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More than a yolk: the short life and complex times of the plant endosperm

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In most angiosperms, the endosperm is limited to a short phase of the plant life-cycle where it forms part of the seed and plays a major role in its development. Our understanding of this terminally differentiated organ has accelerated in recent years, with discoveries in the fields of phylogeny and developmental genetics shedding light on its evolutionary origin, function and development. Here we explore various conserved and unique features of plant endosperms, including the establishment of distinct functional domains that regulate nutrient transfer from the maternal parent to the developing embryo. We also review data from *Arabidopsis* and maize that confirm the existence of complex genetic mechanisms operating during endosperm development. Importantly, these findings confirm that, in addition to nourishing the embryo, the endosperm fulfils several other key functions including that of fertilization 'sensor'; detecting and aborting the fertilization products of incompatible or wide hybridizations between related species.

More than a century ago, Sergius Nawaschwin [1] and Léon Guignard [2] independently discovered double fertilization in angiosperms, in which the union of two sperm cells with the egg and central cell results in the formation of the embryo and endosperm, respectively. Whereas the embryo eventually develops into the mature plant, the 'life-history' of the endosperm is confined to the seed stage. However, correct development of the endosperm is essential for successful embryogenesis. Because of its inaccessibility and ephemeral nature, the endosperm has long been regarded as a simple nurse tissue, and consequently its biology has, until recently, stimulated little interest.

> **As an individual, [the endosperm] is unique for it regularly has three ancestors and never leaves any descendants (P. Weatherwax, 1930) [3]**

In this review, we examine the many conundrums surrounding the plant endosperm and assess whether recent advances in phylogeny, comparative biology and developmental genetics have aided general understanding of this organ. For example, we analyse whether we are any nearer to elucidating the evolutionary origin of the endosperm – does the endosperm really represent a modified supernumerary embryo, or is it an extension of the embryo sac (female gametophyte)? Furthermore, we consider several characteristics of the endosperm that are shared among a wide-range of basal and higher angiosperms, such as conservation of function and developmental patterning.

Considering the breadth of this topic, a review of this length cannot provide a comprehensive account of endosperm biology in all groups. For this reason we focus primarily on development of NUCLEAR-TYPE endosperms (see Glossary), which are prevalent in angiosperms, and to a lesser extent on CELLULAR- and HELOBIAL-TYPE endosperms. Molecular and genetic data largely obtained from studies in maize (*Zea mays*) and *Arabidopsis thaliana* are also discussed in an attempt to unravel the unique genetic systems operating in triploid endosperms. Collectively, these findings are identifying the endosperm as being of great significance, not only as a paradigm for plant developmental mechanisms, but also as a key participant in seed development, hybridization and speciation.

The evolutionary origin of the endosperm – further clues or more red herrings?

At the turn of the 20th century, contrasting hypotheses were presented to explain the evolutionary origin of the

Glossary

ANITA: the phylogenetic grade encompassing the *Amborellaceae, Nymphaceaceae, Illiciales, Trimeniaceae* and the *Austrobaileyaceae* clades.
Cellular-type: type of endosperm that develops via a series of mitotic divisions always coupled with cytokinesis.
Cellularization: the partitioning of a multinucleate cell into individual mononucleate cells.
Dosage-dependent gene expression: the effect on the expression of a given gene due to the effective number of copies of this gene in the genome.
Genomic imprinting: an epigenetic mechanism that determines the expression or repression of genes according to parental origin.
Helobial-type: type of endosperm in which the first division of the pro-endosperm gives rise to two daughter cells, one of which undergoes cellular-type development, whereas the other follows nuclear-type development.
Nuclear-type: most common form of endosperm. Development involves a limited or permanent phase of free-nuclear (syncytial) division without cytokinesis.
Nucleocytoplasmic domains: the arrangement of individual nuclei into cytoskeletally defined compartments contained within a common cytoplasm.
Polycomb-group: a class of genes originally described in *Drosophila melanogaster*, whose gene products form protein complexes that act as repressors of gene expression.
Syncytium: a mass of cytoplasm containing several nuclei enclosed within a single plasma membrane.

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endosperm; one held that the endosperm represented a delayed continuation of female gametophyte development [4], the other considered this structure to have originated as an altruistic secondary embryo [5]. These ideas sparked off a series of investigations into the reproductive development of a variety of angiosperms and, although neither proving nor disproving either theory, these studies pointed to a biparental, triploid ancestral condition for the endosperm [6]. Recent re-evaluations of angiosperm phylogeny are challenging this interpretation. Currently the clades comprising the ANITA grade are contenders for the earliest angiosperm lineages [7], and the members of this grouping characteristically possess a four-celled and four-nucleate female gametophyte – including a genetically haploid central cell that upon fertilization is expected to yield a biparental, diploid endosperm [8]. Curiously, within this group, *Amborella* alone possesses a seven-celled and eight-nucleate female gametophyte that contains a genetically diploid central cell [9]. At present, it is not known whether double fertilization and hence development of a triploid endosperm takes place. It therefore remains uncertain whether diploidy represents the ancestral condition for endosperm and it is important that further comparative studies are extended to all members of the ANITA grade, and in particular to *Amborella*.

Does the endosperm truly represent a recapitulation of either embryogenesis or female gametophyte development? Most angiosperms possess either nuclear or cellular-type endosperms. Cellular-type endosperms are mainly restricted to the more basal angiosperms and therefore might represent the ancestral ontogeny [10]. Certainly, the similarity between cellular-type endosperm development and embryogenesis favours the notion that the modern endosperm might have originated as a rudimentary supernumerary embryo. However, further work is required to determine if cellular-type endosperms have a common origin before reaching such conclusions.

By comparison, the nuclear-type endosperm is most common in the angiosperms. Many aspects of nuclear-type endosperm development appear to have been repeatedly and independently sequestered from pre-existing mechanisms found in female gametophytes and other reproductive cells of land plants, including gymnosperms [11]. Like female gametophytes, nuclear endosperms undergo multiple rounds of free-nuclear or syncytial divisions occurring in rapid, coordinated waves, and a degree of nuclear migration [12–15]. Further resemblance to female gametophyte development is evident during the closing stages of syncytial development, when nuclei become arranged into NUCLEOCYTOPLASMIC DOMAINS via the establishment of radial microtubule arrays [15]. CELLULARIZATION is subsequently achieved through the formation of open-ended tube-like structures termed alveoli. However, alveoli are only a feature of nuclear-type endosperms and gymnosperm female gametophytes [15]. Collectively, these data point to a female gametophytic origin for the endosperm. Interestingly, gymnosperms do not possess true endosperms, but instead develop characteristically enlarged female gametophytes that accumulate nutrients before fertilization. In this respect, the endosperm can be considered a functional homologue of the gymnosperm female gametophyte because it too provides nutrients for the growing embryo. It is likely that the appearance of the endosperm in angiosperms provided numerous evolutionary advantages, including a reduction in female gametophyte size and a significant conservation of maternal resources until fertilization has taken place.

Endosperm functional domains are conserved across the angiosperms

Despite the apparent diversity of the angiosperms, the putative functional domains of plant endosperms are remarkably similar and often cytologically distinguishable. These include a maternal–filial interface, an embryo–endosperm interface, an epidermis and, in some angiosperms, a separate storage tissue (Figure 1).

The endosperm is structurally adapted to ensure efficient translocation of nutrients from the mother plant to the developing embryo. In monocot and eudicot endosperms, nutrient uptake (mostly in the form of sucrose) occurs at the maternal–filial interface, notably in the specialized haustoria or in chalazal domains, which are characterized by the presence of many organelles (particularly mitochondria and rough ER) and specialized cell wall projections [16]. Sucrose is mostly converted to hexose sugars in the seed by cell wall bound and soluble invertases. The regulation of sugar flux by invertases is of paramount importance, not only in regulating cell division and grain filling during seed development but also in the formation of specialized cell wall projections. For instance, in *Vicia faba* seeds, cell wall projections are induced and inhibited by hexoses and high sucrose levels, respectively [17,18]. Similarly in maize, there is accumulating evidence that gene expression in the basal endosperm transfer region [henceforth referred to as the 'chalazal endosperm' (Figure 1)] is regulated by sugars [19–21].

A second endosperm domain apparent during early seed development is the micropylar endosperm (MC) – a region immediately adjacent to the embryo [10,22,23] (Figure 1). Considering its position within the seed, this domain might be required to sustain communication with its neighbouring embryo, as well as forming a structural and protective barrier. This interpretation is supported by studies in maize that have identified several genes expressed in the MC domain [otherwise referred to as the embryo-surrounding region (ESR)] that encode putative signalling peptides [24] and antifungal proteins [25]. In addition, an ESR-specific invertase inhibitor, INVINH1, has also been recently isolated [26]. These findings suggest that, at least in maize, the MC domain might play a role in regulating embryo growth during early developmental stages by limiting the supply of hexose sugars. Functional studies of INVINH1 and other proteins specific to the MC domain are now required to establish any developmental role for this tissue.

A principal function of most endosperms is presumed to be the synthesis and accumulation of storage products for the growing embryo. High levels of lipids and proteins are formed in the majority of angiosperm endosperms, whereas starch synthesis is confined to fewer taxa, including the monocots. Unsurprisingly, an inverse correlation exists in different species between the size of the

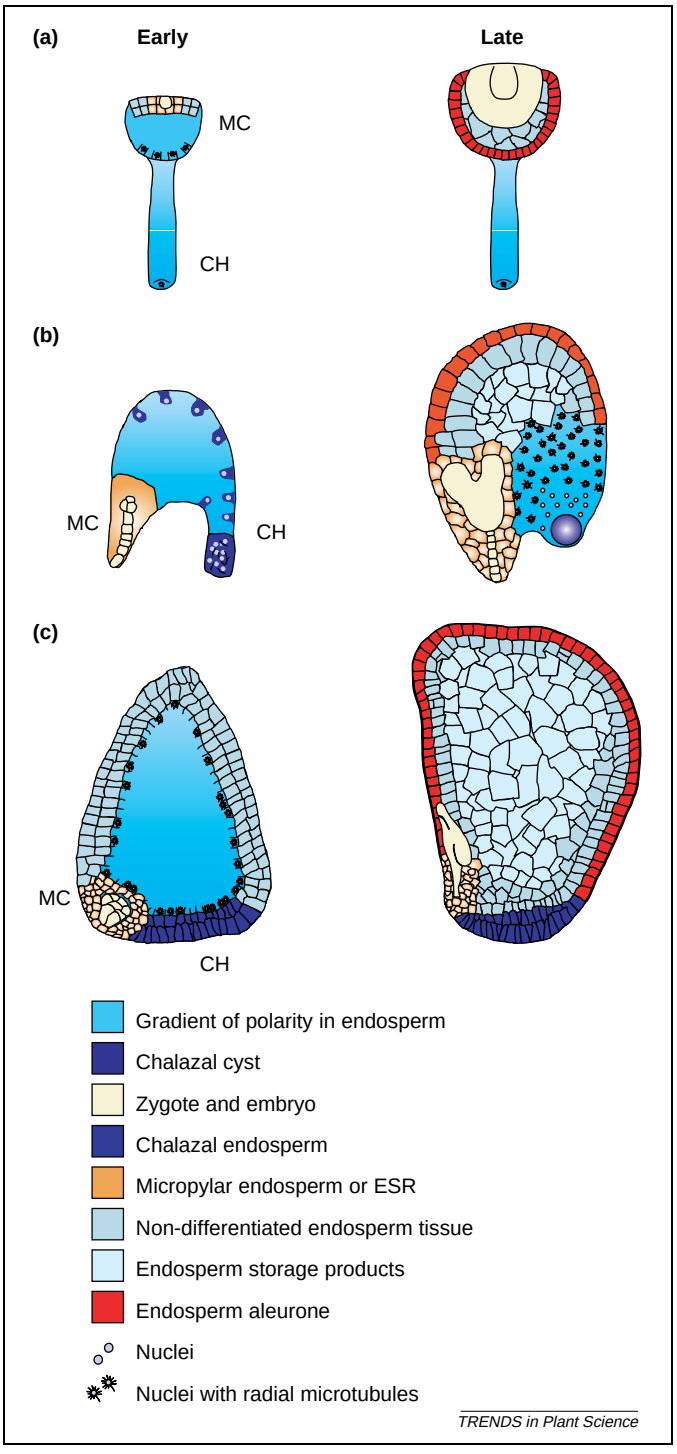

Figure 1. Functionally homologous endosperm domains in basal and higher angiosperms. **(a)** Helobial-type endosperm development in the basal dicot *Cambomba caroliniana*, a member of the Nymphaeales. The micropylar (MC) and chalazal (CH) endosperm domains are established early in development. Following the first division of the pro-endosperm, free-nuclear development of the MC endopserm takes place, with cellularization commencing in the vicinity of the zygote. The CH endoperm remains a uninucleate haustorial tube. Complete cellularization of the MC domain is achieved as the embryo matures. Endosperm cells are large and vacuolated and eventually become replaced by the cotyledons, except for the outer layer, which accumulates lipids [10]. **(b)** Nuclear-type endosperm development in the eudicot *Arabidopsis thaliana*. The MC and CH poles are established early in development, and endosperm cellularization initiates around the embryo in the micropyle. As cellularization proceeds, limited storage products accumulate in the endosperm, which consequently becomes reabsorbed by the embryo, except for the peripheral aleurone layer. **(c)** Nuclear-type endosperm development in the monocot *Zea mays*. The apical and basal pole is established early in development, as is the MC [or embryo-surrounding region (ESR)] and the basal endosperm transfer region, which is functionally homologous to the chalazal endopserm. Centripetal cellularization occurs via the formation of open-ended alveoli until the endosperm cavity is filled. At later stages, the bulk of the maize endosperm synthesizes and accumulates protein and starch. Coincidentally, the outer aleurone layer differentiates and accumulates lipids and proteins in preparation for germination.

endosperm storage tissue and that of the embryo. Thus in monocots, a persistent mass of enlarged cells termed the starchy endosperm (SE) constitutes the bulk of the storage tissue, whereas the majority of the nutrient reserves of dicot seeds are held in the cotyledons – and to a lesser extent in the endosperm, including the surrounding epidermis (Figure 1).

Both persistent (e.g. maize, barley and rice) and transient (e.g. *Arabidopsis*, *V. faba* and *P. sativum*) endosperms possess a defined epidermis or aleurone, which usually consists of a peripheral layer of characteristically small and isodiametric cells with thickened walls (Figure 1) [16,23]. In grasses, and probably in other plant groups, the aleurone contains proteins that repress precocious germination and promote desiccation tolerance. Upon imbibition, hydrolytic enzymes are secreted to mobilize nutrient reserves for seed germination. The persistence of this tissue, in spite of the almost entire reabsorption of the endosperm by the embryo in some dicots, indicates a possible role in seed maturation and germination. Future work should therefore focus on identifying key genes in species other than in the grasses, to establish the importance of the aleurone during seed development.

Endosperm patterning occurs in two main stages

Detailed histological and developmental genetic studies have identified two early events that are conserved in nuclear-, cellular- and helobial-type endosperms (Figure 1). The crucial first phase probably occurs in the central cell and results in the formation of a polarized pro-endosperm. The second phase results in the functional specialization of the maturing endosperm.

The initial phase leads to the establishment of the 'chalazal' and 'micropylar' domains (Figure 1). Importantly, this formative phase seems to be tightly governed by intrinsic maternal morphogenetic factors. To date, several maternally expressed genes have been identified that might be associated with these events. These include a novel maize gene proposed to be involved in basal endosperm patterning [27], and members of the Polycomb-group (Pc-G) complex found in maize and *Arabidopsis* [28–34]. Mutations in any of the Pc-G genes in *Arabidopsis* show a maternal effect and lead to ectopic chalazal endosperm development, which suggests that they are involved in maintaining the early chalazal–micropylar endosperm axis [34,35]. Because the Pc-G complex functions in a context-dependent manner, it is difficult to separate the pre- and post-fertilization effects of disrupting these genes. This dilemma could be overcome by examining the outcome of eliminating or altering Pc-G gene function in the endosperm alone. Alternatively, future screens could be directed at identifying lesions in novel maternal gametophytic genes, which perturb

nuclear migration or polarity in the female gametophyte. This approach would help establish the extent to which maternal gametophytic genes are required for correct endosperm patterning.

There are indications that the first developmental phase continues post-fertilization and into the early syncytium of nuclear endosperms. Observations from reciprocal interploidy crosses in maize and in *Arabidopsis* suggest that polarity is severely perturbed in resulting endosperms, with the 'chalazal' endosperm being affected more than other endosperm domains. Endosperms with an excess of maternal genomes often exhibit an under-developed chalazal domain [30,36,37], whereas those with an excess of paternal genomes display either an over-proliferation of the chalazal pad [37] or displaced 'chalazal'-specific transcripts in the syncytium and cellularized endosperm [30]. In a similar vein, analysis of a monogenic recessive mutation disrupting syncytial endosperm development in maize suggests that chalazal cell fate is terminally determined during a narrow window of syncytial development [38]. These findings are in line with cytological observations in *Arabidopsis* endosperms, which clearly identify a distinct chalazal domain by the 16 nucleate syncytial stage [12,39].

The second phase involves functional specialization in the maturing endosperm. This commonly constitutes formation of the epidermis and the remainder of the endosperm tissue, which eventually give rise to starch and/or protein accumulation (Figure 1). The events associated with this phase have been largely studied in cereals owing to the accessibility of a large persistent endosperm, and appear to involve a range of mechanisms common to other processes in plant development. A series of elegant genetic studies in maize successfully demonstrated that the developmental fates of the aleurone and SE are interchangeable, and hence these tissues are not terminally determined [40]. Moreover, the SE can be considered the 'default' endosperm cell type, a view supported by mutants in which aleurone cells fail to assume their proper fate and instead assume a SE identity [40]. By contrast, peripheral positioning or isolation constitutes an intrinsic part of aleurone development. Furthermore, this process appears to be mediated by a signal transduction cascade involving a leucine-rich repeat (LRR) receptor kinase [41]. Studies in *Arabidopsis* suggest that this LRR receptor kinase is probably localized to the internal plasma membrane of the epidermis where it is able to perceive signals from adjacent neighbouring or underlying cells [42]. Other components of the aleurone developmental pathway have been isolated in maize [43,44] and tobacco [45]. The putative functions of these genes suggest that vesicle trafficking and targeted vacuolar proteolysis are also necessary for aleurone development. Certainly, correct vesicle cycling and trafficking are fast emerging as pivotal for the development of tissue specificity and for a range of cellular processes that occur during normal plant growth, such as cytokinesis, hormone perception and signal transduction [46].

Development of the triploid endosperm is regulated by unique genetic systems

Despite possessing a genetic constitution identical to the embryo, the endosperm follows a different developmental pathway. The diverging fates of both fertilization products can be attributed to parent-of-origin effects resulting from differences in parental gene expression – the majority of which exert a strong maternal bias. These so-called 'effects' are attributed mainly to maternal cytoplasmic factors, DOSAGE-DEPENDENT GENE EXPRESSION and GENOMIC IMPRINTING.

Maternal cytoplasmic factors comprise organellar genes and genes expressed in female gametes, some of which are specific to the egg or central cell [47–49]. To date, genetic studies have demonstrated that several female gametophytic genes are essential for correct embryo and endosperm development [50–52]. Mutations in such genes often result in semi-sterility.

Many plant endosperms are triploid in nature, in that they contain an unequal contribution of two maternal genomes to one paternal genome (2m:1p). The importance of maintaining the correct gene dosage for some parentally inherited alleles has been inferred through studies of reciprocal interspecific [53] and interploidy crosses, which result in abnormal endosperm development (see above). Recently, novel approaches to the molecular isolation of genes that are asymmetrically expressed in the endosperm, including those subject to dosage-dependent regulation, have been attempted in maize [30,54]. These studies identified a large proportion of mainly structural and biochemical genes subject to dosage effects. Dosage-dependent gene regulation has been widely documented in the endosperm for almost a century, yet its molecular basis remains unknown.

Another phenomenon reported to occur in the endosperm is genomic imprinting (Box 1). The range of imprinted genes found in plants to date falls into two categories. The first comprises genes of which a particular allele is expressed according to parental origin in some genetic backgrounds. Genes subject to allele-specific imprinting have only been reported in maize (e.g. the *R1-r: standard* allele of the *r1* gene [55]), and share common features such as belonging to large gene families and expression in the endosperm. Genes in the second category, designated locus-specific imprinting, exhibit a parent-of-origin expression pattern for all alleles in all genetic backgrounds. These genes are commonly expressed only from the maternal allele during early endosperm development (e.g. Pc-G genes) (Box 2). Notably, locus-specific imprinted genes are essential for early stages of seed development. Further, this form of imprinting is conserved in distantly related monocot and dicot plants, which might indicate an ancient form of female gametophytic control over fertilization. If this hypothesis is true, then we would expect to find locus-specific imprinting in all sexually reproducing plants, including those that undergo single fertilization. By contrast, allele-specific imprinted genes have not been ascribed any regulatory or developmental roles. The occurrence of allele-specific imprinting in maize is puzzling, although we speculate that it might have arisen recently owing to

Box 1. How and why does imprinting occur in the endosperm?

Recent data suggest that the central cell differs epigenetically from the egg [77] and that these differences might well be responsible for either the early activation of maternal alleles or the continued silencing of paternal alleles in the endosperm. Precisely why imprinting should occur in the endosperm is open to speculation. One explanation is that imprinting in the endosperm might be a means of extending the haploid status of the female gametophyte into early seed development [78]. Not only would this effectively prevent any deleterious mutations passing from one generation to the next but it would also ensure tight maternal control of seed development.

An alternative, long-standing interpretation was provided by David Haig and Mark Westoby [70] who identified the endosperm as a site where maternal and paternal alleles, because of their differing evolutionary 'interests', are engaged in conflict. In particular, these authors contend that it is in the interest of the maternal sporophyte to distribute resources equally among her several progeny, whereas paternal interests are best served by extracting the maximum amounts of resources for individual offspring. Imprinting could therefore provide the 'medium' through which paternal and maternal alleles would strive to take advantage of one another during early seed development. Perhaps significantly, the imprinted Polycomb-group genes affect early stages of endosperm development, including endosperm patterning (see above) and cell proliferation – both anticipated 'targets' of parental conflict. Other imprinted genes have been implicated in the transfer of nutrients from the maternal tissue [62], and in the synthesis of storage proteins [63]. Additional evidence for parental conflict is the presence of 'endosperm size factors' [79], to date found only in maize, where they occupy defined chromosomal domains. Their paternal inheritance must be essential for correct endosperm development given that seed size is substantially reduced in their absence.

the dynamic nature (e.g. duplications and rearrangements) of the maize genome.

It is not known how many genes are expressed in the endosperm according to parental origin. A recent report has suggested that a total or partial inactivation of the paternal genome occurs in the period immediately following fusion of the sperm with the central cell [56]. The concept of complete paternal silencing remains controversial, not least because paternally inherited transgenes are expressed soon after fertilization [57–59]. Further, it is also unclear if paternally inherited genes are indeed silenced post-fertilization or whether silencing occurs during male gametogenesis. In this connection, it is worth noting that in maize, the maternal genome is largely demethylated in the endosperm when compared with the paternal [60], and more detailed studies have revealed an inverse correlation between DNA hypomethylation of maize sequences and their preferential maternal allelic expression in the endosperm [61–63]. Although potentially significant, this correlation should be regarded with some caution because these studies were performed on hybrid endosperms in which both gene expression and DNA methylation could be modified by extraneous genomic interactions.

More than a yolk: endosperm function in embryogenesis, hybridization and speciation

In the majority of angiosperms, including most apomictic plants, the endosperm is required for successful embryogenesis, particularly during early developmental stages. In many cases, embryo development proceeds more rapidly once the endosperm has enlarged and differentiated. Thus, with reason, the principal function ascribed to the endosperm has been that of nutritive support during embryogenesis and, in the monocots, seedling germination. Other than playing a major role in providing resources for the developing embryo, there is increasing evidence that identifies the endosperm as playing a more fundamental part in reproductive development. One way in which this is achieved is by acting as an intermediary between the embryo and the surrounding maternal sporophytic tissue. Several mutations disrupting communication between the maternal integument and the endosperm have been shown consequently to affect development of the seed or surrounding maternal sporophyte [64–68]. Another way is through the possession of unique genetic systems that render the endosperm 'sensitive' to genomic balance. Thus, although embryogenesis will often commence following crosses between distant relatives or lines of differing ploidy, endosperm development will abort. Abortion of the endosperm has been mainly attributed to alterations in gene dosage and to an imbalance between the maternal and paternal populations of imprinted alleles [37,53,69–71]. This 'genomic equilibrium' is usually fixed, but in some species can be altered to promote the success of interspecific hybrids [53,72]. Significantly, the stringent requirement for correct parental genomic balance in the endosperm can also be overcome through mechanisms by which epigenetic patterns are established, such as DNA methylation [73]. Therefore, if we consider polyploidy and hybridization as major forces in the evolution of flowering plants and stimuli for the appearance of invasive new species [74,75], then epigenetic and dosage-dependent mechanisms operating in the endosperm, which regulate these processes, must have played, and continue to play, a crucial role in angiosperm evolution.

Conspectus

In recent years, a combination of developmental genetics, molecular analyses and evolutionary studies has elevated the flowering plant endosperm from a straightforward, terminally differentiated nurse tissue to an intricate and largely maternally regulated organ that exerts a profound influence on reproductive success. To further unravel the complexities of the endosperm, we must focus on the identification of genes that are necessary for its development. Equally important, attention must be given to identifying more female gametophytic genes that play a crucial role in regulating endosperm development. Success to date has been limited, not least because of the lethality of such mutations leading to poor or no transmission to the next (diploid sporophytic) generation. However, the development of more effective genetic screens, such as enhancer or gene trap lines expressing visible markers and novel mutagenesis strategies [76], should overcome this present constraint.

Another outstanding issue that needs to be tackled concerns the molecular mechanisms involved in establishing and maintaining imprinting in the endosperm. Further efforts should be employed to decipher the specific changes in DNA methylation and chromatin configuration occurring at the parental alleles of imprinted genes.

Box 2. Imprinting mechanism(s) in the *Arabidopsis* endosperm

Imprinting is probably established before fertilization and, although not yet fully understood, the imprinted state of individual genes must be maintained by a range of different mechanisms. To date, four imprinted genes have been well characterized in *Arabidopsis*. The first three comprise the Polycomb-group (Pc-G) *FIS* class of genes, which are required to suppress precocious endosperm development in the absence of fertilization [29,32,33]. The fourth imprinted gene, *FWA*, encodes a homeodomain-containing transcription factor with unknown function [49]. For all these *Arabidopsis* genes, only the maternal allele is active in the endosperm, whereas both parental alleles are expressed in the embryo, except for *FWA*, which is solely expressed in the central cell and endosperm.

A turning point in understanding how imprinting is established in plants came with the discovery of several DNA-modifying enzymes (Figure I). *DEMETER* (*DME*) has recently been shown to activate both maternal *MEA* and *FWA* expression in the central cell [48,49]. Interestingly, DME is similar to REPRESSOR OF SILENCING 1 – a DNA glycosylase able to excise 5-methylcytosines *in vitro* [80]. It is thus plausible that DME acts to demethylate *MEA* and *FWA* in the central cell through a similar mechanism. Evidence for this is provided by the latest finding that the DNA glycosylase domain of DME is necessary for the activation of *MEA* [81]. By contrast, DNA METHYLTRANSFERASE 1 (MET1) is primarily involved in methylation of CpG sites. By using *met1* mutants, it has been shown that DNA methylation regulates *MEA* and *FWA* imprinting [49,82]. Thus, the antagonistic effects of MET1 and DME in the female gametophyte can control imprinting of at least two genes – *FWA* and *MEA*.

Other recent studies point to the existence of several layers of imprinting control. For instance, mutation at the *decrease in DNA methylation* (*ddm1*) locus causes a range of effects including a reduction in global DNA methylation, paternal *MEA* activation in the endosperm, and consequent suppression of the maternal *mea* seed phenotype [83]. Intriguingly, *DDM1* encodes a chromatin-remodelling factor [84], thus suggesting that interplay between chromatin conformation and DNA methylation is able to regulate the expression of some imprinted genes.

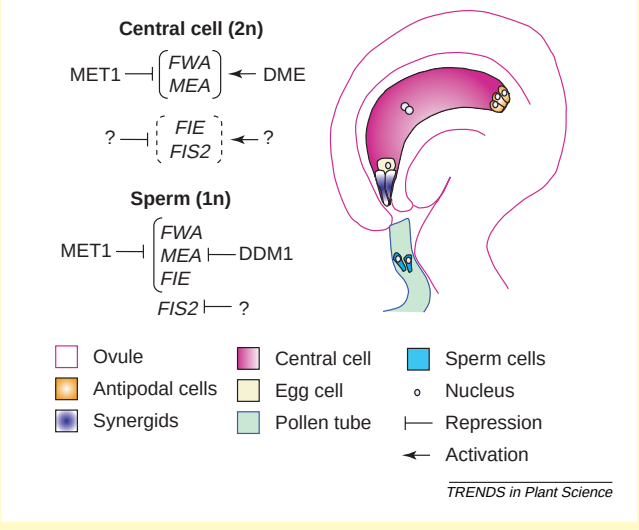

Figure I.

Intriguingly, non-coding RNAs are associated with the imprinted expression pattern of some mammalian genes, and hence future research should be directed at attempting to identify non-coding RNAs that might also regulate imprinting in the plant endosperm.

Finally, from the findings reviewed here, it is clear that much important information has been gleaned from *Arabidopsis* and maize compared to other, less-studied species. However, it is anticipated that comparative studies across the range of angiosperms will hold the key to gaining exciting and rewarding insights into the molecular and evolutionary basis of endosperm development.

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