mutants reveal a role for nucleoside tri- and diphosphate hydrolysis in pollen tube growth (Steinebrunner *et al.* 2003). An interesting phenotype was observed in the T-DNA insertion *kinky pollen* (*kip*). Despite strong aberrations in pollen tube growth the ultrastructural zonation of organelles showed the same features as the wild-type. The only difference is the absence of callose plugs, yet, the callose deposition along pollen tubes occurs normally. The *KIP* gene encodes a protein predicted to be targeted to the secretory pathway (Procissi *et al.* 2003). The role of calmodulin-binding protein was demonstrated in the *no pollen germination1* T-DNA mutation. NPG1 is the first protein mediating calcium/calmodulin action described genetically despite numerous reports on the role of calcium and calmodulin in pollen germination and tube growth. *NPG1* is not necessary for microsporogenesis and gametogenesis but is essential for pollen germination (Golovkin and Reddy 2003). A similar phenotype with a different origin was observed in *sec8* mutants. AtSEC8 encodes an exocyst component that facilitates vesicle docking at the plasma membrane during exocytosis and is expressed in pollen and vegetative tissues (Cole *et al.* 2005). The *pok* mutant exhibits very short pollen tubes and slow growth. The POK protein is conserved from lower to higher eukaryotes and its homologue in yeast (Vps52/SAC2) is Golgi-localised and involved in retrograde vesicle traffic between the early endosomal compartment and the trans-Golgi network. This suggests a more general role for POK in polar growth beyond pollen tube elongation (Lobstein *et al.* 2004). In the case of transcription factors, a T-DNA insertion mutation in *Arabidopsis* TFIID *AtTAF6* specifically affects pollen tube growth resulting in significantly reduced male transmission (Lago *et al.* 2005). AtTAF6 may have a role as a selective basal transcriptional factor acting on the regulation of a specific subset of gametophytic genes.

Although the detection and identification of mutants affecting the progamic phase is more challenging in comparison to those affecting the earlier developmental phase, a significant collection of mutations that affect pollen germination, pollen tube growth and guidance has been assembled and a growing list of genes are being identified. Most genes identified represent known signalling, metabolic, and transport pathways that highlight essential functions required for cellhomeostasis and polar growth.

6. MALE GAMETOPHYTE TRANSCRIPTOMICS

Until recently, gene-by-gene characterization has led to the identification of approximately 150 pollen-expressed genes from different species, with strong evidence for pollen-specific expression in about 30 (reviewed in Twell 2002). The availability of new high-throughput technologies has enabled analysis of the haploid transcriptome on a global scale. Three initial studies, exploiting serial analysis of gene expression (SAGE) technology (Lee and Lee 2003) and 8K Affymetrix AG microarrays (Honys and Twell 2003, Becker *et al.* 2003), provided analyses for mature pollen based on approximately one-third of the *Arabidopsis* genome. Further refinement was enabled by the availability of Affymetrix 23K *Arabidopsis* ATH1 arrays. Currently, there are three publicly available independent data sets for the male gametophyte. The first contains microarray data covering four stages of male gametophyte development (uninucleate microspores, bicellular pollen, tricellular pollen and mature pollen) for ecotype Landsberg erecta (Honys and Twell 2004). The two remaining datasets were obtained from mature pollen grains from ecotype Columbia (Zimmermann *et al.* 2004, Pina *et al.* 2005). The major impact of these studies lies in the massively increased knowledge of the complexity and dynamics of haploid gene expression throughout single-cell development in plants (Honys and Twell 2004).

Three independent mature pollen transcriptomic datasets (two in duplicate and one in triplicate) represent ample potential for qualified estimates of the number of genes active in the male gametophyte and their functional categorization. First, comparative analyses of all male gametophyte and reference sporophytic transcriptome datasets led to the finding that the differences observed between individual datasets did not correlate with ecotype used. Therefore experimental conditions seem to be more important than the ecotype itself and previously published speculations about significant pollen transcriptome differences between ecotypes (Pina *et al.* 2005) are not supported. Second, we can now estimate the total number of genes expressed in mature pollen to lie between 5,000 and 7,000 genes. Normalized datasets based on both MAS4 and MAS5 detection algorithms can be accessed and downloaded at the *Arabidopsis* Gene Family Profiler (aGFP) site (http://aGFP.ueb.cas.cz).

Comparative analyses of the male gametophytic transcriptomes are being performed against an increasing number of sporophytic datasets. Unless otherwise stated, the following results were obtained using transcriptomic data from Landsberg erecta (4 developmental stages, 8 individual GeneChips; Honys and Twell 2004) as all corresponding samples originated from plant populations grown under identical conditions. Reference sporophytic datasets were obtained through the AffyWatch service (Craigon *et al.* 2004) and represent 62 experiments (154 individual chips) that provide transcriptomic data for 16 different organs or cell types[1]. All datasets were normalised together using the same algorithm[2].

Considering all developmental stages, 14,037 genes gave a positive expression signal in the developing male gametophyte. In individual stages the number of active genes decreased from 11,615 (microspores, UNM) and 11,961 (bicellular pollen, BCP) through 8,831 (tricellular pollen, TCP) to 7,264 (mature pollen, MPG). Currently, only a relatively low number of genes show strictly male gametophyte-specific expression (5.6% of all male gametophyte-expressed genes). This number is decreasing further as new sporophytic datasets emerge. The estimated number of strictly pollen-specific genes is therefore significantly lower than expected from previous studies (Honys and Twell 2003 2004, Becker *et al.* 2003, Pina *et al.* 2005). However, those remaining male gametophyte-specific genes are characterised by very high expression signals, highlighting their importance and their potential as targets for functional analysis.

1 The complete list of dataset codes is available from authors.

2 MAS4 detection algorithm, normalisation of all arrays to the median probe intensity level, calculation of model-based gene-expression values using Perfect Match-only model (dChip 1.3 software, http://www.dchip.org; Li and Wong 2001), exclusive treatment of replicates for identification of expressed genes.

Analysis of the overlap between gametophytic and sporophytic expression and the relative levels of expression in both generations clearly demonstrated marked differences. Moreover, there were striking differences between early and late male gametophytic transcriptomes. Male gametophyte development and function are under the control of at least two successive global gene expression programs, early and late, with the switch point prior to PMII (Honys and Twell 2004). Early genes follow the general trend of reduction in complexity. On the contrary, a number of very abundant late genes are activated after PMII and the majority of highly expressed late genes show pollen-specific expression. This switch supports the previously published uniqueness of the late male gametophytic transcriptome even when compared with with the early male gametophyte (Honys and Twell 2003 2004). Scatter plot analyses of transcriptomic datasets originating from several resources illustrate the developmental switch between early and late male gametophytic developmental programs (**Fig. 5**). Moreover, gene expression early in gametophyte development is significantly more similar to the sporophyte than to the late male gametophyte. This could suggest a significant contribution of pre-meiotic gene expression to the early gametophytic program. On the contrary, genes specifically activated after PMII are likely to play important roles in post-pollination events.

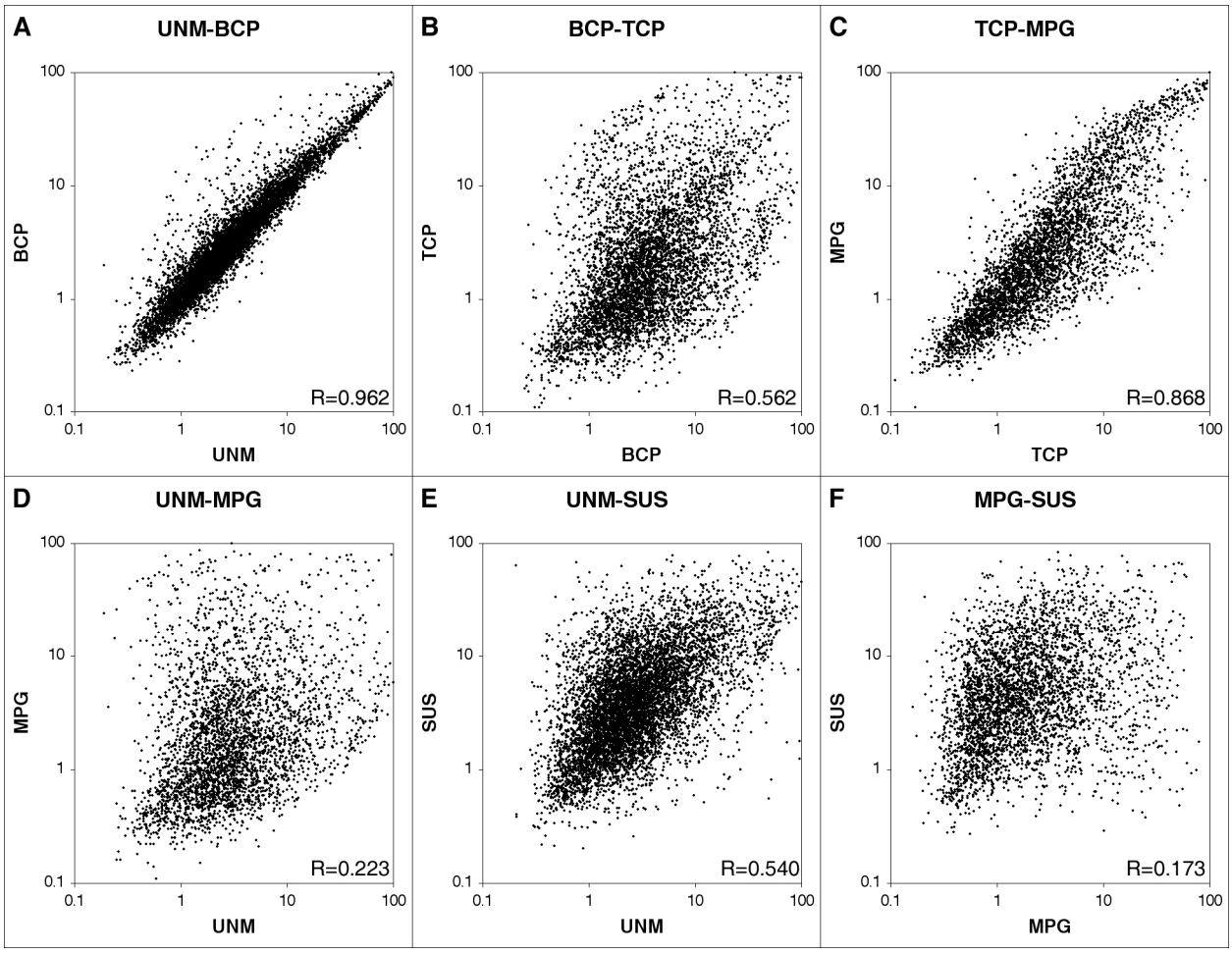

Fig. 5 Scatter plots comparing relative gene expression in pairs of developmental stages. The expression levels of individual genes were normalized using a logarithmic scale of 0 to 100 and genes co-expressed in pairs of transcriptome datasets were plotted. (**A**) UNM versus BCP stage; (**B**) BCP versus TCP stage; (**C**) TCP versus MPG stage; (**D**) UNM versus MPG stage; (**E**) UNM versus suspension cultured cells (SUS); (**F**) MPG versus SUS. R value represents correlation coefficient.

To evaluate the functional divergence of both developmental programs, the dynamics of male gametophyte-expressed mRNAs between gene function categories was analysed. Twelve gene function categories were defined as described previously (Honys and Twell 2003). The distribution of microspore-expressed genes among functional categories was similar to that of rosette leaves. Among rare exceptions were genes involved in signaling and stress-responses (~1% under-represented) and genes involved in transport (~1% over-representation). Moreover, the replacement of a number of expressed genes with their relative signal intensity led to a dramatic expansion in the contribution of genes involved in protein synthesis from 5-14%. This highlights the importance of protein synthesis initiated during early male gametophyte development. A different figure was obtained when only genes specific to the male gametophyte were analysed (423 genes). Highly expressed, but mostly constitutive genes involved in protein synthesis disappeared and were replaced by the gene categories, cell wall metabolism and transport (Honys and Twell 2004).

The transcriptome of mature pollen grains differs markedly from all other tissues and is accompanied by a significant reduction in complexity (Honys and Twell 2003 2004, Becker *et al.* 2003, Lee and Lee 2003, Pina *et al.* 2005). Although the down-regulation of most microspore-expressed genes is a general trend, a significant group of genes are specifically up-regulated late during pollen development. This reduction in complexity and the parallel activation of different gene sets are not uniform. The most overrepresented gene categories were cell wall metabolism, cytoskeleton and signaling. Other important functional categories such as transport and stress-related mostly contained less abundant non-pollen-specific genes. What was new and surprising was the extent of transcription of mRNAs forming those up-regulated gene function categories. There was an enormous increase in the average signal per gene in these categories. In sporophyte and early male

gametophyte the average signal/gene was within the range 300-500, whereas in late male gametophyte this value increased to approximately 1,000. With the exception of the protein synthesis category all other gene function groups were more abundantly represented among pollen-specific mRNAs. In particular, the average signal/gene for pollen-specific genes involved in cell wall metabolism reached nearly 6,000. All these findings unequivocally confirm the bias of male gametophytic gene expression towards functional specialization involving storage, signaling and rapid pollen tube growth (Honys and Twell 2004, D. Honys and D. Twell, unpublished data).

It is well established that transcriptional processes play important roles in global and specific gene expression patterns during pollen maturation. On the contrary, pollen germination in many species has been shown to be largely independent of transcription but vitally dependent on translation (Twell 1994 2002). *Arabidopsis* was shown to follow this general trend (Honys and Twell 2004) and there is compelling evidence that many mRNAs and mRNPs are stored in preparation for translation during tube growth (Honys *et al.* 2000, Twell 2002). Developmental transcriptomic studies prove the existence of a large number of stored mRNAs in mature pollen (Honys and Twell 2004). Most abundant late pollen-expressed transcripts up-regulated after pollen mitosis II fall into this category and represent a stored mRNA charge. These studies also revealed that it is not only mRNAs that are stored, but also pre-formed protein synthesis machinery. Most protein synthesis genes are down-regulated in mature pollen and *Arabidopsis* pollen tube growth is strictly dependent on translation (Honys and Twell 2004). Further evidence for functional protein storage in mature pollen comes from particular *seth* mutants affecting progamic development in which mRNAs are expressed early during pollen development, but whose protein products are essential for pollen germination and tube growth (Lalanne *et al.* 2004a).

7. CONCLUSIONS

We have presented an overview of important cellular processes in male gametophyte development together with recent advances in genetics and genomic approaches that are advancing the field significantly. Genetic approaches have identified a growing number of gametophytic mutants affecting discrete steps during the developmental or progamic phase unraveling some of the key molecular processes involved. The key impact of pollen transcriptomic studies is that they provide the first comprehensive genome-wide view of the complexity of gene expression during reproductive cell development. This work demonstrates extensive (>95%) haplo-diploid overlap, providing enormous potential for the gametophytic selection of sporophytic traits. These studies reveal at least two successive global gene expression programs. The early program is more similar to the sporophyte and follows a trend of reduction in complexity associated with termination of cell proliferation. On the contrary, a number of late genes are activated after PMII many of which show pollen-specific expression patterns and are likely to play important roles in post-pollination events. Male gametophyte development is associated with the expression of around 600 putative transcription factors offering insight into the potential complexity of regulatory networks involved in gametophytic transcription. Moreover, during post-pollination development there is a diminished role for transcription and an important role for mRNA and protein storage that is poorly understood. Transcriptome analysis has revealed a striking overrepresentation of cell wall metabolism, cytoskeleton and signaling genes in preparation for the progamic phase. This quantum leap in gene-centered knowledge of pollen development highlights the functional specialization of this pathway and offers many new opportunities for the dissection of cellular processes that control male reproductive success.

The quest to achieve a comprehensive description of the cellular, molecular and genetic events that control pollen development is not only of fundamental interest, but may find application in the production and genetic improvement of crops. Current knowledge is applied in several areas of crop production including pollination and crop yield optimisation, hybrid seed production and in the production of economic products such as honey and pharmaceuticals. The recent progress in mutational and genome-wide analysis now provides enormous potential to develop new strategies for molecular intervention and modification of pollen development and functions.

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