Male gamete biology in flowering plants

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Abstract
Flowering plant reproduction is characterized by double fertilization, in which two diminutive brother sperm cells initiate embryo and endosperm. The role of the male gamete, although studied structurally for over a century at various levels, is still being explored on a molecular and cellular level. The potential of the male to influence development has been historically underestimated and the reasons for this are obvious: limitations provided by maternal imprinting, the much greater cellular volume of female gametes and the general paucity of paternal effects. However, as more is known about molecular expression of chromatin-modifying proteins, ubiquitin pathway proteins and transcription factors in sperm cells, as well as their ability to achieve effect by intaglio expression, passing transcripts directly into translation, the role of the male is likely to expand. Much of the expression in the male germ line that appears to be distinct from patterns of pollen vegetative cell expression may be the result of chromosomal level regulation of transcription.

Structure, timing and physiology in flowering plant reproduction
In flowering plants, double fertilization launches two dramatically distinct developmental programmes. One of the two sperm cells in a successful pollen tube fuses with the egg cell to form the canonical zygote and embryo; a second brother sperm cell fuses with the central cell to form the male portion of the unique and precocious nutritive endosperm. This unique complement is part of a temporal choreography that is conducted most conspicuously in floral parts that are paradoxically sporophytic and asexual, but also govern the timing of gametophytic events [1]. Much of this timing is simply a matter of opportunity, as indicated by the conspicuous displays of chasmogamous flowers, whereas the clues are cryptic in cleistogamous flowers and, in the absence of a conspicuous visual signal, nevertheless produce receptive gametes that are timed within the flower to accomplish fertilization.

The diversity of structural organization of gametophytes is well described in previous reviews [2,3], but these gamete-producing lineages also express their diversity in the timing of various maturational cues. Maturation may be delayed or hastened to match male development to female development, and the duration of the progamic phase (from pollination to fertilization) is adapted to optimize receptivity of male and female gametes. Communication at the gametophytic level (fertilization) is adapted to optimize receptivity of male and female gametes. Much of this timing is simply a matter of opportunity, as indicated by the conspicuous displays of chasmogamous flowers, whereas the clues are cryptic in cleistogamous flowers and, in the absence of a conspicuous visual signal, nevertheless produce receptive gametes that are timed within the flower to accomplish fertilization.

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Gametes themselves are the most critical functional element of the evolutionarily highly reduced gametophytes. The male gametes originate as part of a ‘male germ unit’ in which the male germ lineage of the generative and later sperm cells associate with the VN (vegetative nucleus) (Figure 1). The male germ unit forms a functional assemblage that provides for the co-transmission of male gametes at the time of their optimal receptivity [8,9]. Flowering plant sperm cells have a relatively diminutive cytoplasm, a prominent nucleus and, frequently, an elongated projection that appears to be associated physically with the VN [10]. Rather than leading the male gametes, however, the ‘tail’ often follows the lead of the VN, and the male gametes are non-motile, rather being conveyed by actin–myosin interactions that act on an enveloping pollen tube plasma membrane [11].

The female gametes are flanked by sister cells known as synergids, which aid in the attraction, receipt and transmission of male gametes into the embryo sac. The egg and the central cell are the female fusion target cells, which together with the two synergids form the functional entity known as the ‘female germ unit’ [9]. These cells display characteristic features such as cellular polarity, prominent nuclei and nucleoli, an appearance of quiescence in the female gamete before fertilization, but are otherwise ultrastructurally unremarkable.
Figure 1 | Schematic representation of the male germ lineage within a flowering plant pollen tube illustrates the leading sperm cell (S_{lm}) associated with the VN and trailing (S_{ua}) sperm cell. The S_{ua} produces ECBs at its trailing end and VCBs (unlabelled arrows) laterally. The S_{lm} produces VCBs from the tip of the cytoplasmic extension (arrowheads) and the lateral cytoplasm (unlabelled arrows). A freed VCB (asterisk) is visible in the trailing cytoplasm of the vegetative cell (VC). G, Golgi body; L, lipid body; M, mitochondrion; NU, nucleolus; PTW, pollen tube wall; V, vacuole; W, sperm cross-wall. Magnification approx. ×5000. Modified with kind permission from Springer Science+Business Media: Protoplasma, Sperm cells in pollen tubes of Nicotiana tabacum L.: three-dimensional reconstruction, cytoplasmic diminution, and quantitative cytology, vol. 168, 1992, pp. 172–183, Yu, H.S., Hu, S.Y. and Russell, S.D., Figure 20. © Springer-Verlag 1992.

Overall, the male and female gametes have a relatively simple cellular structure that provides useful clues about their functional competence, but few cues about their receptivity. Female gametophytes in angiosperms have variable genetic input from the products of meiosis, known as the megaspores, and one, two or four megaspores may contribute to the embryo sac [2]; this creates a genetic wealth of diversity with regard to megagametophyte formation [12]. The evolutionary origin of the embryo sac suggests a diploid endosperm with cellular plasticity with regard to ancestral forms [13] and plasticity with regard to identity and placement of accessory cells in embryo sacs. Interestingly, among modular organizational units in the female gametophyte [14], it appears that the position of the egg cell and synergids in the ovule is mediated by an auxin gradient signal, as a reversal of the auxin gradient causes the opposite quartet of nuclei to form the female germ unit [15]. Male gamete determination seems to be independent of sporophyte cues.

Molecular repertoire of flowering plant sperm cells

Although male gametes were initially believed to lack independent molecular expression, studies in the last decade have uncovered unique male germ lineage genes, RNA sequences, promoters and repressor elements [16]. The diversity of transcripts produced by sperm cells evident in ESTs (expressed sequence tags) and genomic microarray analysis [17] reveal that transcribed products are abundant. Germline-specific promoters show high specificity of action [18], and pathways required for processing transcripts are present [19,20]. Translation of some sperm transcripts has been verified in generative and sperm cells [21], but proteomic validation will be needed to determine how many such transcripts are expressed. If expression in sperm cells resembles that in pollen tubes of tobacco [22], some ribonucleoprotein-associated mRNAs may be retained for expression later in development.

To date, one study is available comparing the transcriptome of the pollen tube with that of sperm cells [17]. Sperm cells are small compared with pollen tube and somatic cells, are dependent on the tube for transportation and nutrition, and their cytoplasm is actively lost during maturation [23], yet they may produce a quarter more transcripts than the tube and as many as some sporophyte tissues. Common themes of expression in the male germ lineage have included the enrichment of pathways involving chromatin-modifying proteins [24,25], ubiquitin pathway proteins [26], position in the cell cycle [27] and various metabolic products (Table 1), as well as some unique transcription factors.
Most abundant transcripts in Ssu and Svn of Plumbago zeylanica, based on BLASTx of GenBank® non-redundant protein database

Table 1

<table>
<thead>
<tr>
<th>Putative identity</th>
<th>AGI (Ssu/Svn)</th>
<th>E-value (Ssu/Svn)</th>
<th>Clones (Ssu)</th>
<th>Clones (Svn)</th>
<th>Putative function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ubiquitin ligase</td>
<td>At5g42190/At1g75950</td>
<td>6 × 10^{-42}/3 × 10^{-30}</td>
<td>33</td>
<td>34</td>
<td>Protein degradation</td>
</tr>
<tr>
<td>Putative phosphatase</td>
<td>At1g17710</td>
<td>10^{-61}/4 × 10^{-55}</td>
<td>27</td>
<td>8</td>
<td>Unclassified</td>
</tr>
<tr>
<td>Ubiquitin-conjugating enzyme</td>
<td>At3g52560</td>
<td>6 × 10^{-71}/4 × 10^{-56}</td>
<td>12</td>
<td>3</td>
<td>Protein degradation</td>
</tr>
<tr>
<td>High-mobility group protein</td>
<td>At1g20693</td>
<td>5 × 10^{-42}/10^{-42}</td>
<td>11</td>
<td>13</td>
<td>Chromatin modelling</td>
</tr>
<tr>
<td>Histone H3</td>
<td>At5g10980</td>
<td>7 × 10^{-72}/2 × 10^{-71}</td>
<td>8</td>
<td>8</td>
<td>Chromatin modelling</td>
</tr>
<tr>
<td>Heat-shock protein</td>
<td>At5g59720/At4g27670</td>
<td>10^{-55}/3 × 10^{-33}</td>
<td>7</td>
<td>6</td>
<td>Cell rescue, defence and virulence</td>
</tr>
<tr>
<td>Actin-depolymerizing factor</td>
<td>At1g01750/At4g25590</td>
<td>9 × 10^{-67}/3 × 10^{-52}</td>
<td>5</td>
<td>6</td>
<td>Protein binding/cytoskeleton</td>
</tr>
<tr>
<td>ABC (ATP-binding cassette) transporter family protein</td>
<td>At3g10670</td>
<td>10^{-14}</td>
<td>3</td>
<td>-</td>
<td>Cellular transport, facilitation, targeting</td>
</tr>
<tr>
<td>F-box family protein</td>
<td>At4g03220</td>
<td>3 × 10^{-36}/4 × 10^{-79}</td>
<td>2</td>
<td>6</td>
<td>Protein degradation</td>
</tr>
<tr>
<td>Polyubiquitin</td>
<td>At4g02890/At5g20620</td>
<td>4 × 10^{-79}</td>
<td>2</td>
<td>4</td>
<td>Protein degradation</td>
</tr>
<tr>
<td>Exopolygalacturonase</td>
<td>At3g14040</td>
<td>3 × 10^{-16}/10^{-21}</td>
<td>1</td>
<td>4</td>
<td>Carbohydrate metabolism</td>
</tr>
</tbody>
</table>

TEs (transposable elements) are abundant in the sperm cells of grasses [28], where their percentage abundance may approach the relative abundance of TEs in the grass genome. TEs appear mobilized in the vegetative cell of Arabidopsis pollen according to transposon display, but no such evidence exists in sperm cells [29]. TE activation is normally suppressed by methylation of cytosine residues at the DNA level, and the male germ lineage appears to remain sufficiently methylated to prevent TE activation. The production of abundant small RNA species in the male lineage is an interesting parallel with animals [30]. The 24 nt siRNA (small interfering RNA)-mediated heterochromatin formation pathway is down-regulated in the pollen vegetative cell, whereas microRNA and trans-acting siRNA pathways are functional. Silencing elements in the form of tasiRNAs (trans-acting siRNAs) synthesized in the vegetative cell and imported into the sperm cells have been proposed as a mechanism capable of suppressing TE transcripts from activating sperm-based mobile elements [29].

Little direct structural support is available for this, but the occurrence of ECBs (encuolated cytoplasmic bodies) and VCBs (vesicle-containing bodies) during male germ unit maturation is evidence of communication between vegetative and male germ cells, although their fate is unclear (Figure 1). The clearest evidence is that sperm cells simply lose cytoplasmic volume through this mechanism. In tobacco, accelerated cytoplasmic loss from the VN-associated sperm (Ssu) leads to sperm dimorphism [31]. Mechanistically, such cytoplasmic reduction has been cited as a means by which paternal cytoplasmic inheritance, as well as volume, could be reduced in the male germ lineage [32]. The presence of distinctly different transcriptional profiles between the pollen vegetative cell and sperm cells, depicted by Principal Component Analysis [17], suggests that such contents of vesicles are not shared, but rapidly degraded, and are not taken up by either (movement of small RNA species in this environment has not been examined). The pollen vegetative cell displays down-regulation of siRNA species associated with maintenance of heterochromatin; the VN is accordingly depauperate of heterochromatin. Small RNA species are known to activate RNAi (RNA interference)-related pathways and heterochromatin formation and are abundant in the male germ lineage, with involvement in the epigenetic maintenance of the germ lineage in plants [30].

mRNAs exchanged between gametophytes and the next generation

Communication between the synergid and pollen tube has been the subject of elegant experimentation and molecular studies [6,33], as has activation of development [34] and molecular mechanisms of male gamete identity [35], which are also discussed elsewhere in this issue of Biochemical Society Transactions. An area of less attention has been the expression and function of the gametes, particularly the male gamete [21], and its potential post-fusion role.

Recently, Bayer et al. [36] characterized an IRAK (interleukin-1 receptor-associated kinase)/Pelle-like kinase gene, called SHORT SUSPENDER (SSP), which is transcribed in the male gametophyte, but translated and expressed in the newly fused zygote. Zygotes receiving the defectivessp gene through the paternal lineage failed to divide asymmetrically and did not form the disproportion typical of apical versus basal cells in the proembryo. Thus initial
Cell–cell Communication in Plant Reproduction

**Figure 2** | Identification of differentially expressed genes in Sua and Svn

(a) Scatter plot of >2-fold up-regulated ESTs identified by SSH and microarray by sperm type, Sua and Svn. Cy3, indocarbocyanine; Cy5, indodicarbocyanine. (b and c) Functional categorization from SSH cDNA libraries provide dissimilar functional profiles for Sua (b) and Svn (c). See Tables S7 and S8 of [42] for further annotation. Modified from [42] with permission.

The transmission of already present paternal transcripts into the egg, reported also in tobacco (but without functional validation of protein expression) [39], is not a case of imprinting, but is limited to the lifespan of the transcript. We compare this with an intaglio effect, as the transcripts (or translated products) exist as a template to which the ink of translation is impressed, resulting in transient products that are neither renewed nor truly inherited. These products are present only during the lifespan of the transcripts and their products. Maternal control is the general rule during reproduction and after fusion [12], but a male intaglio effect that delivers critical transcripts (or even translated products) to the newly fused products of fertilization may dramatically contract the time needed for initial pattern establishment in a newly constituted embryo or endosperm cell.

*Plumbago zeylanica* is an interesting model for flowering plant fertilization because the two sperm cells are known to be dimorphic, and each of the two sperm cells undergoes preferential fertilization, thus, if there are fusion-targeted male effects, they may be evident in this plant. One sperm cell, which is physically associated with the VN (Svn), contains numerous mitochondria and infrequent plastids, whereas the sperm cell unassociated with the VN (Sua) contains abundant plastids and fewer mitochondria [40]. During fertilization, the plastid-rich sperm cell fuses with the egg cell (Sua), and the mitochondrially enriched sperm cell (Svn) fuses with the central cell [41], triggering the formation of embryo and endosperm respectively. Cell populations were separated and PCR-amplified cDNA libraries constructed to examine potential differential transcription, with and without SSH (suppression subtractive hybridization) [42]. ESTs, custom microarray plots and real-time RT (reverse transcription)–PCR indicate clearly that the two sperm cell types express divergent, complex and unique transcriptional profiles (Figure 2).

The Sua, which targets the egg cell, has a greater abundance of transcripts relating to transcription, translation and protein modification, and thus appears to reflect a profile similar to anticipated patterns of expression in the embryo. Some ESTs encode Sua sequences relating to cellular signalling, including a number of calcium-related kinases. The Svn, which targets the central cell and forms the endosperm, displays greater abundance of transcripts relating to metabolism and phytohormone biosynthesis, particularly including energy/storage product-related transcripts and multiple copies of IPT (isopentenyl transferase), which frequently serves as the controlling enzyme for cytokinin biosynthesis. Although cytokinin causes negligible response in pollen [43], cytokinin levels are strongly elevated during endosperm proliferation and appear to control endosperm...
growth and development [44]. These IPT gene sequences appear to be transmitted during fertilization, and preliminary results indicate that they are expressed in the embryo sac (X. Gou, X. Wei, T. Yuan and S.D. Russell, unpublished work). In the endosperm, such paternal transcripts of IPT may contribute toward heightened cytokinin production, and thus precocious nuclear division and metabolic activation. The Svn, which targets the central cell and forms the endosperm, may represent a paternal effect in endosperm similar to that in embryos [36]. Relative to overall expression, the Svn appears to have a more strongly divergent programme than the Sua, as many up-regulated genes from the SSH cDNA library of the Svn lacked homology with other sequences in GenBank® and may be evolutionarily specialized, as it also displays few close homologues with the Arabidopsis sperm transcriptome [42].

Sperm cell recognition

Control of gametic fusion events and the occurrence of preferential fusion in angiosperms is strong evidence that sperm cells undergo recognition. Although few flowering plants with cytoplasmic heteromorphy have been described [45], essentially all angiosperms studied to date display polarized male germ units. Examined using in vitro fertilization, sperm cells in maize proved to be interchangeable during fertilization [46]. Patterns are not so clear, however, when sperm cell formation is suppressed by a number of genes critical to the division of the precursor generative cell. In the case of cdc2a mutants, a ‘sperm’ cell was formed that fused only with the egg cell, generating a signal that triggered endosperm proliferation [47]. This cell-cycle-regulating gene, known also as CDKA;1, is required to enter both mitosis and DNA synthesis. Another gene, DUO1, is involved with sperm cell identity control, encoding a novel R2R3 MYB transcription factor [35]. In the mutant duo1 the undivided generative cell undergoes DNA synthesis, but remains sterile, indicating that full sperm function is not achieved by the mutant [48]. In generative cells containing the msi1 mutation, a single sperm cell produced without a fusion preference [49]. In contrast, when diphtheria toxin A subunit is expressed in the generative cell, sperm cell maturation is inhibited, and the single sperm cell fuses preferentially with the central cell [18]. Potential two recognition systems exist: one that recognizes male gamete cells in general and the other discriminating specific gametic fusion targets, egg and central cell, separately [50]. Tools to evaluate and resolve long-standing questions of cell–cell communication in the critical molecular events of double fertilization may soon be available.

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References


