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The sporophyte-to-gametophyte transition: The haploid generation comes of age

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Abstract

Flowering plants alternate between two multicellular generations: the diploid sporophyte and haploid gametophyte. Despite its small size, the gametophyte has significant impacts on plant genetics, evolution, and breeding. Each male pollen grain and female embryo sac is a multicellular organism with independent gene expression, a functioning metabolism, and specialized cell types. In this review, we describe recent progress in understanding the process in which the haploid genome takes over expression from its diploid parent – the sporophyte-to-gametophyte transition. The focus is on pollen, but similar concepts may also apply to the female gametophyte. Technological advances in single-cell genomics offer the opportunity to characterize haploid gene expression in unprecedented detail, positioning the field to make rapid progress.

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Gene expression, Pollen, Gametophyte, Gene regulation, Single-cell RNA-seq, Haploid selection.

Introduction

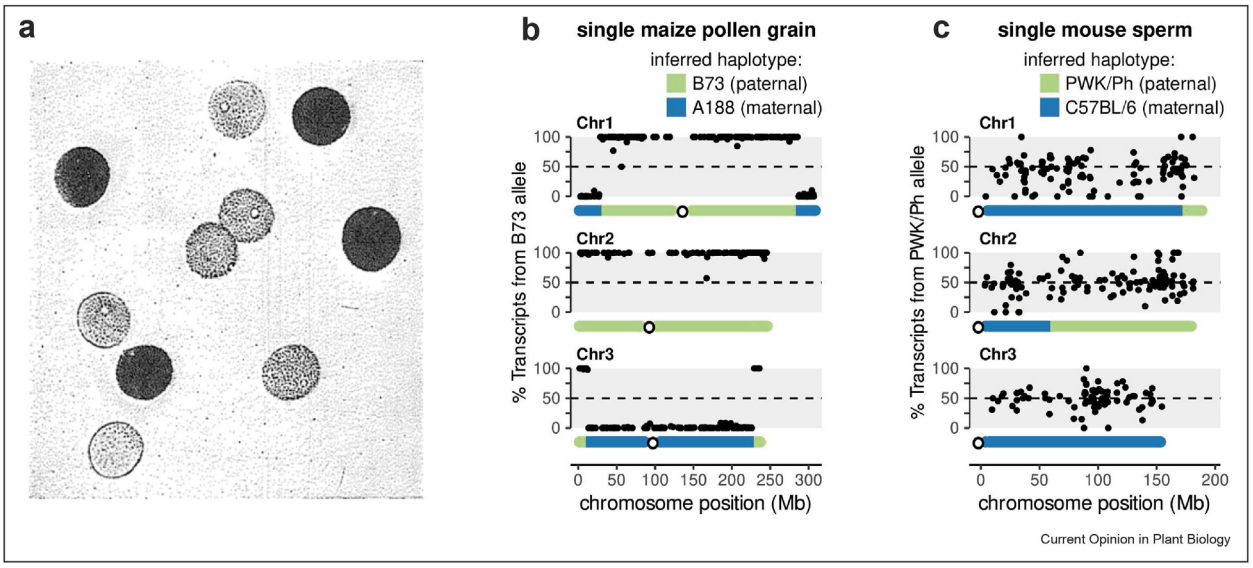
In angiosperms, most of the gametophyte life cycle occurs within developing flowers and is heavily supported by the sporophyte. The mature gametophyte is small, containing only 3 cells for male pollen and 4–15 for the female embryo sac [1]. However, the gametophyte is not a passive carrier for genetic information, as there is active gene expression from the haploid genome. Distinct phenotypes can routinely be seen segregating among pollen grains from a single plant [2], and transcripts for a majority of genes are detectable in the gametophyte [3–5]. As a result, a large fraction of the genome is exposed to selection during the haploid phase [6–9].

This review focuses on the timing of haploid expression in the gametophyte, with an emphasis on male pollen. We discuss parallels between early gametophyte expression and the maternal-to-zygotic transition (MZT), an analogous shift in expression from parent-to-offspring in the embryo. There are far too many fascinating aspects of gametophyte biology to cover here; we point the reader to excellent reviews on allied topics including male [10–12] and female [1] gametophyte development, pollen gene expression [5], epigenetic regulation in the gametophyte [13–16], evolutionary implications of the gametophytic phase [6–8], broader consequences of haploid selection [9], and the maternal-to-zygotic transition in plants [17–20] and animals [21–24]. In this review, we use the phrase 'haploid expression' to mean the portion of gene expression, whether transcript or protein, originating from the haploid gametophyte genome.

Expression from the haploid pollen genome

Active gametophytic gene expression has important consequences for plant genetics and evolution because it connects haploid genotype to phenotype [25]. Gametophytes from a single plant can be genetically heterogeneous because of the shuffling of alleles during meiosis and the occurrence of new mutations during development. The capacity for selection is particularly high in pollen, as there are large population sizes (e.g. 10 million pollen grains per maize plant) and extensive competition during dispersal and fertilization. Furthermore, recessive mutations can immediately show a phenotype in the haploid phase. For example, Figure 1a shows a micrograph of rice pollen stained with iodine [2]. The parent plant was heterozygous for a recessive waxy allele, and the waxy (unstained) phenotype can be seen segregating among pollen grains from this single plant. This example demonstrates another important feature of pollen expression: it can affect alleles that have phenotypes in other stages of the life cycle. The waxy allele in Figure 1a is also responsible for the glutinous phenotype of "sticky" rice, and thus has an additional, agronomically important phenotype in endosperm [2,26].

Figure 1



Gene expression in pollen originates from the haploid genome. **a)** Micrograph of rice pollen stained for amylose starch using iodine. The pollen is from a single heterozygous plant segregating for the waxy (unstained) phenotype. From Ref. [2], reproduced with permission from Springer Nature. **b)** Allelic bias of transcripts from a single isolated maize pollen grain, plotted for the first three chromosomes. The inferred pollen haplotype is below. Data from Ref. [3]. **c)** Allelic bias of transcripts from a single isolated mouse sperm. Data from Ref. [33] (SRA accession SRR12790356), mapped to the GRCm39 mouse genome and plotted as described in Ref. [3].

A large fraction of the genome is expressed in the gametophyte. Transcripts for 40–60% of predicted genes are detectable during pollen development [3–5,8], most of which (~90%) are also expressed in the sporophyte and likely represent genes important for both life cycle phases. There are developmental trends in gene expression, with reduced transcript diversity in mature pollen compared to earlier haploid stages [5]. In mature pollen, most expression originates from the haploid genome [3,27,28], with carryover transcripts and proteins from the sporophyte being rare. Widespread haploid expression is reflected in the frequency of gametophytic phenotypes. Mutant alleles with strong gametophytic defects are routinely found in genetic screens [29,30], and even relatively small chromosomal deletions cannot be transmitted through pollen [31,32].

Distinguishing expression originating from the haploid genome

Given the small size of the angiosperm gametophyte, haploid expression has historically been difficult to follow except for a handful of genes with readily accessible phenotypes [2,27,28]. Bulk methods cannot unambiguously separate haploid-derived expression because steady-state transcript and protein levels are regulated at many steps. Fortunately, genomic technologies are now sensitive enough to assay even single haploid cells [3,33], and single pollen grains and precursors can be isolated manually with established techniques [3,34,35]. In single pollen grains from F_1 hybrid plants, haploid expression is distinguishable because it comes from a single allele (Figure 1b), while diploid-derived expression is biallelic in origin [3]. Allele-specific pollen expression data is currently only available for the transcriptome [3], but similar techniques could be paired with proteomics [36] or ribosome profiling [37] to study gametophytic protein expression and translational regulation.

Comparison to animals

The scale of haploid expression is vastly different between plants and animals. This can be clearly seen by comparing recent allele-specific RNA-seq data of single maize pollen grains [3] to mouse sperm [33]; while pollen grains showed strong, genome-wide allelic bias reflecting the haploid genotype (Figure 1b), most transcripts in mouse sperm were derived from both parental alleles (Figure 1c; biological reasons for this reviewed in Ref. [9]). The contrast is even stronger based on phenotypes, as many animal species routinely produce functional gametes with severe genomic abnormalities [9]. For example, in healthy human adults, 5.3% of sperm and 16.1% of oocytes lack entire chromosomes [38,39]. Aneuploidy has been observed for every autosome and both sex chromosomes [39], and aneuploid sperm are capable of fertilizing an egg [38]. Thus, the haploid genome is not strictly required for the haploid phase in animals.

However, phenotypes less severe than "death" can still have important impacts on evolutionary timescales, and this is true in the haploid phase just as it is in the diploid one. There are examples of animal genes with experimentally confirmed transmission ratio distortion [9]. Furthermore, a subset of mouse genes showed statistically significant allelic bias in sperm; this subset was enriched in selective sweep regions, suggesting increased selective pressure [33]. Finally, haploid transcription might have impacts beyond the immediate production of gene products, as there is evidence that transcription in animal sperm decreases the genic mutation rate by activating transcription-coupled DNA repair [40].

The sporophyte-to-gametophyte transition (SGT) and gametophyte genome activation (GGA)

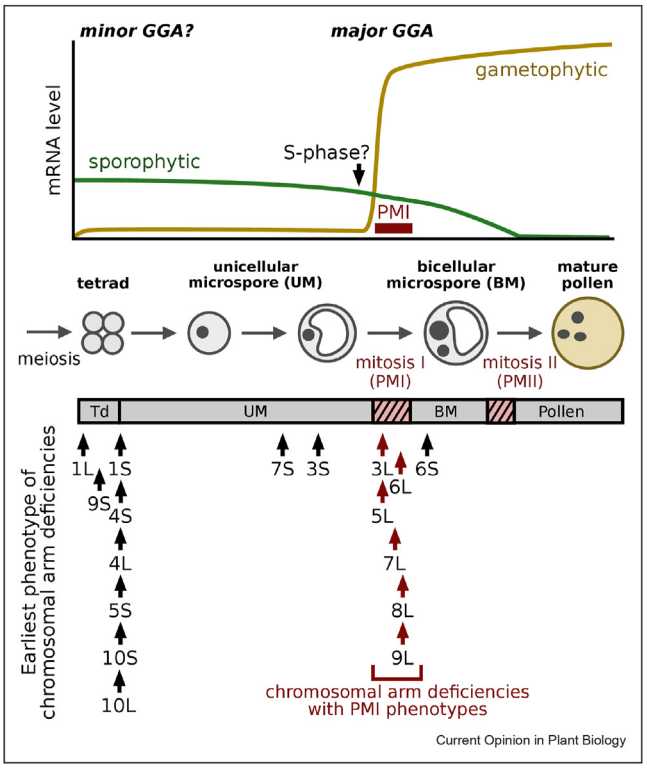
It has long been suspected that pre-meiotic transcripts persist in the haploid phase of plants [41], leading some microspore-expressed genes to be under sporophytic control. Recent evidence indicates that this is a global phenomenon, as pre-meiotic (biallelic) transcripts are retained for the majority of genes until ~11 days after meiosis in maize [3]. This is analogous to the maternal-to-zygotic transition (MZT) in animals, in which the zygotic genome is initially transcriptionally quiescent and early embryonic development is under maternal genetic control [21]. Here, we define the SGT and GGA in direct analogy to MZT and ZGA: SGT is the complete process in which pre-meiotic, sporophytic gene products are degraded and replaced with gametophytic products; GGA specifically refers to the activation of the haploid gametophyte genome, an important component of SGT.

Timing of the SGT in maize pollen

A recent study used allele-specific RNA-sequencing to follow haploid expression throughout maize pollen development [3]. Sporophytic transcripts persisted throughout the unicellular microspore (UM) stage, up to 11 days after meiosis. This was followed by a rapid and global shift to monoallelic expression near the time of pollen mitosis I (PMI). Monoallelic expression was driven primarily by new transcription and genome activation: pre-meiotic transcripts could still be detected after PMI, but a massive increase in transcripts from the haploid genome overwhelmed the low level of residual sporophytic transcripts.

By integrating these allele-specific expression data with classic genetic studies, a more complete model of the SGT can be derived (Figure 2). In particular, Kindiger and colleagues characterized the consequences of 17 of 20 chromosomal arm deficiencies during pollen development [31]. They found that hypoploid phenotypes clustered at two stages: around the time of tetrad dissolution and during PMI (Figure 2, bottom). This

Figure 2



Working model of the SGT during maize pollen development. Bottom: timing of the earliest phenotype for chromosome deficiencies in 17 of 20 maize chromosomal arms, from Ref. [31].

second cluster can be readily explained if GGA occurs just before PMI, as any chromosome deficiencies that are still viable quickly lead to defects after widespread GGA. The earlier cluster suggests that some haploid expression is required long before PMI. No haploid-expressed genes were observed prior to PMI in the recent allele-specific expression study [3], but if such genes were a rare subset they could have been missed. By comparison, in many animal species the MZT has been resolved into a multi-step process with multiple waves of ZGA [21]. It will be important to establish the identity of haploid expressed genes at the start of pollen development, potentially representing a minor, early wave of GGA. Given that 10 chromosomal arm deficiencies led to phenotypes before PMI [31], it would suggest ~12 genes are both expressed and required before PMI[1] (95% confidence interval: 7–15 genes).

Why would the major wave of GGA be delayed until PMI? One hypothesis is that this delay protects the male germline from transposons. PMI is the cell division that separates the somatic vegetative cell from the

[1] Binomial distribution assuming each chromosome arm has approximately the same number of genes and essential gametophyte genes are randomly distributed between them.

gametophyte germline (generative cell). In many animal species, ZGA is actively delayed in primordial germ cells relative to the soma [21,42]. This is thought to reduce the rate of DNA damage by making the chromosomes less accessible to mutagens. The selective pressure to keep the microspore genome inaccessible may be particularly high because of the prevalence of active transposons in plants. In committed somatic cells, transposons are evolutionarily aligned with their hosts: anything that decreases host fitness will reduce the chance of the transposon's own survival. In the germline, however, there is evolutionary conflict between transposon and host [42], as a decrease in host fitness can be offset if it allows the transposon to replicate.

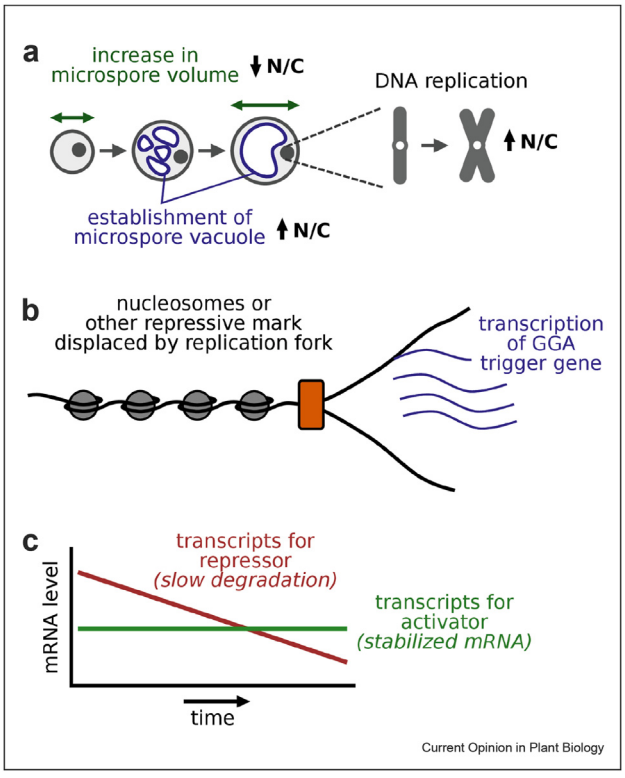
Timing of the SGT in other species

The timing of ZGA varies widely among animal species, occurring before the first cell division in some (e.g. mouse) and not until there are hundreds of cells in others (*Xenopus*) [21]. It is not yet clear if there is similar variation in GGA and SGT among plants. Many species have large differences in chromatin status between UMs and later stages [15,43–45], suggesting that PMI may mark an important, conserved shift in pollen gene expression. However, transcriptional changes between the uni- and bi-cellular stages are modest in *Arabidopsis* [5,46,47], which has led to the conclusion that the SGT occurs later in *Arabidopsis* compared to maize [47]. We argue that this conclusion is hasty without allele-specific sequencing data from single gametophytes, as it is possible for the source of transcription to change (from diploid to haploid) without a substantial shift in relative transcript abundance. In maize, many genes that do not show a substantial change in relative transcript abundance at PMI still shift from biallelic to monoallelic in origin [3]. Further data will clarify this issue. If the scope and timing of the SGT does vary between species, it would provide a mechanism for plants to control how much haploid selection they face [48].

Potential mechanisms of GGA

What might be the initial trigger for GGA? In some species, isolated microspores can be cultured in minimal media and develop into mature, viable pollen [49]. If GGA has not already occurred at the time of isolation in these cultures, it would suggest that genome activation is initiated by the gametophyte itself and does not require a signal from the surrounding anther tissue. A classic model for the MZT in animals is that ZGA is triggered as the nuclear to cytosolic (N/C) ratio increases during the early embryonic cell divisions, titrating out specific DNA-binding proteins that initially repress transcription [24]. A similar model is possible for GGA, as even before the first cell division (PMI) several features of microspore development potentially alter the N/C ratio (Figure 3a). Another possible trigger for GGA may be DNA replication (Figure 3b), perhaps by displacing nucleosomes or a repressive factor at the replication fork. Finally, post-transcriptional regulation may play a particularly important role prior to GGA. Translation regulation and RNA processing affect genes important for meiotic exit [50] and pollen development [51,52]; similar regulation may serve as a trigger for GGA (Figure 3c).

Figure 3



Potential mechanisms to initiate GGA. **a)** GGA may be triggered after a hypothetical inhibitor or activator is titrated by changing N/C ratios during microspore development. The microspore increases in size and becomes filled with a large vacuole, both of which affect the free cytosolic volume available to proteins in opposite directions. The nuclear DNA content doubles during DNA replication. **b)** DNA replication itself may trigger GGA by resetting epigenetic marks or displacing histones and other proteins. **c)** Post-transcriptional regulation may activate a factor to set off a wave of gametophytic transcription.

Conclusions and future perspectives

Widespread haploid expression in the gametophyte has significant consequences on plant genetics and evolution [9,25]. It is now clear that the gametophyte can be provisioned with pre-meiotic gene products for a substantial portion of its life cycle [3]. The ability to perform genomics on single-cells makes it possible to distinguish haploid from diploid gene products throughout gametophyte development. The timing of haploid expression in plants is only beginning to come into focus, and many open questions remain. First, how

is translation regulated during the SGT? In maize, there are substantial changes to the proteome at PMI [53], and it will be important to determine how the protein complement changes during the shift to gametophytic expression. Second, when does the SGT occur in the female gametophyte? Is this also during the first mitotic division and does it occur as suddenly as in maize pollen? Third, how do mutants with known gametophytic defects disrupt the SGT? This information will help place existing pollen developmental genes into the SGT pathway. Finally, there is need for molecular biologists, evolutionary biologists, and breeders to collaborate to better understand the consequences and opportunities for haploid selection in plants.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data used in Figure 1b-c is available through the Sequence Read Archive and described in the original publications.

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