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Embryo–Endosperm Interactions

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Abstract

In angiosperms, double fertilization triggers the concomitant development of two closely juxtaposed tissues, the embryo and the endosperm. Successful seed development and germination require constant interactions between these tissues, which occur across their common interface. The embryo–endosperm interface is a complex and poorly understood compound apoplast comprising components derived from both tissues, across which nutrients transit to fuel embryo development. Interface properties, which affect molecular diffusion and thus communication, are themselves dynamically regulated by molecular and physical dialogues between the embryo and endosperm. We review the current understanding of embryo–endosperm interactions, with a focus on the structure, properties, and function of their shared interface. Concentrating on *Arabidopsis*, but with reference to other species, we aim to situate recent findings within the broader context of seed physiology, developmental biology, and genetic factors such as parental conflicts over resource allocation.

Contents

1. INTRODUCTION

Gametophyte: gamete-producing generation of the plant life cycle; develops from haploid spores, which are the product of meiosis

The production of seeds, which ensure the protection, nourishment, and dispersal of the main product of sexual reproduction—the embryo—by the maternal tissues, undoubtedly made a major contribution to the remarkable evolutionary success of seed plants, which currently dominate most terrestrial ecosystems. Among the great diversity found in the fossil record, only five lineages of seed plants remain; four are gymnosperm lineages, and the fifth contains the angiosperms. In extant and extinct gymnosperms, and probably in the common ancestor of angiosperms and gymnosperms, the nutritive tissues surrounding the developing embryo are formed directly by the haploid female gametophyte (megagametophyte) (105). Gymnosperm megagametophytes can be

large and may contain several archegonia, each of which harbors a fertilization-competent egg cell, allowing polyembryony in some species.

One of the major innovations that set apart the angiosperm lineage from gymnosperms is the acquisition of fertilization competence in a second cell of the much-reduced female gametophyte. This innovation underlies double fertilization. During this process, the two sperm cells carried within the pollen grain, which are haploid mitotic siblings, fuse with the haploid egg cell and a second fertilization-competent cell (the central cell, usually dihaploid) of the female gametophyte, which develops enveloped within the ovule. These fusion events trigger the concomitant proliferation of two distinct fertilization products, the embryo and the endosperm. These two fertilization products develop enveloped within the integument-derived maternal seed coat (testa) in most angiosperm species (92, 115).

The exact sequence of events that led to the acquisition of double fertilization is a subject of considerable discussion (130). However, its consequence was to tether the proliferation of the major nutritive tissue within the seed—the endosperm—to egg cell fertilization. The evolutionary advantages of this dramatic developmental switch have been extensively discussed elsewhere (5, 59, 129) and are not the subject of this review. However, the introduction of a male genome into the endosperm, a major site of nutrient flux between the mother plant and the developing embryo, is thought to have exacerbated genomic conflicts over resource allocation, which in turn may have contributed to the rapid radiation of the angiosperms (35, 67, 99, 100, 129). Double fertilization has thus changed the nature of seed development, making the angiosperm seed a much more complex structure in which three distinct organisms, with differing genetic compositions, cohabit and develop concomitantly in a limited space. The coordination in the development of these three organisms is crucial for the production of viable seeds and requires a precise and complex dialogue, especially between the two fertilization products. This topic is the focus of this review.

The first step toward an understanding of the embryo–endosperm interaction was the characterization of the developmental events that occur throughout embryo and endosperm development. Multiple highly detailed and beautifully illustrated monographs dating back over more than a century have described the development of both tissues in numerous angiosperm species (4, 77, 109). However, these analyses rely on fixed and sectioned plant materials and provide little dynamic insight into developmental communication. In reality, although indispensable for seed development, the seed coat and associated tissues significantly impede in vivo observation and manipulation of the embryo and endosperm in most species, explaining why most of our current knowledge regarding the molecular regulation of endosperm–embryo communication is derived from studies in the model plant species *Arabidopsis thaliana*. In addition to providing unparalleled genetic resources, *Arabidopsis* produces numerous small seeds with a simple structure and reproducible developmental trajectory. However, it is important to bear in mind that *Arabidopsis* seed development has distinct characteristics that are not necessarily shared with other angiosperm species. The physical and molecular interactions between the embryo and the endosperm, for example, appear to differ profoundly among Brassicaceae (exemplified by *Arabidopsis*), grasses (exemplified by maize), and legumes (such as peas). Furthermore, these model species likely represent only a fraction of the mechanistic diversity in embryo–endosperm interactions across the angiosperms. Therefore, in discussing the mechanistic and functional basis of embryo–endosperm interactions, we concentrate on recent findings in *Arabidopsis* while using them as a basis for a broader reflection on the possible divergence of communication mechanisms.

Understanding the complex interactions between the embryo and the endosperm requires a vision of the evolutionary origin of extant processes that is difficult to obtain. These interactions result from a long evolutionary history that integrated different parameters, such as environment pressure, parental conflicts, or, in agronomically important species, the human selection of specific

Megagametophyte: gametophyte that develops from a female spore (megaspore) in heterosporous plants; in angiosperms, can be described as the embryo sac

Archegonium: gametophytic structure that bears egg cells in basal land plants, including some gymnosperms

Ovule: structure that forms the seed after fertilization; composed of integuments, nucellus, megagametophyte, and funiculus (stalk) at maturity

Embryo: in angiosperms, the organism generated by fusion of the egg cell and a sperm cell

Endosperm: in angiosperms, the transient tissue generated by fertilization of the megagametophyte central cell by a sperm cell

Integuments: in angiosperms, ovule-derived organs that envelop the nucellus and megagametophyte and give rise to the seed coat after fertilization

attributes (such as size or content of specific metabolites). It is within this complex evolutionary context, and in light of recent and novel findings, that this review aims to discuss the nature and consequences of embryo–endosperm communication.

Plasmodesmata: membrane-lined transparietal channels that link the cytoplasm, plasma membranes, and endoplasmic reticulum of neighboring plant cells

Symplastic communication: communication between two plant cells via cytoplasmic connections provided by plasmodesmata

Apoplastic communication: communication between two plant cells through the diffusion of chemical cues across the extracellular space

Symplastic isolation: lack of symplastic communication between two cells due to the absence, removal, or occlusion of plasmodesmata

2. DOUBLE FERTILIZATION: CHANGING COMMUNICATION PATHWAYS

2.1. The Egg Cell and Central Cell Before Fertilization: Two Connected Gametes

In *Arabidopsis*, the embryo and the endosperm are derived from the fertilization of the egg cell and the dihaploid central cell, respectively. Both the egg cell and the central cell originate from the cellularization of the multinucleate female gametophyte coenocyte. From its inception, the egg cell shares a common interface with the central cell at its chalazal pole. In some species, hooks and ingrowths increase the size of this interface, suggesting the need for extensive apoplastic exchanges (73). In numerous angiosperm species, the cell wall between the egg cell and the central cell has been described as discontinuous, giving rise to large areas in which the plasma membranes of the egg and central cell are apparently directly juxtaposed, potentially facilitating gamete fusion (73, 117, 133). In *Arabidopsis*, however, the cell wall between the egg and central cell is continuous but of irregular thickness and with deep furrows (157). The apoplastic composition of this interface remains a mystery. However, it is perforated by plasmodesmata that connect the egg and central cell cytoplasms (39, 80, 142). This symplastic connection has been experimentally demonstrated by the injection of molecular tracers into the central cell of the embryo sac–apparent species *Torenia fournieri* Lind., and more recently in *Arabidopsis* (49, 69, 148). Although the diffusion limits of these connections are variable, they allow, for instance, the movement of small RNAs produced by the central cell, which maintain the repression of egg cell transposable elements by promoting methylation (49, 74).

On the basis of these findings, it seems probable that the egg and the central cell can communicate through both symplastic and apoplastic channels. Symplastic communication is essential for the maturation of both cells and is a prerequisite for normal development of the endosperm and the embryo (163, 170). Assessing the requirement for apoplastic communication at this stage of development is more complex, due to both the presence of symplastic communication and a lack of informative mutants.

2.2. Postfertilization Symplastic Isolation of the Embryo and Endosperm

Egg cell fertilization is associated with a rapid change at the embryo–endosperm interface. In vitro studies of isolated maize egg cells showed a rapid extrusion of material within 30 s of fertilization. This material forms a continuous wall within the following 20 min (97). Callose was found in the newly formed cell wall of the zygote in several Ericaceae species (168), suggesting that the rapidly exocytosed material could be callose. Rapid extrusion of material may prevent the fusion of more than one sperm cell with the egg cell but also isolates the newly formed sporophyte from the surrounding endosperm and the degenerating synergids.

Studies in both *Arabidopsis* and *T. fournieri* Lind. embryo sacs showed that symplastic movement of molecules between the endosperm and the embryo is strongly reduced or even absent after fertilization (**Figure 1**), suggesting a restriction or rupture in symplastic communication (49, 69). This symplastic isolation requires either removal or blocking of the preexisting plasmodesmata. In *Arabidopsis*, plasmodesmata between the embryo and the endosperm disappear after fertilization (111). Later in development, the embryo appears to remain symplastically isolated from the

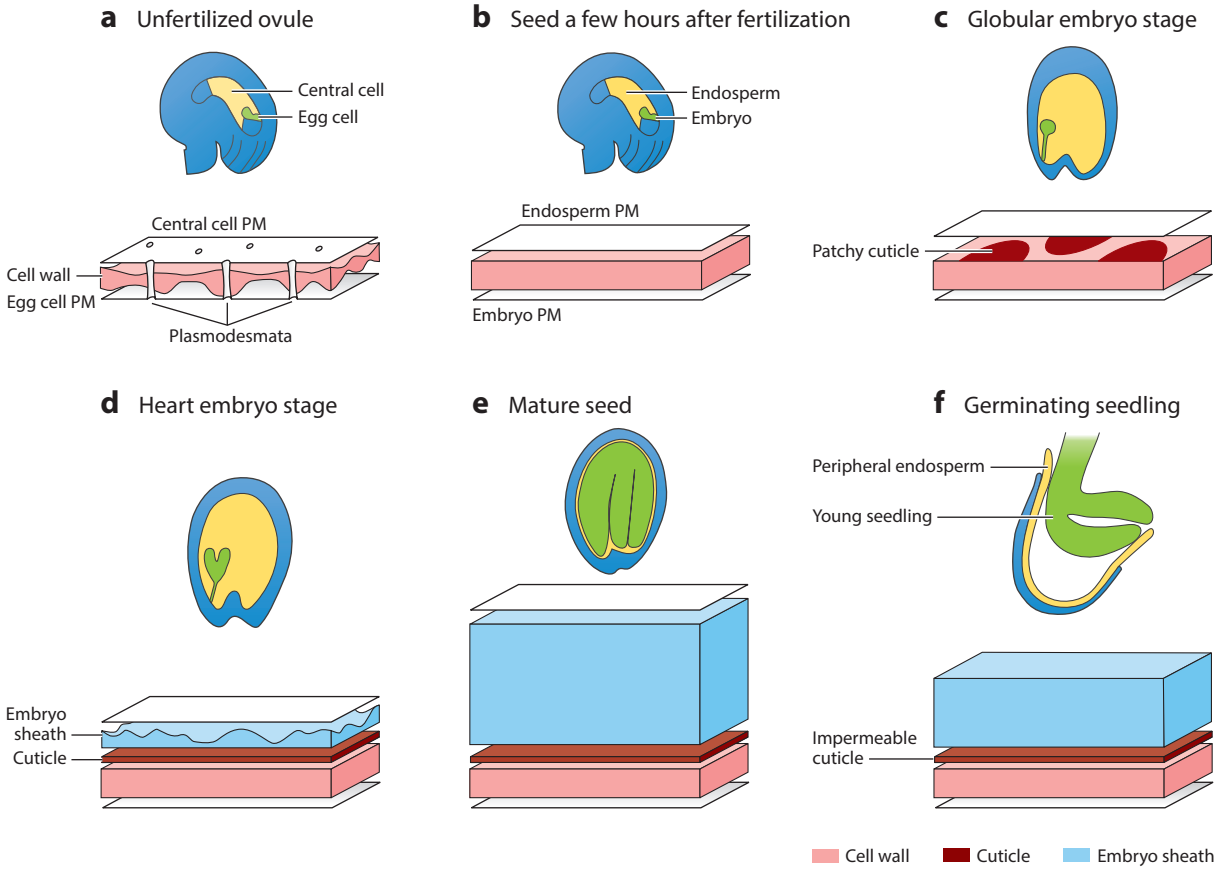

Figure 1

Structure of the embryo–endosperm apoplast at different stages during seed development in *Arabidopsis*. (*a*) In an unfertilized ovule, the central cell and the egg cell are separated by an irregular cell wall perforated by plasmodesmata. (*b*) After double fertilization, a rapid secretion of cell wall material thickens the cell wall between the embryo and the endosperm (*pink*). (*c*) From the early globular stage onward, the embryonic cuticle (*red*) is secreted by the embryo, first in a patchy pattern and then (*d*) in a continuous thin layer. Material produced by the endosperm cells is secreted at the embryo–endosperm interface and accumulates on the outer face of the embryo cuticle. (*e*) This material eventually forms the embryo sheath (*light blue*). (*f*) After germination, a continuous layer of embryo sheath remains on the cotyledon and hypocotyl surfaces, while the cuticle is remodeled to form the impermeable seedling cuticle. Abbreviation: PM, plasma membrane.

endosperm, as demonstrated by the absence of cytoplasmic green fluorescent protein diffusion toward the endosperm (147). Consistent with this finding, the literature contains no reports of the presence of plasmodesmata at the embryonic surface in *Arabidopsis*. Together, these observations suggest that, in these species at least, the embryo and endosperm become symplastically isolated soon after fertilization.

The presence of symplastic barriers between the embryo and the endosperm is variable. Intriguingly, in some Fabaceae species and several Crassulaceae species, the presence of plasmodesmata at the embryo–endosperm interface has been reported after fertilization. However, they are restricted to the basal cell of the two-celled embryo and, later, to the suspensor (46, 82, 93, 94, 137). Microinjection of tracers into the basal suspensor cell of *Sedum acre* (Crassulaceae) revealed unidirectional symplastic movement from the basal suspensor cell toward the endosperm. Unlike

Suspensor: transient embryonic tissue that connects the embryo to the maternal tissues and is involved in embryo nutrition

Embryo proper: portion of the young angiosperm embryo that generates most of the tissues of the mature embryo/seedling

the situation in *Arabidopsis*, movement from the embryo proper toward the basal suspensor cell was not detected, indicating that the suspensor may be symplastically isolated in species where symplastic connections are maintained between the endosperm and suspensor (169). This idea is further supported by the results of elegant feeding experiments in *Phaseolus coccineus* and *Phaseolus vulgaris*, which show that although sucrose movement from the endosperm to the suspensor (which in these species could be either apoplastic or symplastic, due to the presence of plasmodesmata) is passive, movement from the suspensor into the embryo proper requires energy, suggesting that this movement occurs against an endogenous concentration gradient and is likely apoplastic in nature (179).

In summary, double fertilization strongly influences how the egg cell/embryo and central cell/endosperm can communicate. In some species, symplastic communication between the embryo and the endosperm is maintained in the suspensor, but in these species, symplastic communication between the embryo proper and the suspensor may be blocked or strongly reduced. No evidence of postfertilization symplastic communication [either direct or indirect (via the suspensor)] between the embryo proper and the endosperm appears to exist, suggesting that apoplastic communication likely plays a major role in embryo development.

3. APOPLASTIC MOVEMENT BETWEEN THE EMBRYO AND ENDOSPERM

3.1. Molecules Involved in Embryo–Endosperm Communication

The tight coordination between embryo and endosperm development implies an extensive dialogue throughout seed development. Although deciphering the whole picture will likely remain a challenge for decades, some pieces of the puzzle have already been discovered. For example, signaling peptides have been found to diffuse across the embryo–endosperm apoplast to mediate communication between these tissues during seed development. These signaling peptides include the EMBRYO SURROUNDING FACTOR (ESF) peptides (**Table 1**) secreted first by the central cell and then by the endosperm after fertilization, which promote suspensor elongation via the activation of *WOX8* expression (30). Another example involves the TWISTED SEED 1 (TWS1) peptide (44, 55), which is discussed in more detail in the following section.

Hormonal communication is also thought to occur between the two tissues. Research has focused mainly on auxin, a key factor necessary for the patterning of early embryo development in *Arabidopsis* (60). Although the apical–basal polarization of the early embryo depends on a basal (suspensor)–apical (embryo proper) auxin flux, the origin of the auxin involved in this early patterning event is enigmatic. A recent study provided evidence that maternal tissues (the integuments) produce auxin that can be imported across the interface between the basal cell of the embryo and the integuments (131). Auxin also plays a key a role in the initiation of endosperm development by stimulating nuclear division (6, 52), and the export of endosperm-derived auxin coordinates the postfertilization development of the endosperm with that of surrounding maternal tissues (the integuments) (52, 53). If, as proposed, auxin biosynthesis in the integuments is necessary for the establishment of embryo polarity (131), it follows that endosperm-derived auxin that is produced postfertilization, which in turn stimulates auxin production in the seed coat (51), could be implicated at least indirectly in early embryo patterning. In support of this idea, *Arabidopsis* plants lacking the auxin biosynthetic enzymes YUCCA 1 (YUC1), YUC4, YUC10, and YUC11 show embryo defects, whereas the *yuc1 yuc4* double mutant shows no abnormal phenotype. In light of the fact that *YUC10* and *YUC11* are expressed specifically in the endosperm, Figueiredo & Köhler (53) proposed that the embryo phenotype is caused by a lack of auxin production in the endosperm. However, whether auxin—and other hormones—diffuses directly through the

Table 1 Proteins referred to in this review

Protein	Identifier	Type	Reference(s)
AAD1/5 (ACYL-ACYL CARRIER PROTEIN DESATURASE 1/5)	AT5G16240/ AT3G02630	Δ9 stearoyl-ACP desaturase	88
ACR4 (ARABIDOPSIS CRINKLY 4)	AT3G59420	Receptor-like kinase	120, 135, 165
AGL62 (AGAMOUS-LIKE 62)	AT5G60440	Type I MADS domain transcription factor	71, 84
ALE1 (ABNORMAL LEAF-SHAPE 1)	AT1G62340	Subtilisin-like serine protease	44, 153
ALE2 (ABNORMAL LEAF-SHAPE 2)	AT2G20300	Receptor-like kinase	154
ATML1 (MERISTEM LAYER 1)	AT4G21750	Homeodomain protein	120, 135
(At)CAT6 (CATIONIC AMINO ACID TRANSPORTER 6)	AT5G04770	Cationic amino acid transporter	68
CIF1/2 (CASPARIAN STRIP INTEGRITY FACTOR 1/2)	AT2G16385/ AT4G34600	Precursor of a sulfated CIF-like peptide	40, 123
(At)DEK1 (DEFECTIVE KERNEL 1)	AT1G55350	Plant-specific CALPAIN protease (phytocalpain)	110
ESF1.1/2/3 (EMBRYO SURROUNDING FACTOR 1.1/2/3)	AT1G10747/ AT1G10745/ AT1G10717	MEG family cysteine-rich peptide precursor	30
(At)EXP2 (EXPANSIN A2)	AT5G05290	EXPANSIN (cell wall protein)	23, 136, 175
FAB2 (FATTY ACID BIOSYNTHESIS 2)	AT2G43710	Stearoyl-ACP desaturase	88
GSO1 (GASSHO1/ SCHENGEN 3)/GSO2	AT4G20140/ AT5G44700	LRR kinase	44, 159, 171
IKU1 (HAIKU1)	AT2G35230	VQ motif–containing protein	164
IKU2 (HAIKU2)	AT3G19700	LRR receptor–like kinase	107
HDG11/12 (HOMEODOMAIN GLABROUS 11/12)	AT1G73360/ AT1G17920	Homeodomain protein	160
ICE1 (INDUCER OF CBF EXPRESSION 1	AT3G26744	MYC-like bHLH transcription factor	28, 38, 83
KRS (KERBEROS)	AT1G50650	STIG1 family cysteine-rich peptide precursor	41, 119
KOD (KISS OF DEATH)	Unknown	Small cytoplasmic peptide	14
MINI3 (MINISEED 3)	AT1G55600	WRKY DNA-binding protein	107
PDF2 (PROTODERMAL FACTOR 2)	AT4G04890	Homeodomain protein	135
RGL2 (RGA-LIKE 2)	AT3G03450	GRAS family transcription factor	104
AtSUC5 (SUCROSE-PROTON SYMPORTER 5)	AT1G71890	Sucrose-proton symporter	7
SSP (SHORT SUSPENSOR) (AT2G17090)	AT2G17090	RLCK II family kinase	8
SWEET11/15	AT3G48740/ AT5G13170	Sucrose efflux transporter proteins	26
TPST (TYROSYLPROTEIN SULFOTRANSFERASE)	AT1G08030	Tyrosylprotein sulfotransferase	34, 40, 44
TWS1 (TWISTED SEED 1) (AT5G01075)	AT5G01075	Precursor of a sulfated CIF-like peptide	44, 55
UMAMIT25/28 (USUALLY MULTIPLE ACIDS MOVE IN AND OUT TRANSPORTERS 25/28)	AT1G09380/ AT1G01070	Nodulin MtN21-like transporter family protein	121
WRI1 (WRINKLED 1) (AT3G54320)	AT3G54320	AP2/EREBP transcription factor	88, 158

(*Continued*)

Table 1 (*Continued*)

Protein	Identifier	Type	Reference(s)
YUC1/4/10/11 (YUCCA 1/4/10/11)	AT4G32540/ AT5G11320/ AT1G48910/ AT1G21430	Flavin monooxygenase	6, 52, 131
(Os)YUC11 (YUCCA11)	Os12g08780	Flavin monooxygenase	173
(Zm)YSL2 (YELLOW STRIPE LIKE2)	Zm00001eb 248990	Metal–NA transporter family protein	181
ZOU/RGE1 (ZHOUPI/RETARDED GROWTH OF EMBRYO 1)	AT1G49770	bHLH family transcription factor	91, 177
(Zm)ZOU (ZHOUPI/Opaque11)	Zm00001eb 082900	bHLH family transcription factor	50, 64

Abbreviations: bHLH, basic helix-loop-helix; LRR, leucine-rich repeat; MEG, maternally expressed gene; NA, nicotianamine.

embryo–endosperm apoplast in *Arabidopsis* is still debated, and the exact contribution of the endosperm auxin to the embryo development remains to be clarified.

In maize, the situation is slightly different. Auxin responses, measured by the activity of the DR5 reporter, are absent from the embryo until the transition stage (~7 days after pollination). At this point, DR5 signals appear at the top of the embryo. A strong DR5 signal in the surrounding endosperm suggests that auxin may be exported directly from the endosperm to the embryo (25), where its redistribution may be involved in the establishment of the apical–basal axis (42, 56, 57). Furthermore, an endosperm auxin maximum has been proposed to orient embryonic growth (25). Functional studies of appropriate auxin-defective mutants are needed to test these hypotheses.

3.2. Two Routes for Nutrients to Reach the Embryo: The Trans-Endosperm and the Suspensor Pathways

Throughout its development, the embryo requires nutrients such as carbohydrates, amino acids, and ions to support its development. In addition, many angiosperm species store significant quantities of nutrients in the embryo that can be remobilized and used during seedling establishment. Although the embryos of some species appear to be capable of limited photosynthetic activity (2, 15, 132, 144), the vast majority of nutrients used and stored by embryos are imported from maternal tissues, making seeds major energy and nutrient sinks (1, 155).

Once nutrients are released into the integument apoplast, the exact pathway they take to reach the embryo is unclear, but two main possibilities exist. One possible route involves direct maternal–embryo transport at the apoplastic interface that links the base of the suspensor to the maternal apoplast. Suspensors vary dramatically in size among species, and although the suspensor–maternal interface is limited to one cell in *Arabidopsis*, giant bulbous suspensors found in some legumes or members of the Crassulaceae permeate maternal tissues in an almost parasite-like manner (93, 109, 180). In both giant and small suspensors, a transcriptomic analysis revealed enrichment in the expression of genes associated with transport and carbohydrate metabolic processes, highlighting a universal role as a nutrient highway (27). Although significant quantities of nutrients are likely to be directly absorbed from maternal tissues by the embryo in species with semipersistent giant suspensors (95), in most species, including *Arabidopsis* and maize, the suspensor path is not sufficient to fully supply the embryo, especially during later stages of development, after

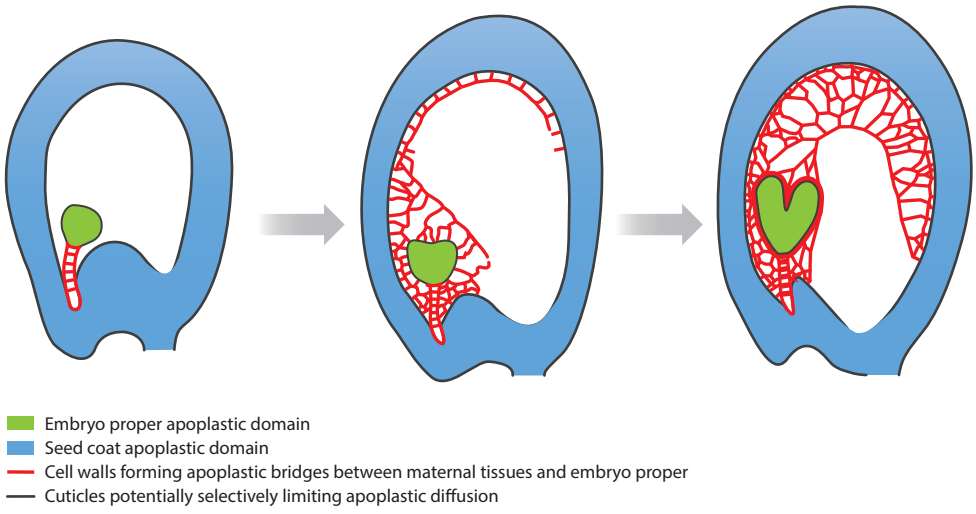

Figure 2

Endosperm cellularization generates apoplastic bridges connecting the embryo proper to the seed coat apoplastic domains in *Arabidopsis*. In the young seed, before endosperm cellularization, the only apoplastic interface between the maternal tissues and the embryo proper is the suspensor. Endosperm cellularization increases the number of apoplastic junctions connecting the maternal tissues with the embryo proper. From the heart stage onward, the embryo–endosperm apoplastic space is further modified by endosperm deposits forming the nascent embryo sheath. Cuticles that could potentially selectively limit the movement of some molecules are depicted in black.

suspensor degeneration, and when the demands of the embryo are greatest. Therefore, nutrient transfer between the maternal tissues and the embryo necessarily involves exchanges across the embryo proper–endosperm interface.

The second pathway involves trans-endosperm transport, implying that sugars are taken up by the endosperm at the interface between the endosperm and the integuments, diffused across the endosperm, and subsequently exported from the endosperm and taken up by the embryo (118). The endosperms of the vast majority of angiosperms go through an initial phase of mitotic nuclear divisions in the absence of cytokinesis, leading to the formation of a multinucleate coenocyte (124). It seems likely that, during this stage, nutrients are imported into the endosperm cytoplasm from maternal tissues and then reexported to the embryo surface by transmembrane transporters (**Figure 2**). This hypothesis is supported by the observation that the sucrose exporter AtSUC5, expressed in the coenocytic endosperm, is necessary for early embryo expansion (7). In addition, the fact that mutants with reduced suspensor length tend to show a delay in early embryo development (3) could suggest that the surface area of the early embryo that is in contact with the coenocytic endosperm determines nutrient uptake capacity, at least in *Arabidopsis*. Subsequent cellularization of the endosperm dramatically changes the nature of trans-endosperm molecular movement by creating apoplastic bridges between maternal tissues and the embryo that bypass the endosperm cytoplasm. This topic is discussed further in Section 4.

Deciphering the exact pathways taken by sugars, amino acids, and other nutrients into the early embryo will necessitate detailed studies of transporter localizations and activities within the endosperm suspensor and embryo. Such studies represent a significant technical challenge due to the internal position of the tissues of interest.

Embryonic cuticle: lipid-rich structure secreted by the embryonic epidermis into its external apoplast; acts as a selective apoplastic barrier during seed development

4. CONTROL OF MOLECULAR DIFFUSION ACROSS THE EMBRYO–ENDOSPERM INTERFACE

4.1. Genesis of the Embryonic Cuticle

During early embryonic development, the outermost cell layer of the embryo proper differentiates into a protoderm, which subsequently gives rise to the epidermis (81). Plant epidermal cells are highly polar and form a continuous cell layer that can be developmentally modified to optimize interactions between the plant and the surrounding environment. This is also true of the embryonic epidermis, which covers the embryo proper and is modified during embryogenesis to support embryo development and viability.

A major characteristic of epidermal cells is their ability to secrete unesterified hydroxy fatty acids, which become polymerized on the outer surface of the epidermal cell wall, together with other molecules, in order to form a continuous film called the cuticle (78, 178). The secretion of cuticular components and their transport through the epidermal cell wall are highly polar, and because epidermal cells propagate through anticlinal cell divisions, once the cuticle has been established, it is continuously "inherited," forming a scaffold that can be extended and reinforced as tissues expand. However, during early embryogenesis, no cuticle exists on the surface of either the suspensor or the embryo proper, and the embryonic cuticle must form de novo (31, 37). In *Arabidopsis*, osmophilic patches start to become apparent at the embryo proper–endosperm interface at the globular stage of embryogenesis (**Figures 1** and **3**). These patches may be a consequence of the secretion of cutinosomes (i.e., vesicles containing cuticle components), which have been reported to traverse the outer epidermal cell wall of the embryo before depositing their contents (150). These patches rapidly coalesce and form a continuous cuticle by the late globular/early heart stage of embryogenesis (31, 151) (**Figures 1** and **3**). Genes encoding key enzymes known to be involved in cuticle biosynthesis are expressed in the embryo epidermal cells (31). However, how cuticle components condense into a polymerized and continuous film at the embryo–endosperm interface remains a mystery. Although the suspensor is devoid of cuticle, a recent study (9) showed that the root tip is also surrounded by a thin cuticle layer, albeit with properties different from those of the layer covering the rest of the embryo. This finding indicates that, after the suspensor degenerates at the torpedo stage, the whole embryo is encapsulated by a continuous cuticular membrane (9, 87).

Over the past few decades, analyses of mutants involved in embryonic cuticle formation have allowed the identification of many genes involved in embryonic cuticle establishment. These genes have been extensively studied in *Arabidopsis* and can be classified into three categories (**Figure 3**). The first class consists of genes implicated in epidermis specification, such as those encoding the ATML1 and PDF2 transcription factors ARABIDOPSIS CRINKLY 4 and ABNORMAL LEAF-SHAPE 2 receptor-like kinases (RLKs) and the CALPAIN DEK1 (110, 135, 154, 165). Because cuticle production is a unique characteristic of epidermal cells, changes in the specification of this cell type necessarily alter cuticle formation.

The second class of genes encodes proteins involved in the biosynthesis of the embryonic cuticle and expressed in the epidermis (likely under the direct or indirect control of proteins involved in epidermal specification). Despite the extensive literature on postembryonic cuticle biosynthesis (reviewed in 178) and the fact that many genes known to be involved in the biosynthesis of functional cuticles postembryogenesis are expressed in the embryo epidermis from the globular stage onward (31), few single mutants have shown convincing embryonic phenotypes. However, a recent study (88) determined that FAB2, AAD5, and AAD1, three enzymes involved in the desaturation of fatty acids, are involved in the production of a functional embryonic cuticle (**Figure 3**).

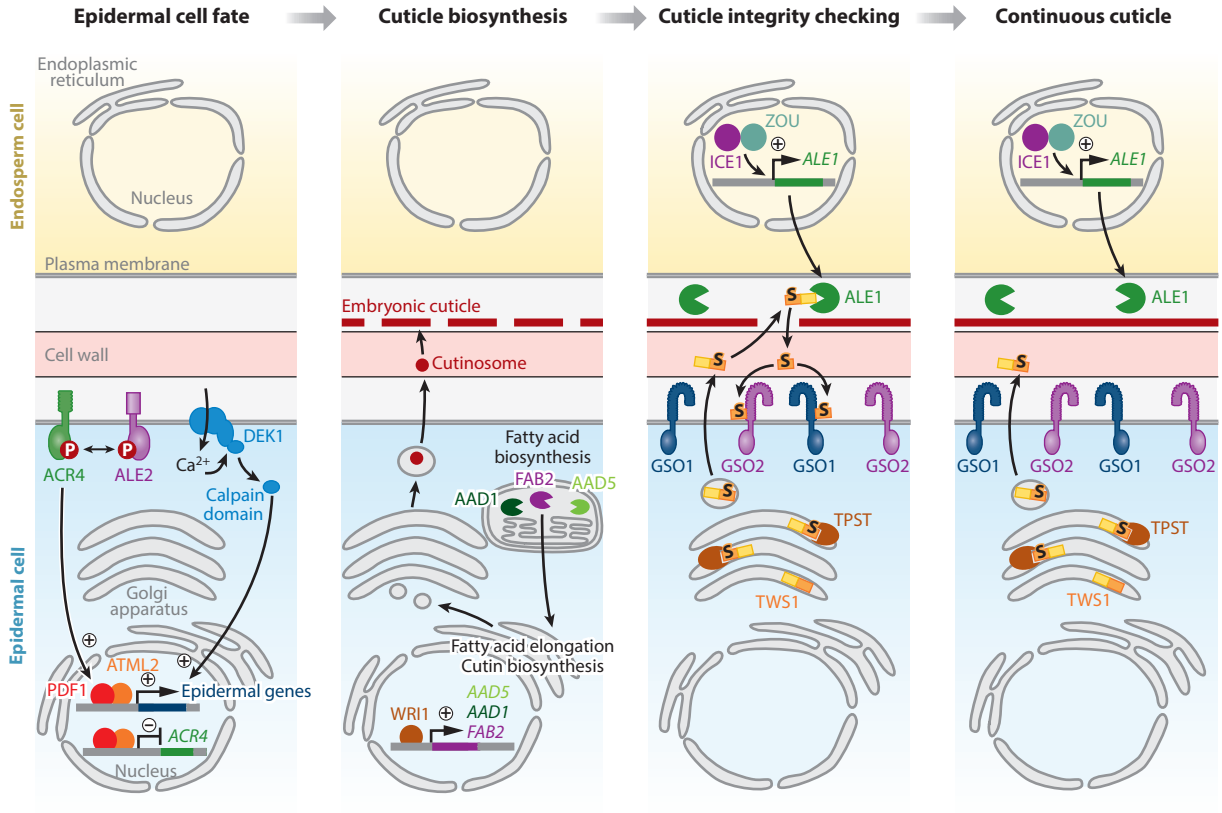

Figure 3

Steps required for formation of the embryonic cuticle in *Arabidopsis*. The main molecular actors and their corresponding pathways are shown for each step. First, the outermost cell layer of the embryo proper differentiates into an epidermis. Then, the epidermal cells start to produce cuticle components that are secreted into the apoplast to form a discontinuous, patchy cuticle. A bidirectional exchange of signals between the embryo and the endosperm enables the detection of the gaps in this structure and mediates their closure, thus ensuring cuticle integrity. After cuticle gap closure is complete, the pathway is shut down unless new gaps appear during embryo expansion. Abbreviations: P, phosphorylation; S, tyrosine sulfation.

The third class of genes is involved in the control of embryonic cuticle integrity. Loss of function of these genes does not directly affect either epidermis specification or cuticle biosynthesis. However, in mutants, the embryonic cuticle remains discontinuous and uncondensed even at late developmental stages (31, 135), suggesting a loss of the ability to perceive and repair cuticle gaps. A recent study has shown that these genes are involved in the bidirectional embryo–endosperm communication required to achieve cuticle integrity monitoring (44). In the endosperm, a subtilase (serine protease) called ABNORMAL LEAF-SHAPE 1 (ALE1) is produced around the embryo. *ALE1* expression depends on the activity of an endosperm-specific basic helix-loop-helix transcription factor complex comprising ZHOUPI (ZOU) and ICE1 (38, 153, 171, 177). In the embryo, the two redundant leucine-rich repeat (LRR) RLKs GASSHO1/SCHENGEN 3 (GSO1) and GSO2 are produced in the embryonic epidermis, where they localize in a nonpolar fashion in the epidermal cell membrane (55, 159). The final pathway component, TWS1 (55), is the precursor of a sulfated peptide. Inactive sulfated proTWS1 peptides are secreted by the embryo into its apoplast. If the embryonic cuticle is discontinuous, proTWS1 can escape to the endosperm,

where it is activated by proteolytic removal of a C-terminal inhibitory domain by ALE1. The active peptides then diffuse back into the embryo, bind the GSO1 and GSO2 receptors on the embryonic surface, and trigger a cellular response leading to cuticle gap filling. Once the cuticle is continuous, ALE1 and proTWS1 are physically separated and signaling stops. New gaps appearing during embryonic growth can be repaired by this pathway, as pathway components remain expressed until embryo growth arrests (44) (**Figure 3**).

The establishment of a functional embryonic cuticle is crucial for the mature embryo, as cuticle integrity prevents catastrophic water loss from the seedling upon germination. However, the cuticle integrity pathway described above is active at the globular embryo stage in *Arabidopsis*. Why does the embryo need to establish a functional embryonic cuticle so early in seed development? And what are the consequences in terms of embryo–endosperm interactions?

Embryonic axis: in cereals, contains the shoot and root apical meristems, the mesocotyl, the embryonic leaves, the coleoptile, and the coleorhiza

Scutellum: abaxial embryonic organ found in cereals and involved in nutrient uptake from the endosperm, particularly during germination

4.2. The Embryonic Cuticle: Filter or Barrier?

That the embryo cuticle restricts the apoplastic movement of peptides is demonstrated by the very mechanism that leads to integrity establishment. Interestingly, a potential function in restricting peptide movement was proposed on the basis of the synergistic genetic interactions of mutants affected in cuticle integrity, as well as mutants with compromised epidermal identity, which led to the suggestion that the presence of cuticle defects could cause inappropriate leakage of developmentally important ligands from the embryo (120, 135). Although the identity of the ligands involved remains an enigma, the cuticle could ensure the stability of embryonic patterning and/or cell differentiation.

However, even if early cuticle formation is developmentally important, it seems possible that it could impede other processes, including nutrient uptake from the surrounding endosperm, which might explain why the suspensor is not covered in cuticular material. However, in *Arabidopsis*, the suspensor degenerates long before nutrient uptake and storage in the embryonic tissues is complete, or even maximal. Additionally, the small suspensor would rapidly become insufficient to explain the observed nutrient flow into the embryo. In *Arabidopsis*, analysis of embryonic cuticle permeability to toluidine blue, a low-molecular-weight hydrophilic dye, revealed that although embryonic surface permeability decreases throughout embryogenesis in wild-type plants, it remains relatively high in comparison to that of postgermination organs, with the dye penetrating in a matter of minutes even in fully expanded embryos (9, 31, 34). Furthermore, although the root pole of the embryo is covered by a structurally intact cuticle, it is considerably more permeable to toluidine blue than the cuticle present on other areas of the embryo (9, 31). Given these observations, it appears unlikely that the embryonic cuticle would represent a significant barrier to metabolites such as sugars and amino acids, whose molecular weight is roughly comparable to that of toluidine blue. Although nutrient influx might be higher at the root pole, this represents a very small surface area, and the expression of transporters throughout the embryonic epidermis suggests that nutrients can transit over the entire embryonic surface, at least in *Arabidopsis* (167). Thus, the embryonic cuticle of *Arabidopsis* probably acts more as a filter than as a hermetic barrier.

Although an intact cuticle covering the entire embryonic surface is necessary for the survival of epigeal plants upon germination, the properties and even the existence of this apoplastic filter can vary dramatically between species and even between organs. In cereals, for instance, part of the embryonic axis (mesocotyl and coleoptile) is surrounded by a thick, impermeable cuticle (45, 64, 98), while the scutellum is highly permeable despite reports of the presence of a cuticle-like structure on the scutellum surface (29, 161). This difference could be related to the function of the scutellum surface, which facilitates endosperm nutrient uptake both during embryogenesis and during germination and never emerges from the seed coat or becomes a photosynthetic

organ. Similarly, in *Vicia faba* and in pea, in which cotyledons actively take up sugars and amino acids during seed development (156, 167) but, again, do not emerge from the seed coat during germination, the cotyledon epidermis differentiates transfer cell–like properties but does not produce a clearly defined cuticular layer (16–18).

5. ENDOSPERM CELLULARIZATION: THE EMBRYO TAKES THE UPPER HAND

Endosperm cellularization is a poorly understood process that involves partitioning of the nuclei, cytoplasm, and vacuole of the endosperm coenocyte into individual uninucleate cells. Early endosperm development involves nuclear divisions in the absence of cytokinesis, leading to the formation of a single polynucleate coenocyte with a large central vacuole (21, 124, 146). In *Arabidopsis*, cellularization generally initiates when the embryo is at the early heart stage of development, around the time when rapid embryo growth and early reserve accumulation initiate. Cellularization has been proposed to play a critical role in embryo development, because in *Arabidopsis* mutants in which the endosperm fails to cellularize the embryo generally arrests at the heart stage, leading to seed abortion (71). In addition, regulation of the initiation of endosperm cellularization appears to be a key target of parental imprinting, being retarded by the paternal genome and promoted by the maternal genome, supporting the idea that cellularization, at least in *Arabidopsis*, could significantly affect resource allocation to the developing embryo (49, 63, 96, 99, 100, 138).

5.1. Endosperm Cellularization Around the Embryo: Bridging the Gap

In many species, including *Arabidopsis*, endosperm cellularization initiates in the micropylar region, in which a densely cytoplasmic endosperm fully envelops the developing embryo. An elegant study by Otegui & Staehelin (126) suggests that a process occurs involving the progressive genesis of syncytial-type cell plates that form progressively from the midplane between sister and nonsister nuclei. Such plates can then fuse with the endosperm and embryo cell wall, effectively forming direct links between these two apoplastic spaces. This observation is consistent with research in other species, suggesting that cell walls directly link the endosperm and embryo surface, even in species where the endosperm is considered to be noncellularized, such as pea (112). Whether these links reflect the presence of individual cells, or simply localized cell wall connections, remains to be resolved through the application of three-dimensional imaging techniques. Nonetheless, these connections may play important roles in ensuring embryo nutrition.

Two mechanisms are generally evoked. The first is that apoplastic connections anchor the embryo in a zone of the endosperm where nutrient transfer from maternal tissues (presumably via the endosperm) is optimized, for example, close to areas of maternal export (proposed in some legumes, where the maternal cell wall differentiates transfer cell–like characteristics) (112). The second proposed mechanism is that, by forming physical bridges directly linking the maternal apoplast to the embryonic apoplast, endosperm cellularization effectively extends the direct apoplastic interface between the maternal and embryonic apoplast, permitting the embryo to bypass the endosperm and absorb nutrients directly from maternal tissues (71) (**Figure 2**).

5.2. Regulation of Endosperm Cellularization

Endosperm cellularization, as described above, is promoted by the maternal genome and retarded by the male genome. FERTILIZATION INDEPENDENT SEED POLYCOMB REPRESSIVE COMPLEX 2 (FIS PRC2) acts in the female gametophyte to repress seed development in the absence of fertilization and subsequently acts maternally in the endosperm to promote cell cycle arrest and cellularization (52, 66, 71, 89, 90, 106). As a result, in seeds arising from female

gametophytes lacking a functional FIS PRC2, the endosperm fails to cellularize, and the embryo arrests at the heart stage of development. One of the key targets of PRC2, with respect to the regulation of endosperm cellularization, is the MADS-box transcription factor AGAMOUS-LIKE 62 (AGL62), which represses endosperm cellularization (84). Loss of AGL62 function leads to seed lethality associated with extremely early endosperm cellularization, which is proposed to be an indirect consequence of seed coat expansion failure (51). Loss of function of the maternally encoded FIS PRC2 leads to a convincing partial rescue of the *agl62* endosperm cellularization defect and restores seed viability (71).

Another key factor regulating endosperm cellularization is the plant hormone auxin. Interploidy crosses with an excess of paternal genomes, which show cellularization defects similar to those observed in seeds lacking maternal FIS PRC2, show increased and prolonged auxin production in the endosperm (6). Furthermore, increasing auxin production in the endosperm leads to delayed cellularization, while during normal seed development cellularization occurs only once auxin levels in the endosperm have dropped. Auxin appears to act either parallel to or downstream of proteins such as AGL62, itself a regulator of auxin transport (51), to regulate cellularization (6). A recent study in rice (173) has shown not only that *OsYUC11* is necessary for endosperm development but also that, as is the case for the *Arabidopsis YUC10* gene (52, 72), *OsYUC11* is expressed predominantly from the male genome during early seed development (173).

Finally, although endosperm cellularization has been proposed to require mitosis (21, 146), it also appears to be linked with a cessation of nuclear proliferation. While endosperms lacking maternal FIS PRC2, like those with a paternal genome excess, undergo excess nuclear proliferation (65) and fail to cellularize, mutants with reduced proliferation, such as those defective in the poorly understood signaling pathway involving the proteins HAIKU1 (IKU1), IKU2, and MINISEED3/WRKY10, cellularize earlier than wild-type seeds (62, 107, 164).

6. INVADING THE ENDOSPERM

6.1. Embryo Invasion of the Endosperm and Cell Elimination

In most angiosperm species, the embryo grows invasively into the surrounding endosperm. The endosperm is remodeled and progressively eliminated to allow the embryo to expand (113). In *Arabidopsis*, most of the endosperm is removed by a lytic process. At maturity, only the peripheral endosperm layer remains intact and alive. This lytic cell death is suppressed in both *ice1* and *zou* mutants, which harbor a persistent endosperm in addition to their cuticle phenotype (91, 177). ZOU is specifically expressed in the endosperm, while its partner, ICE1, is extensively expressed both within and outside the seed (28, 38, 83, 102). A constitutively active ICE1 protein enhances endosperm degradation (38, 58), but in contrast to other cell death–regulating transcription factors, neither ICE1 nor ZOU can directly trigger programmed cell death in the tissues where they are overexpressed (28, 32, 61, 83, 177). This observation suggests that they act upstream of cell execution "setting the scene." In *zou* mutants, cell wall thinning and cell separation observed in the endosperm of wild-type seeds do not occur. Consistent with this finding, many cell wall remodelers that are usually expressed in the endosperm are not expressed in *zou* mutants (58, 166). Thus, ZOU may regulate endosperm cell wall softening, which in turn allows invasive embryo growth, a hypothesis supported by biophysical analysis of *zou* mutant seeds (58). In mutants where embryo growth arrests very early, endosperm cells do eventually die but the cell content is not cleared (58, 172).

In plant species with a so-called persistent or semipersistent endosperm, embryo invasion remains limited to a subdomain of the endosperm. In cereals, most of the endosperm persists to maturity. However, as in *Arabidopsis*, only the peripheral layer, called the aleurone, remains alive

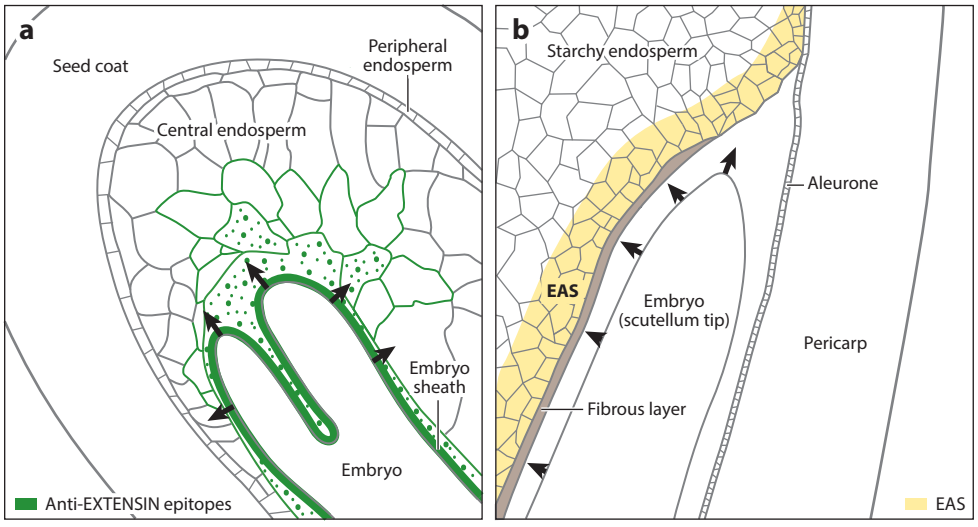

Figure 4

Embryo invasion of the endosperm in (*a*) *Arabidopsis* and (*b*) maize. (*a*) In *Arabidopsis*, embryo invasion leads to almost complete removal of the central endosperm cells. Prior to embryo invasion, components rich in epitopes specifically detected by anti-EXTENSIN antibodies are produced specifically by endosperm cells surrounding the embryo and are deposited at the embryo–endosperm interface to form the embryo sheath, which subsequently mediates the physical separation of the embryo and endosperm. (*b*) In maize, embryo invasion crushes endosperm cells, leaving undigested wall that accumulates in a fibrous layer. In a limited number of EAS layers, the embryo partially regulates the expression of a specific transcriptomic network. Arrows indicate the direction of embryo expansion. Abbreviation: EAS, endosperm adjacent to the scutellum.

(134). The central starchy endosperm undergoes programmed cell death that preserves the cell corpses, but the contribution of the embryo to this process appears minimal. However, around the embryo, two developmentally and cytologically distinct zones of endosperm cell elimination can be distinguished. The first is the embryo-surrounding region (ESR), a small patch of densely cytoplasmic cells surrounding the young embryo that is proposed to have both nutrient transfer and developmental roles (42, 64, 125). The embryo rapidly outgrows this zone and invades the bulk endosperm above. The ESR is eliminated at around 14 days after pollination in wild-type plants, and ESR elimination correlates with that of the embryonic suspensor, suggesting that the two processes may be linked (64). The elimination of the ESR depends on the activity of the maize ZHOUPI ortholog ZmZOU (also known as Opaque11) (50, 64). Once the embryo has emerged from the ESR, cell death can be observed around the expanding scutellum, in a zone called the endosperm adjacent to the scutellum (EAS) (**Figure 4**). Interestingly this process does not involve the complete removal of cell corpses, as observed in the ESR, and cell wall remnants persist at the embryo–endosperm interface (43, 64, 139). ZmZOU/Opaque11 does not appear to be involved in either EAS elimination or, indeed, starchy endosperm programmed cell death. The extent to which the elimination of the ESR or of the EAS depends upon embryo growth is not clear.

6.2. Endosperm Elimination and the Formation and Function of the Embryo–Endosperm Interface

Why is the endosperm in the maize EAS not fully degraded? One possibility relates to the fact that, unlike the situation in *Arabidopsis*, where cotyledon cuticle integrity is a requirement for

Apoplastic barrier: extracellular structure in plants that limits molecular diffusion

Embryo sheath: endosperm-derived, glycoprotein-rich structure deposited on the *Arabidopsis* embryo surface during seed development

seedling survival upon germination, in maize the scutellum remains enclosed within the seed upon germination. As a result, the maize scutellum, like the cotyledons of hypogeal dicotyledonous species such as pea, does not suffer from the antagonistic requirements of forming an apoplastic barrier while acting as a major conduit for nutrient exchange. Indeed, the presence of functional apoplastic barriers of the surface of these structures remains unproven. Nonetheless, the lack of such a barrier could also render the developing embryo susceptible to attack from lytic enzymes involved in endosperm elimination, possibly explaining the lack of complete cell wall breakdown in the EAS of the maize endosperm.

Surprisingly, the idea that endosperm breakdown is required to release nutrients to the developing embryo or, indeed, to facilitate nutrient transport from maternal tissues to the developing embryo during development, although widely assumed, has not been rigorously tested or demonstrated. However, the expression of membrane-localized transporters in the endosperm surrounding the developing embryo suggests that this is the case (26, 43, 121). For instance, the sugar exporters SWEET11 and -15, which are required for normal seed filling in *Arabidopsis*, are expressed in the ESR of *Arabidopsis* endosperm (7, 26). The route taken by other nutrients toward the embryo is still poorly understood in *Arabidopsis* but may resemble that of sugars. In *Arabidopsis*, AtCAT6, UMAMIT25, and UMAMIT28 are expressed in the late endosperm, but their early expression and exact contribution to embryo filling remain to be clarified (10, 68, 121). In maize, both amino acid transporters and SWEETs are strongly expressed in the EAS (43). Other endosperm-expressed transporters may transfer various other nutrients toward the embryo, such as biotin (via AtSUC5) in *Arabidopsis* (128) and iron (via ZmYSL2) in maize (181).

6.3. Endosperm-Derived Materials Help Build the Embryo Sheath in *Arabidopsis*

As described above, in *Arabidopsis* the bulk endosperm is completely degraded. Nonetheless, some materials derived from the endosperm accumulate around the embryo and contribute to its surface structure and function. Notably, the embryo sheath is progressively built up outside the embryonic cuticle. The embryo sheath has an organized and homogeneous structure with a regular thickness, suggesting that its formation involves active assembly on the embryo surface (**Figure 1**). The embryo sheath is strongly and specifically recognized by anti-EXTENSIN antibodies and has thus been proposed to include a three-dimensional EXTENSIN-based scaffold (41, 119). EXTENSINs can self-assemble in vitro through isodityrosine cross-linking (22, 101). EXTENSIN epitopes are first detectable immunologically at the mid to late heart stage of embryogenesis, when endosperm breakdown initiates (119). They initially localize within vesicles in the endosperm cells surrounding the embryo and are secreted to the apoplast before accumulating at the embryo surface. The secretion of sheath materials may precede endosperm degeneration, with progressive glycosylation subsequently rendering them detectable by specific antibodies. However, the lack of sheath production in *zou* mutants supports a link between endosperm degradation and embryo sheath formation (119). At maturity, the embryo sheath forms a thick envelope around the embryo and is very tightly associated with the embryo surface (**Figures 1** and **4**).

6.4. Function of the Embryo Sheath

Identification of the *Kerberos* (*krs*) mutant, which is defective in embryo sheath formation, has led to a deeper understanding of the function of this structure in *Arabidopsis*. KRS is a cysteine-rich peptide produced in the endosperm surrounding the embryo from the heart stage onward. Expression of *KRS* depends entirely on ZOU function (119). However, unlike *zou* mutants, *krs* mutants show no defects in endosperm elimination. Furthermore, genetic analyses suggest that *KRS* acts in a pathway that is parallel to the cuticle integrity pathway. In *krs* mutants, the sheath (as detected by

anti-EXTENSIN antibodies) is completely absent, and physical separation between the growing embryo and the endosperm does not occur (119). Abnormal embryo–endosperm adhesion during development leads to misorientation of embryo growth in some seeds. After germination, seed coat shedding from the cotyledons (**Figure 1**) is also impaired (41). The observed adhesion between the embryo and endosperm, both before and during germination, suggests that the embryo sheath acts as an antiadhesive structure. Consistent with this idea, the adhesion of germinating cotyledon surfaces to silica beads, as measured by atomic force microscopy, is considerably stronger in *krs* mutants than in the wild type (41).

Our understanding of the mechanism of sheath formation, biochemical composition, and deposition remains rudimentary. However, although the formation of EXTENSIN epitopes in the embryo-surrounding endosperm of *Arabidopsis* occurs normally in mutants lacking cuticle integrity pathway components, the deposition of a structured embryo sheath on the embryo surface depends entirely on this pathway and is lost both in *ale1* and in *gso1 gso2* double mutants (119). This finding suggests that an embryo-derived factor, acting downstream of GSO signaling, could be implicated in sheath deposition on the embryonic surface, adding further complexity to the dialogue between the embryo and the endosperm necessary for embryonic surface formation.

Does the sheath exist in other species? The presence of a hyaline layer around the embryos of Brassicaceae species suggests that this structure is conserved throughout this family (162). In pea, an embryo sheath around the developing embryo has been described (112), although its origins remain unclear. In more distant species, such as the black nightshade (*Solanum nigrum*), material from limited endosperm breakdown in the so-called zone of separation and secretion has been reported to form a thick, fibrous structure around the embryo. This structure could have a function similar to that of the *Arabidopsis* embryo sheath in separating the embryo and endosperm (19, 20). Finally, in castor bean, where endosperm cells die on the cotyledon surface after germination, a slimy layer of crushed cells has been proposed to facilitate the separation of the two tissues (141).

In summary, the invasion of the endosperm by the embryo is often accompanied by interface modifications involving components of the dying endosperm, which may participate in the physical separation of the two structures. How these modifications affect embryo–endosperm communication remains to be investigated. However, the physical detachment of the two tissues could be important for extending the apoplastic space between the embryo and the endosperm, facilitating nutrient circulation and uptake. At present, very little is known regarding the diffusion properties of these interfaces, and this role remains speculative at best.

7. THE FINAL ACT: EMBRYO–ENDOSPERM COMMUNICATION AND GERMINATION

Embryo–endosperm communication does not end at seed maturity. In many angiosperm seeds, part of the endosperm remains alive during seed quiescence and plays critical roles in germination. Recent studies suggest that these roles involve complex physical and metabolic interactions with the embryo as well as hormone- and peptide-mediated communication.

7.1. Role of the Endosperm in Controlling Seed Dormancy

The endosperm is critical in maintaining seed dormancy. This topic has been extensively reviewed elsewhere, including in this volume (79), so we provide only a relatively brief overview.

Seed dormancy is a complex concept and covers a range of phenomena affecting the ability of a seed to germinate despite favorable environmental conditions (24, 54). *Arabidopsis* seeds show a shallow primary seed dormancy that is established during seed development and progressively lost postshedding, increasing the potential of seeds to respond to environmental conditions favorable

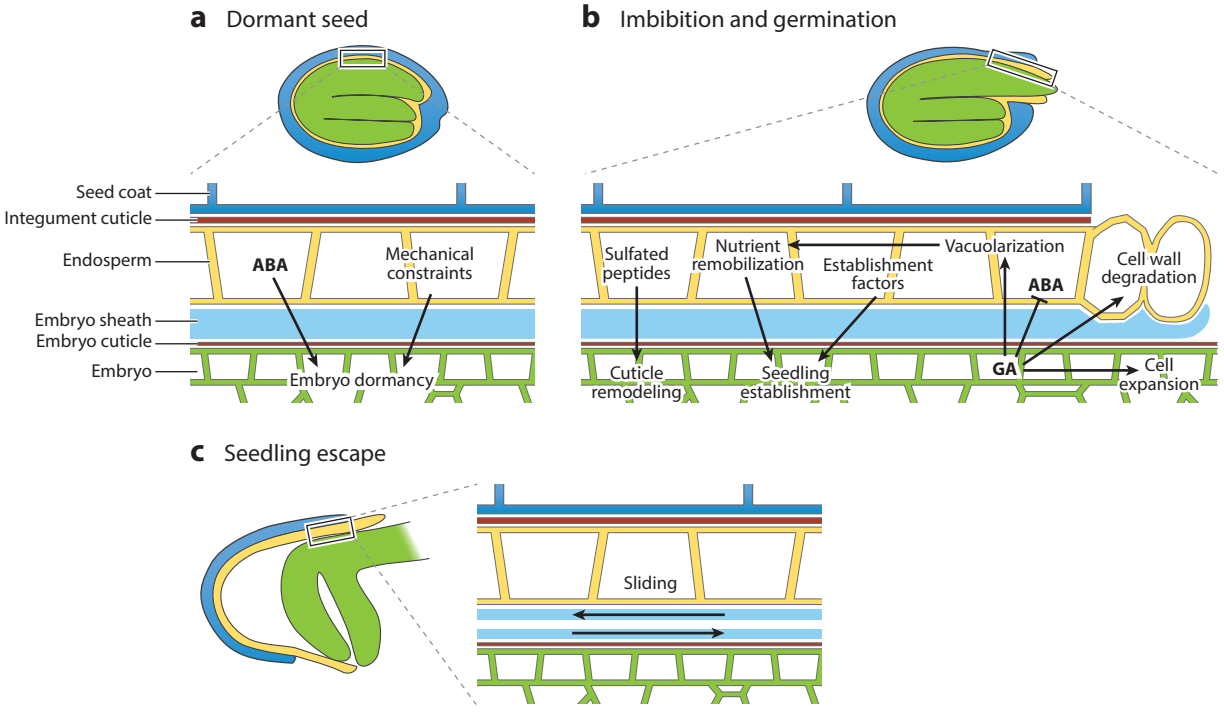

Figure 5

Embryo–endosperm dialogue during germination and seedling establishment in *Arabidopsis*. (*a*) In dormant seeds, both mechanical constraints and chemical cues (especially ABA) from the endosperm maintain embryo dormancy. (*b*) During imbibition and germination, the embryo triggers peripheral endosperm vacuolization and thus remobilization of nutrients in a GA-dependent fashion. Together with other, unknown endosperm-derived factors, this process promotes seedling establishment. Sulfated peptides from the endosperm regulate cuticle remodeling, increasing cuticle impermeability. At the root tip, the embryo triggers GA-dependent endosperm cell wall remodeling, thus allowing the root tip to emerge. (*c*) Finally, during seedling escape, the endosperm-derived embryo sheath, through its antiadhesive properties, facilitates seedling emergence. Abbreviations: ABA, abscisic acid; GA, gibberellic acid.

for germination. It has been described as the culmination of the seed maturation process. The importance of the endosperm in maintaining seed dormancy in *Arabidopsis* has been demonstrated through both genetic studies and dissection studies in which the embryo and endosperm are separated and then physically reunited (i.e., bedding assays) (reviewed in 24, 54). The fact that the endosperm-specific ZOU transcription factor is critical in determining the depth of primary dormancy reinforces this view (108). Interestingly, in legumes, many of which do not contain a persistent endosperm layer, dormancy is physical rather than physiological and is mediated by the impermeable seed coat (13, 54).

Bedding assays are a powerful tool with which to dissect tissue communication during germination and have led to a convincing model in which endosperm-derived abscisic acid (ABA), produced at high levels in dormant imbibed seeds, mediates the maintenance of embryo dormancy (34, 104) (**Figure 5**). Transporters potentially involved in the export of ABA from the endosperm and its import into the embryo have been identified (85). Endosperm-mediated ABA production is stimulated by the DELLA protein RGL2. In nondormant seeds, RGL2 is degraded via a gibberellic acid (GA)– and embryo-dependent process, suggesting retrograde communication. Dormancy has been proposed to prevent GA-dependent RGL2 degradation through a lack of production of GA signaling components (11, 104).

Although several potential GA transporters are expressed during germination and affect seed germination (33, 86, 152), conclusive evidence of an embryonic origin of the GAs perceived in the endosperm during *Arabidopsis* seed germination remains sparse, as GA biosynthesis can also occur in the endosperm (122, 127, 174). Other embryo-derived signals, including mechanical signals (see Section 7.2, below), could also be involved in coordinating germination (114, 136). In cereals, a role for embryo-derived GAs in controlling endosperm behavior, including amylase secretion by the aleurone and subsequent cell death during seed germination, has also been proposed (12). Consistent with this hypothesis, both amylase production and cell death progress from the embryo-containing zone to more distant zones of the endosperm. In *Ricinus*, where endosperm remains on the cotyledons after germination, cell death initiates proximally to the embryo before expanding outward (141). Again, this finding suggests that the embryo promotes endosperm cell death, although the signal involved is unclear.

In *Arabidopsis*, the proposed movement of hormones between the endosperm and the embryo raises an important question: How, if the embryo already has an intact cuticle, do these hormones traverse the embryo–endosperm interface? As discussed above, the cuticle of the mature embryo, although physically intact, remains relatively permeable to hydrophilic molecules, which may partly explain this exchange (9, 31). In addition, a recent study showed that the changes in embryo cuticle structure and composition that render the cuticle less permeable occur during the early stages of germination. These changes depend on the presence of the endosperm and are mediated by the production of sulfated peptides, including TWS1-related CIF1 and CIF2 (potentially perceived by the GSO1 and GSO2 receptors) as well as PSY1 (potentially perceived by PSY1 RECEPTOR). This impermeabilization is proposed to render the embryo less sensitive to endosperm-derived ABA, thus decreasing endosperm-imposed germination inhibition. Consistent with this idea, germinating wild-type embryos rapidly lose their ABA sensitivity, whereas this sensitivity is retained in embryos defective in cuticle biosynthesis. Note, however, that *tws1* mutants closely resemble *gso1 gso2* double mutants (44), suggesting that the proposed GSO1/GSO2-dependent roles of CIF1 and CIF2 during germination are not sufficient to rescue strong defects in embryonic cuticle development generated prior to seed maturation. However, this recent research clearly shows that peptide-mediated communication between the endosperm and the embryo could play an important role in maintaining the integrity of the embryonic cuticle during the germination of wild-type seeds.

7.2. Mechanical Communication Between the Embryo and the Endosperm During Germination

In addition to peptide and hormonal cross talk, mechanical interactions between the embryo and endosperm have long been proposed to play important roles in the regulation of seed germination. In several species, endosperm softening and cell expansion, culminating in endosperm rupture, allow the growth of the embryonic root during the germination process. The mechanical properties of the micropylar endosperm are a key target for regulation. Specific cell wall modifications, mediated either enzymatically or through the production of reactive oxygen species, occur specifically in this region (47, 48, 103, 149, 182). Furthermore, genotypes in which the composition of the endosperm is altered due to the loss of function of specific enzymes (such as alterations in xyloglucan metabolism, pectin methylesterification status, or mannan breakdown or loss of expression of parietal proteins such as EXPANSINs) affect germination (75, 76, 140, 143, 145, 175).

Do changes in hormone production (such as GA production) and the expression of cell wall–modifying enzymes in the micropylar endosperm depend on the growth of the embryo? Several studies have suggested that this might be the case (23, 36, 76, 114). A detailed transcriptomic

analysis of different seed compartments during *Arabidopsis* germination showed that, in support of the idea that a mechanical cue induced by expansion of the embryo axis could be perceived in the endosperm, touch-induced genes are strongly upregulated in the endosperm prior to radical emergence (36). An analysis of the expression of cell wall–modifying genes, such as that encoding the EXPANSIN AtEXP2, supports the hypothesis that embryo expansion is crucial in triggering GA-dependent gene expression in the endosperm, which in turn modifies the physical properties of this tissue (136).

7.3. Importance of the Endosperm in Seedling Establishment

In addition to regulating seed germination, the endosperm promotes seedling establishment postgermination (**Figure 5**). This role may in part reflect the importance of the endosperm as a nutrient storage compartment from which nutrients are remobilized upon germination to fuel seedling growth until full autotrophy is established. Even seeds in which the endosperm is almost entirely degraded, such as in *Arabidopsis*, store nutrients in residual endosperm that are remobilized and taken up by the embryo in the form of sugars and amino acids upon germination (116, 127, 158). As discussed above, nutrient mobilization in the endosperm requires hormonal and potentially mechanical dialogues between the embryo and endosperm, which have been extensively reviewed elsewhere (e.g., 23, 176) and are not discussed further here due to space constraints. Interestingly, however, recent studies have highlighted other roles for the endosperm in ensuring seedling establishment. Very recent research (34) has shown that the endosperm is critical in ensuring a successful embryo–seedling transition, including both normal photomorphogenic and skotomorphogenic development, and that, in the absence of endosperm-derived cues, both primary root development and seedling chloroplast development are seriously compromised. These defects appear not to be caused by a lack of endosperm-derived nutrients, suggesting that other signals are involved. Interestingly, similar defects in seedling establishment have been reported in the germinating seeds of mutants unable to produce or perceive GA, suggesting that, again, the endosperm-derived signal could be GA dependent (70).

Defects in seedling establishment have also been observed in mutants lacking the endosperm-derived embryo sheath in *Arabidopsis*. Loss of the sheath impedes cotyledon escape due to adhesion between the two tissues (41). Seedling establishment defects, including a failure to establish autotrophic growth, correlate with cotyledon trapping within the seed coat and, unlike the phenotypes associated with the removal of the endosperm prior to germination, can be alleviated by the addition of sucrose to the culture medium during germination. These findings highlight the importance of timely cotyledon release from the surrounding endosperm cells in the establishment of young seedlings.

SUMMARY POINTS

1. The communication pathways between the embryo and endosperm change dynamically as seeds develop and differ between different angiosperm species, with particularly variable roles for the suspensor. The early symplastic isolation of the embryo proper from the endosperm seems to be a universal feature.
2. The embryo–endosperm interface is the site of energy import into the embryo. Embryo-derived signals are important for resource release from the endosperm both postgermination and potentially during development.

3. The presence of an intact apoplastic barrier (cuticle) on the surface of the embryos of epigeal species requires a peptide-mediated dialogue between the embryo and endosperm. This barrier plays protective roles both during seed development and at germination but could also restrict both communication and nutrient absorption.
4. Cellularization of the endosperm appears to play a critical role in ensuring the nutrition of the developing embryo. Even in species with an endosperm that remains essentially uncellularized, bridges formed by endosperm-derived cell walls connect the embryo to the maternal apoplast.
5. The invasive growth of the embryo into the endosperm, both during development and during germination, is associated with cell death in this tissue and further remodeling of the embryo–endosperm interface, which may facilitate nutrient uptake.
6. The significance of embryo–endosperm interactions during seed development highlights the importance of distinguishing these two very distinct tissues when trying to understand fundamental aspects of seed biology.

FUTURE ISSUES

1. What are the precise composition and structure of the apoplastic interface between the embryo and the endosperm, and what are the molecular mechanisms underlying its modification during seed development and germination?
2. Peptides and hormones are known to be involved in embryo–endosperm communication. Are other chemical signals also implicated?
3. Can the biophysical properties of the apoplastic interfaces between the embryo and the endosperm at different stages of seed development, particularly with regard to the diffusion of nutrients and signals, be accurately measured?
4. How are forces exerted by the embryo on the endosperm perceived and interpreted?
5. To what extent are molecular mechanisms identified in *Arabidopsis* and other model species conserved in crops, and do they represent targets for long-term crop improvement strategies?

DISCLOSURE STATEMENT

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