Nuclear Endosperm Development in Cereals and *Arabidopsis thaliana*

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INTRODUCTION

The nuclear endosperm of monocots, including the cereal species maize, rice, barley, and wheat, represents humankind's most important renewable source of food, feed, and industrial raw materials. In addition, the endosperm is an attractive system for developmental biology studies. Similar to the embryo, the endosperm is the result of a fertilization process and therefore may be considered an organism in its own right. Endosperm development has been the subject of several recent reviews (DeManson, 1997; Becraft et al., 2000; Becraft, 2001; Olsen, 2001; Brown et al., 2002; Berger, 2003). This review emphasizes the main developmental aspects of nuclear endosperm development in cereals and *Arabidopsis thaliana*, including evolutionary origin, coenocyte development, endosperm cellularization, cell fate specification, and differentiation.

After fertilization in what is the most common type of endosperm development, the nuclear type, the initial endosperm nucleus divides repeatedly without cell wall formation, resulting in a characteristic coenocyte-stage endosperm. Cellularization occurs via the formation of radial microtubule systems (RMS) and alveolation. Many dicot species, including commercially important crop plants such as soybean, cotton, and Arabidopsis, also have a nuclear endosperm, although the endosperm is consumed in part by the embryo during seed maturation. During its development, striking similarities exist between the endosperm in these groups, particularly with respect to the cellularization phase. Insight into the genetic specification of the different processes in endosperm development (e.g., coenocyte formation, alveolation, cell fate specification, and differentiation) may ultimately reveal how developmental subprograms are integrated into a master program specifying the entire endosperm body plan. This knowledge will not only benefit basic science but also strengthen efforts to improve cereal grain quality.

THE MEGAGAMETOPHYTE AND THE CENTRAL CELL OF CEREALS AND ARABIDOPSIS

The nuclear endosperm of angiosperms, including the monocot cereals and the dicot Arabidopsis, develops from the central cell of the megagametophyte after the process of double fertilization

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(Figure 1), resulting in a diploid embryo and a triploid endosperm (for definition of terms used throughout the text, see Table 1). There are two main reasons why megagametophyte development is of importance in understanding endosperm development. First is the likelihood of developmental cues being established that affect the path of endosperm development. Second, the debate on the evolutionary origin of the nuclear endosperm itself is based on the evolution of the megagametophyte. The *Polygonum* type of megagametophyte found in cereals and Arabidopsis results from a differentiation process in which the nucleus of the surviving megaspore first undergoes one mitotic division without cell wall deposition between sister nuclei, followed by nuclear migration to opposite ends of the megaspore. Two additional rounds of mitoses without cell wall deposition result in two groups of four cells near each pole of the developing megagametophyte (Figures 1A and 1B). One nucleus from each pole (the polar nuclei) migrates to the center of the embryo sac, while cell walls are deposited around the remaining three nuclei at each pole in a process that is believed to involve RMS (see below) (Russel, 1993) (Figure 1C). This process results in a seven-cell, eight-nuclei megagametophyte or embryo sac in which the central cell receives two nuclei that may either remain separate until fertilization, as in maize (Figure 1D), or fuse before fertilization, as in Arabidopsis (Figure 1E) (Webb and Gunning, 1990; Mansfield et al., 1991; Schneitz et al., 1995; Christensen et al., 1997; Drews et al., 1998). The central cell, which develops into the endosperm after fertilization, fills the majority of the volume of the embryo sac and consists of a proximal mass of cytoplasm and a thin line of cytoplasm surrounding a large central vacuole (Figure 2A).

Not surprisingly, genetic evidence shows that mutants affecting maternal ovule tissues also affect endosperm development. A priori, these maternal effects are expected to be of two types, gametophytic (effects expressed in the megagametophyte itself) and sporophytic (effects expressed in maternal plant tissues) (for an overview and references, see Drews et al., 1998; Grossniklaus and Schneitz, 1998; Chaudhury and Berger, 2001). A special group of Arabidopsis female-gametophytic maternal genes that affect endosperm (and embryo) development that have received considerable attention in recent years include *Mea/Fis1* (*Medea/Fertilization-independent seed1*), *Fis/Fis2*, and *Fie* (*Fertilization-independent endosperm*)/*Fis3* (Ohad et al., 1996; Chaudhury et al., 1997; Drews et al., 1998; Grossniklaus and Schneitz, 1998; Grossniklaus et al., 1998; Chaudhury and Berger, 2001). The products of these genes are similar to Polycomb group proteins, which regulate the expression of genes through epigenetic silencing in *Drosophila*
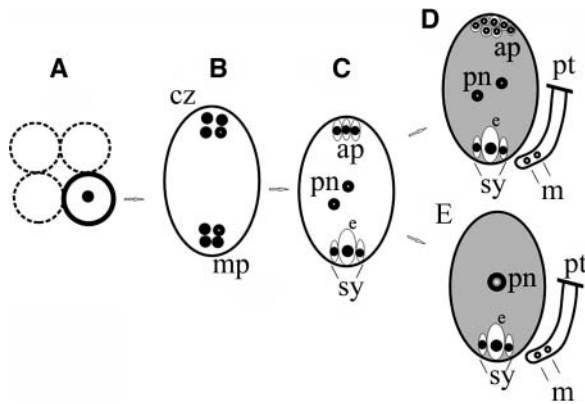
Figure 1. Development and Structure of the *Polygonum*-Type Embryo Sac of Maize and Arabidopsis.

(A) The embryo sac develops from the surviving haploid megaspore resulting from female meiosis.

(B) After three rounds of mitosis without cell wall formation between sister nuclei, two groups of four nuclei form at the micropylar (mp) and chalazal (cz) poles of the young megagametophyte.

(C) Three of the nuclei at each pole are walled off and become the egg cell (e), the synergids (sy), and the antipodal cells (ap). One nucleus from each pole migrates to the center (polar nuclei; pn) of the megagametophyte.

(D) The haploid polar nuclei of maize remain separate until fertilization. The shaded area of the megagametophyte represents the central cell that develops into the endosperm after fertilization. One of the haploid male nuclei (m) from the pollen tube (pt) enters the central cell, and the three nuclei fuse to form the triploid primary endosperm nucleus.

(E) The two polar nuclei of the Arabidopsis central cell fuse before fertilization. The antipodal cells are eliminated by programmed cell death. Figure modified from Drews et al. (1998).

melanogaster (Pirrotta, 1998). During early embryogenesis of *D. melanogaster*, these genes play a role in maintaining established patterns of gene expression, and it is likely that they play a similar role in the endosperm. Recently, the Polycomb group protein Medea was shown to regulate seed development by controlling the expression of the MADS box gene *Pheres1* (Kohler et al., 2003). For a detailed discussion, see review by Gehring et al. (2004) in this volume.

THE EVOLUTIONARY ORIGIN OF NUCLEAR ENDOSPERM

Double fertilization, the process by which one male gamete fertilizes the egg cell to produce a diploid zygote and the second gamete fertilizes the diploid central cell to give rise to the triploid endosperm (Figures 1D and 1E), was discovered more than 100 years ago (Nawaschin, 1898; Guignard, 1901). Since then, it has been regarded as a defining characteristic of angiosperms and has set the stage for the debate on the evolutionary origin of endosperm (Friedman, 1995). Soon after its discovery, two proposals for the origin of the endosperm were advanced, both of which are still valid because the issue remains unresolved (reviewed by Friedman, 1998). The first hypothesis proposes that the endosperm represents an altruistic, modified second embryo that during angiosperm evolution provided a selective advantage by nurturing the embryo proper. The second hypothesis states

that the endosperm represents extended development of the megagametophyte (i.e., the central cell). Until recently, several lines of evidence suggested that the Gnetales represent the extant seed plants most closely related to angiosperms. Based on this assumption, and the observation that double fertilization results in a diploid supernumerary embryo in *Ephedra* and *Gnetum* species, Friedman and co-workers supported the twin-embryo hypothesis (Carmichael and Friedman, 1995). However, recent discoveries in angiosperm phylogeny do not support Gnetales as an angiosperm predecessor (for details, see Mathews and Donoghue, 1999; Qiu et al., 1999; Soltis et al., 1999; reviewed by Friedman and Williams, 2003), lending little support to the twin-embryo hypothesis.

The revised angiosperm phylogeny has motivated a reexamination of the basic questions in the debate on the origin of the angiosperm endosperm, including the structure of the ancestral angiosperm megagametophyte, the role of double fertilization in angiosperms, and whether the ancestral endosperm was cellular or nuclear. Based in part on a reexamination of basal endosperm gametophytes according to the new angiosperm phylogeny, Williams and Friedman (2002) resurrect an idea proposed by Swamy (1946) among others that a four-celled embryo sac reflects the minimal structure common to all angiosperms and that the *Polygonum* embryo sac evolved from a duplication of this basic module gametophyte (Baroux et al., 2002; Friedman and Williams, 2003). In light of this information, Friedman and Williams (2004) favor the second hypothesis, that the triploid endosperm evolved from a maternal (megagametophyte) endosperm to a biparental tissue by the addition of the male nucleus at a later stage in evolution (see also Friedman and Floyd, 2001; Williams and Friedman, 2002). Finally, based on phylogenetic studies, the ancestral endosperm is believed to have been cellular and the nuclear endosperm is believed to have arisen multiple times, including in the angiosperm lineages of the cereals and Arabidopsis (reviewed by Geeta, 2003).

In spite of recent progress in understanding angiosperm phylogeny, all of the main questions regarding the evolutionary history of the nuclear endosperm remain unresolved. As summarized above, one likely scenario is that the ancestral endosperm of the cereals and Arabidopsis evolved from a maternal cellular megagametophyte tissue to a biparental endosperm by the addition of a male (pollen) nucleus at a later stage. Furthermore, the nuclear endosperm of cereals and Arabidopsis are not homologs in an evolutionary sense and consequently must have evolved independently.

THE ENDOSPERM COENOCYTE OF CEREALS

The morphogenetic events of the early stages of endosperm development in cereals were first detailed in a comprehensive way in barley using confocal microscopy (Brown et al., 1994) and later confirmed in rice (Brown et al., 1996a, 1996b). The first division of the triploid endosperm nucleus in the central cell of barley reveals the hallmark of nuclear endosperm, namely, the absence of a cell plate between separating daughter nuclei (Figures 2A and 2B). The continued absence of cell wall formation in the ensuing mitotic divisions leads to a multinucleate cell (the endosperm coenocyte) (Figures 2C and 2D) and stands in

Table 1. Definitions of Terms

Term	Explanation	Figure
Adventitious phragmoplast	Phragmoplast formed between canopies of microtubules in alveoli toward the central vacuole	5D
Alveolus (plural, alveoli)	Tube-shaped structure consisting of cell wall encasing one nucleus with one open end facing the central vacuole	5C
Anticlinal division	Mitotic division leading to a new cell wall that is perpendicular to the central cell wall	5B
Central cell	Central compartment of the megagametophyte with two nuclei (2n) after the formation of cell walls around the nuclei at each pole	1C
Chalazal pole	Distant pole of the megagametophyte	1B
Coenocyte	A cell with multiple nuclei in the same cytoplasm	2D
Cytoplasmic phragmoplast	Phragmoplast formed between opposing arrays of radial microtubules from neighboring coenocyte nuclei	5B
CZE	The endosperm in the chalazal end of the seed	2F
Embryo sac (megagametophyte)	Structure resulting from female meiosis containing the central cell and the egg cell	1C
ESR	Embryo-surrounding region	7A
Hoop-like cortical array	Array of circular microtubules close to the cell surface	3A
Interzonal phragmoplast	The phragmoplast formed between separating sister nuclei in somatic cells	3E and 3F
MCE	Endosperm in the micropylar end of the seed	2F
Megagametophyte (embryo sac)	Structure resulting from female meiosis containing the central cell and the egg cell	1C
Micropylar pole	The pole of the embryo sac where the pollen tube enters and where the egg cell is located	1B
Miniphragmoplast	Substructure of microtubules forming the cytoplasmic phragmoplast	5M
NCD	Nuclear cytoplasmic domain, a portion of the cytoplasm around one nucleus claimed by the radial microtubule system of that nucleus	4A
PEN	Peripheral endosperm in the central chamber of the seed	2F
Periclinal division	Mitotic division leading to a new wall that is parallel to the central cell wall	4C
Phragmoplast	Array of microtubules with opposite polarity mediating the deposition of a new cell wall between nuclei	3E and 3F
Polar nuclei	Haploid nuclei that migrate from the two poles of the megagametophyte to the center of the central cell	1C
PPB	Preprophase band of microtubules marking the future plane of cell division	3B
RMS	Radial microtubule system emanating from the surface of endosperm nuclei	4A

The figure citations are to the first mentions of each term in the text.

contrast to somatic cells, in which the default mitotic division cycle includes the formation of an interzonal phragmoplast between separating sister nuclei. For an overview of the cytoskeletal components of somatic cells relevant to the discussion of endosperm development, see Figure 3. The interzonal phragmoplast of somatic cells consists of two circular arrays of microtubules of opposing polarity that transport Golgi-derived vesicles containing glucan polymers to the site of cell wall deposition (Figures 3E and 3F). The phragmoplast and its cell plate expand laterally until the cell plate fuses with the parental plasma membrane and cell wall (Staehelin and Hepler, 1996; Heese et al., 1998; Sylvester, 2000; Brown and Lemmon, 2001) (Figures 3G and 3H). The molecular basis for the lack of cell wall formation in nuclear endosperm is unknown. Interestingly, in barley, phragmoplast formation is initiated between dividing sister nuclei (Brown et al., 1994), even forming occasional rudimentary cell walls in wheat (Tian et al., 1998). These observations suggest that interzonal phragmoplast function is suppressed after initiation in the endosperm coenocyte and appear to support the conclusion that cellular endosperm represents the basal state for endosperm. Although many details still are lacking about the regulation of phragmoplast formation and expansion, recent data suggest that the cytoskeletal apparatus is controlled by Cdc2-like kinases and mitotic cyclins (reviewed by Calderini et al., 1998; Pickett-Heaps et al., 1999; Smith, 1999; Sato and Kawashima, 2001). The most direct evidence for a role of mitogen-activated protein kinases in phragmoplast formation comes from tobacco, in which the mitogen-activated protein kinase kinase kinase NPK1 interacts with a phragmoplast-localized kinesin-like protein (Machida et al., 1998). These and other proteins should be used as probes to identify and compare the mechanisms for phragmoplast suppression in nuclear endosperm from different angiosperm lineages.

In maize, the first three mitotic divisions of the endosperm nuclei occur in predictable planes, resulting in eight nuclei positioned in a single plane in the basal cytoplasm of the coenocyte (Figure 2C). From this position, each nucleus divides and daughter nuclei migrate and divide, producing a population of nuclei that spreads to a sector corresponding to one-eighth of the coenocyte surface (Coe, 1978; McClintock, 1984). In maize, continued division produces 256 to 512 nuclei, marking the end of the coenocytic stage (Walbot, 1994) (Figure 2D). In barley, this stage is reached at 3 days after pollination (DAP), and the nuclei enter a mitotic hiatus that lasts for ∼2 days. The molecular control mechanism that causes the arrest in the progression of the cell cycle in barley is unknown.
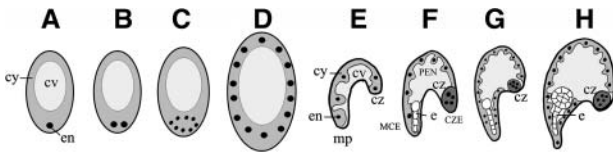
Figure 2. The Endosperm Coenocyte of Cereals and Arabidopsis.

(A) to **(D)** Cereals.
(E) to **(H)** Arabidopsis.
(A) The triploid endosperm nucleus (en) is located in the basal cytoplasm of the central cell. A large central vacuole (cv) fills up most of the volume, surrounded by a thin line of cytoplasm (cy).
(B) The central cell nucleus divides without the formation of a functional interzonal phragmoplast, and no cell wall is formed between sister nuclei.
(C) After three rounds of nuclear divisions, eight endosperm nuclei are located in a single plane in the basal endosperm coenocyte.
(D) The complete endosperm coenocyte contains evenly spaced nuclei in the entire peripheral cytoplasm.
(E) The Arabidopsis endosperm coenocyte has nuclei migrating from the micropylar region (mp) toward the chalazal end (cz), eventually covering the entire periphery of the coenocyte.
(F) and **(G)** As development progresses, the endosperm coenocyte develops three distinct regions: the region surrounding the embryo (MCE), the central or peripheral endosperm (PEN), and the region of the chalazal endosperm (CZE), which contains the chalazal cyst (cz).
(H) At the end of the globular embryo stage, the embryo becomes completely surrounded by cytoplasm.

THE ENDOSPERM COENOCYTE OF ARABIDOPSIS

Dicot nuclear endosperm development has been studied in several Brassicaceae species, including Arabidopsis (Mansfield and Briarty, 1990a, 1990b; Webb and Gunning, 1991; Mansfield, 1994; Van Lammeren et al., 1996; Brown et al., 1999). The central cell containing the endosperm coenocyte of Arabidopsis has three regions that become distinct as the seed grows: the embryo-surrounding region or micropylar endosperm (MCE), the peripheral endosperm (PEN) in the central chamber, and the chalazal endosperm (CZE) (Brown et al., 1999; Boisnard-Lorig et al., 2001; Sorensen et al., 2002) (Figures 2E and 2F). As the embryo sac expands after fertilization, the central vacuole enlarges and the cytoplasm of the endosperm syncytium assumes a peripheral position. At the globular embryo stage, the syncytial cytoplasm of the MCE surrounds the developing embryo, and the multinucleate PEN syncytium is a thin peripheral layer with evenly spaced nuclei (Figure 2H). Using a green fluorescent protein marker that accumulates in cell plates of somatic cells, Sorensen and co-workers (2002) detected cell plate formation between separating sister nuclei in PEN at a low frequency, suggesting that interzonal phragmoplasts were sometimes functional. This observation is interesting in light of the formation of nonfunctional phragmoplasts between sister nuclei in barley and wheat, suggesting that the coenocytic stage in Arabidopsis also results from a suppression of phragmoplast function. Recently, Brown and colleagues (2003) also observed interzonal phragmoplast formation between sister nuclei in the early endosperm coenocyte, suggesting that phragmoplast suppression occurs in a manner similar to that in cereals.

Berger and co-workers divided the syncytial endosperm stage in Arabidopsis into nine substages, each stage representing one of the eight rounds of mitosis (Boisnard-Lorig et al., 2001). At the final stage, the syncytial endosperm contained 200 nuclei. After the initial three synchronous division cycles, the mitotic activity of MCE, PEN, and CZE occurred independently, with nuclei dividing synchronously within domains. Nuclear divisions were never observed directly in the CZE after the three synchronous rounds of division, suggesting that these nuclei undergo endo-reduplication (Boisnard-Lorig et al., 2001).

Molecular markers for different endosperm compartments include the *Fis1/Mea* and *Fis/Fis2* promoters fused to β-glucuronidase (GUS), which represent specific markers of early nuclear endosperm development. GUS activity from these constructs can be detected already in the polar nuclei, the central cell nucleus of unpollinated ovules, and the syncytial endosperm (Luo et al., 2000). After cellularization, activity ceases in the micropylar and peripheral endosperm, being restricted to the chalazal chamber. The green fluorescent protein reporter of the enhancer trap line KS117 is expressed in the chalazal cyst at the heart stage of embryo development, but not in the endosperm nodule (Sorensen et al., 2001). Recently, a set of novel marker lines including markers for the chalazal endosperm as well as the MCE was reported (Stangeland et al., 2003).

ENDOSPERM CELLULARIZATION IN CEREALS

The process of cellularization of the endosperm coenocyte is initiated by the formation of RMS on all nuclear surfaces (Brown et al., 1994) (Figures 4A, 5A, and 5E). The portion of the cytoplasm claimed by these arrays around each nucleus is referred to as a nuclear cytoplasmic domain (NCD) (Brown and Lemmon, 1992). Soon, the microtubules from neighboring nuclei meet, forming interzones in which wall material consisting mainly of callose is deposited (Figure 5B). In barley, the arrays of opposing microtubules from adjacent NCDs are termed cytoplasmic phragmoplasts; these mediate the deposition of the

Figure 3. The Cytoskeletal Cycle of Somatic Cells.

(A) Hoop-like cortical arrays in interphase.
(B) Preprophase band marking the site and orientation of the deposition of the future cell wall.
(C) Metaphase spindle separating the sister chromosomes (not shown).
(D) Shortened spindles in telophase with connecting microtubules between the two poles.
(E) Early phragmoplast in the interzone between the two poles.
(F) Complete interzonal phragmoplast.
(G) Expanding phragmoplast depositing the new cell plate (cw) separating sister nuclei.
(H) Complete cell wall separating the two sister nuclei (cells).
For details, see Staehelin and Hepler (1996), Heese et al. (1998), Sylvester (2000), and Brown and Lemmon (2001).
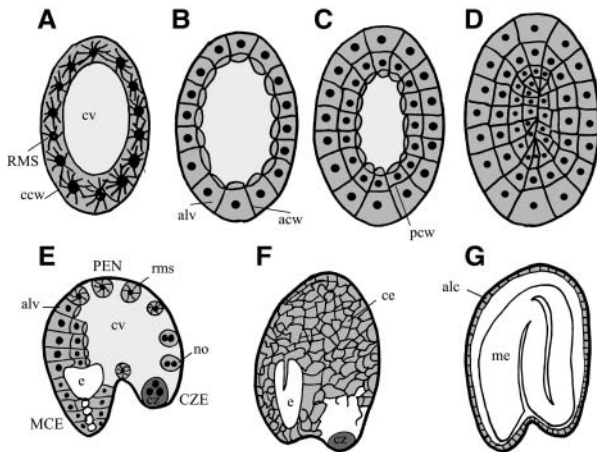
Figure 4. Cellularization of the Endosperm Coenocyte in Cereals and Arabidopsis.

(A) to **(D)** Cereals.
(E) to **(G)** Arabidopsis.
(A) RMS form on nuclear membranes in the cereal endosperm coenocyte. ccw, central cell wall; cv, central vacuole.
(B) Anticlinal cell walls (acw) form tubes or alveoli (alv) around each nucleus with their open ends toward the central vacuole. For details, see Figure 5.
(C) Divisions of alveolar nuclei result in a periclinal cell wall (pcw) that separates the outer layer of cells from a new layer of alveoli.
(D) Repeated periclinal divisions in the innermost layer of alveoli continue until the endosperm is completely cellular.
(E) The endosperm of Arabidopsis at the globular embryo stage showing a cellular MCE, a gradient of stages in the alveolation process in PEN, and endosperm nodules (no) as well as chalazal cyst (cz) formation in CZE.
(F) Completely cellular endosperm (ce).
(G) The endosperm is consumed during seed maturation, leaving only the peripheral aleurone-like cell (alc) layer in a mature embryo (me).
Figure modified from Olsen (2001), Brown et al. (1999), and Boisnard-Lorig et al. (2001).

initial cell walls in the endosperm (Brown et al., 1994). The formation of walls by cytoplasmic phragmoplasts is not unique to endosperm but also is seen in other plant systems, including megagametophyte cellularization, sporogenesis in lower plants, male and female gametophyte development in gymnosperms and angiosperms, and embryogenesis in gymnosperms (reviewed by Brown and Lemmon, 2001). Initially, the endosperm cell walls deposited by the cytoplasmic phragmoplasts form a tube-like structure, or alveolus, around each nucleus, with the open end pointing toward the central vacuole (Figures 4B, 5C, and 5G). In the process that follows, the RMS that encase each nucleus undergo reorganization, anchoring the nuclei to the central cell wall while extending toward the central vacuole in a canopy of microtubules (Figures 5D and 5H). The interzones between adjacent canopies of microtubules, termed adventitious phragmoplasts (Brown et al., 1994), function to extend the alveoli further toward the central vacuole. At the end of the first round of alveoli formation, the nuclei in each alveolus exit mitotic arrest synchronously to divide in a periclinal division plane (the orientation of the new cell wall is parallel with the central cell wall)

(Figures 6A and 6B). Notably, the periclinal cell walls in these mitotic divisions are formed by functional interzonal phragmoplasts that appear for the first time in the cell cycle of the endosperm (Figure 6B). These periclinal cell walls divide the alveoli into a peripheral cell and a new alveolus with its opening toward the central vacuole (Figures 4C and 6C). The repetition of this process four or five times results in a completely cellular endosperm at 6 to 8 DAP in barley and at 4 DAP in maize, wheat, and rice (Figures 4D and 6E).

As in other plant microtubule systems, the molecular basis for RMS formation in nuclear endosperm is unknown (reviewed by Canaday et al., 2000). In contrast to animal cells, in which centrosomes function as microtubule-organizing centers, the site of the initiation of microtubule polymerization in plants is unknown. One possibility is that microtubule polymerization in plants is initiated on nuclear surfaces and that these microtubule precursors are transported subsequently to their final subcellular
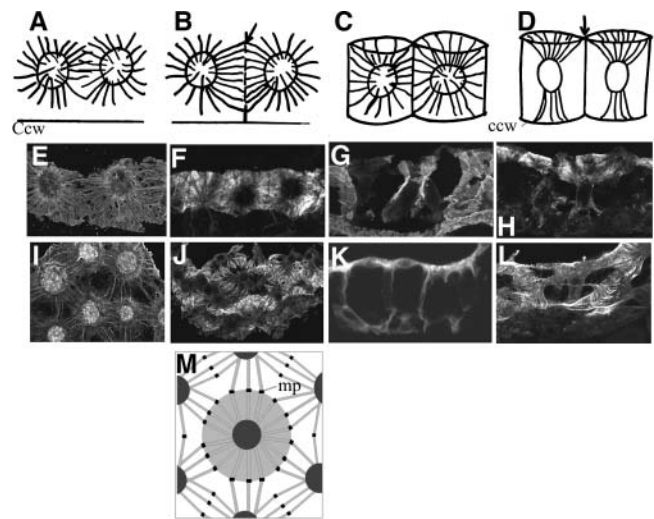

Figure 5. Initial Cell Wall Formation (Alveolation) by Cytoplasmic Phragmoplasts in Cereals and Arabidopsis.

(A) to **(D)** Cytoplasmic phragmoplasts.
(E) to **(H)** Cereals.
(I) to **(M)** Arabidopsis.
(A) Diagram showing RMS on two adjacent cereal endosperm nuclei. For orientation, see Figure 4A. Ccw, central cell wall.
(B) Cytoplasmic phragmoplasts form in the interzones between opposing RMS, mediating cell plate deposition (arrow).
(C) Alveoli form around each nucleus by cytoplasmic phragmoplasts.
(D) Alveoli are extended toward the central vacuole by a canopy of microtubules in a canopy-like fan of microtubules that form adventitious phragmoplasts (arrow). The nuclei are anchored to the former central cell wall by microtubules.
(E) to **(H)** Micrographs from barley depicting the stages diagrammed above each image.
(I) to **(L)** Micrographs from Arabidopsis depicting the stages diagrammed above each image.
(M) Diagram illustrating that cytoplasmic phragmoplasts are composed of substructures termed miniphragmoplasts (mp).
(A) to **(D)** are modified from Olsen (2001), and **(H)** is redrawn from Otegui and Staehelin (2000).
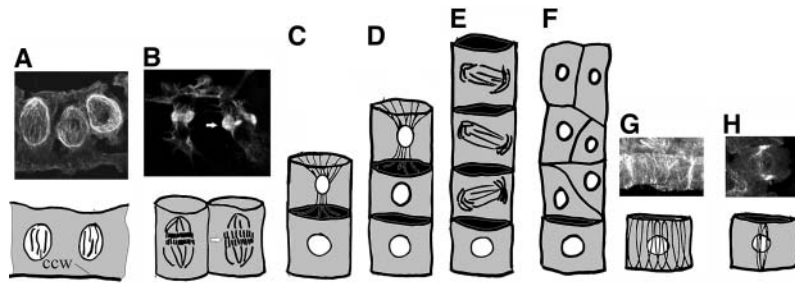
Figure 6. Completion of the Endosperm Cellularization Process and Differentiation in Cereals.

(A) Nuclei in alveoli (see Figure 4B) are surrounded by microtubular networks indicating entry into mitosis. The former central cell wall (ccw) represents the baseline in all panels.
(B) and **(C)** For the first time in the endosperm life cycle, functional interzonal phragmoplasts (arrows) appear between separating sister nuclei, resulting in a periclinal cell wall that divides the alveolus into a peripheral cell **(B)** and a new layer of alveoli **(C)**. For orientation, see Figure 4C.
(D) Repetitions of the alveolation process result in cell files growing in from the periphery that meet in the middle of the former central vacuole (see Figure 4D).
(E) After completion of cellularization, the starchy endosperm cell precursor cells (all except the peripheral layer of aleurone cell initials) divide at random planes.
(F) The cell file pattern in the starchy endosperm is lost soon after several rounds of cell division.
(G) Diagram showing hoop-like cortical arrays in aleurone cells, which represent the first layer of cells formed in the endosperm.
(H) Preprophase bands in an aleurone cell.

destinations. If this is correct, NCDs could be generated by a block in the mechanism(s) that transports microtubules from nuclear surfaces, combined with continued polymerization to grow microtubules to extend the whole radius of NCDs. A better understanding of the mechanisms of microtubule formation in plants should contribute to an understanding of RMS formation and its regulation.

ENDOSPERM CELLULARIZATION IN ARABIDOPSIS

Similar to the cereal endosperm, cellularization of the Arabidopsis coenocyte occurs via formation of RMS and alveolation (Brown et al., 1999; Boisnard-Lorig et al., 2001; Sorensen et al., 2002). The cellularization process starts as a wave in MCE, progressing through PEN and CZE at different rates and with significant variations between the different chambers (Figure 4E). The process of cellularization in PEN is similar, if not identical, to the cellularization process in cereals, and representative images of the different stages in barley and Arabidopsis are shown in Figures 5E to 5L. As in barley, RMS form on nuclear surfaces (Figures 4E and 5I), subsequently forming cytoplasmic phragmoplasts in NCD interzones that mediate alveolar cell wall formation (Figures 5J and 5K). Alveolization initiates at the final round of syncytial mitosis (Sorensen et al., 2002). The leading edge of cytoplasm contains the adventitious phragmoplast that extends the alveoli inward (Figure 5L). Synchronous periclinal mitosis of alveolar nuclei, accompanied by the formation of interzonal phragmoplasts and periclinal cell wall deposition (data not shown), divides the PEN alveoli into a peripheral cell and an internal alveolus (Figure 4E). Completion of endosperm cellularization in Arabidopsis also occurs by repeated rounds of the RMS-alveolation cycle, leading to a cellular endosperm except for the specialized chalazal endosperm cyst (Figure 4F).

The cellularization process in MCE also occurs via RMS and cytoplasmic phragmoplasts, but typical alveoli do not form as a result of spatial constraints in this chamber of the central cell (Figures 2H and 4E). Using labeled cryofixed/freeze-substituted material and high-resolution electron tomography, Staehelin and co-workers (Otegui and Staehelin, 2000; Otegui et al., 2001) have provided a detailed description of cytoplasmic cell plate formation, showing that cytoplasmic phragmoplasts originate at NCD boundaries and consist of substructures that they termed miniphragmoplasts (Figure 5M). On average, six cytoplasmic or syncytial-type cell plates form one hexagon-shaped alveolus. A model for the stepwise formation of the cytoplasmic cell plate was proposed by Otegui et al. (2001). Cellularization in MCE is completed around the embryo, whereas PEN remains syncytial (Sorensen et al., 2002) (Figure 4E). The CZE remains syncytial until late stages of seed maturation (Figure 4F).

How similar are the molecular mechanisms involved in cytoplasmic and interzonal phragmoplast-mediated cell wall formation? Structural similarities between the two types of phragmoplasts include the behavior of the tubular Golgi-derived networks, the appearance of clathrin-coated buds and vesicles, and callose deposition (Brown et al., 1997; Otegui et al., 2001). In addition, Knolle, a protein involved in homotypic fusions of Golgi-derived vesicles, has been shown to play a role both in endosperm cellularization and in cell division in the embryo. In both systems, homozygous mutant *knolle* cells fail to form complete cell walls (Lukowitz et al., 1996; Lauber et al., 1997; Sorensen et al., 2002). Several other mutants with *knolle*-like embryo phenotypes also show an arrest or delay of endosperm cellularization at the syncytial stage, including *hinkel*, which encodes a kinesin-related protein, *open house*, *runkel*, and *pleiade* (gene products unknown) (Sorensen et al., 2002; Strompen et al., 2002). As expected, Arabidopsis mutations that affect microtubule formation or behavior in embryos also affect

endosperm development, including *titan1* and *titan5* (McElver et al., 2000; Steinborn et al., 2002; Tzafrir et al., 2002), *titan7*, and *titan8* (Liu et al., 2002).

In spite of these striking similarities between cytoplasmic and interzonal phragmoplasts, functional differences, such as the mechanism of fusion of the cell plate to form vesicles and the mode of marginal cell plate growth, have been identified (Otegui and Staehelin, 2000; Otegui et al., 2001). Compositional differences between cell plates formed by the two types of phragmoplasts include a lack of terminal fucose residues in xyloglucans and the permanent presence of callose in syncytial cell plates (Liu et al., 2002), possibly making the endosperm walls more suitable for the storage of polysaccharides (Otegui et al., 2001). Genetic evidence for differences between somatic and cytoplasmic cell plates also has been provided in Arabidopsis. In the *spätzle* mutant, the embryo develops normally but PEN cellularization is perturbed, suggesting that *Spätzle* encodes an endosperm cellularization–specific component (Sorensen et al., 2002). In *spätzle* endosperm, PEN contains regularly organized NCDs until the initiation of cellularization, when the nuclei undergo at least one additional mitotic division, resulting in a syncytium with increased NCD density. Subsequently, the number of NCDs in the PEN is reduced continuously while the size of individual nuclei increases, as does the fusion of NCDs, ultimately resulting in a few giant NCDs with one or more giant nuclei. Identification of the *Spätzle* gene product should contribute important insight regarding the mechanisms of NCD formation and cytoplasmic cell plate formation.

ENDOSPERM CELL FATE SPECIFICATION AND DIFFERENTIATION IN CEREALS

The fully developed cereal endosperm consists of four main cell types: the starchy endosperm, the aleurone layer, transfer cells, and cells of the embryo-surrounding region (Figures 7A and 7B). The cereal endosperm has attracted attention from researchers because of its economic importance, and much insight has accumulated about the genes underlying the accumulation of storage products such as proteins and starch. Considerably less is known about the genes that regulate the developmental biology of these cell types, which is the topic of this section. Cell fate specification in cereal endosperm is believed to occur by positional signaling at an early developmental stage (Olsen, 2001) (Figures 7C to 7E). For simplicity, each cell type is described separately below, although cell fate specification occurs simultaneously with the cellularization process described above. How this integration occurs is unknown, but elucidation of the molecular controls for each of the four cell types should lay the foundation for understanding the genetic specification of the entire endosperm body plan.

The Embryo-Surrounding Region

The embryo-surrounding region (ESR) lines the cavity of the endosperm in which the embryo develops and has been studied most extensively in maize (Figure 7A). The exact role of the ESR is unknown, but possible functions include a role in embryo nutrition, the establishment of a physical barrier between the
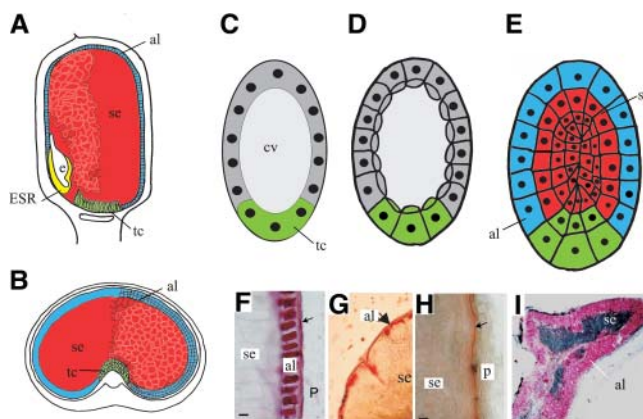

Figure 7. Cell Fate Specification and Development in Wild-Type and Mutant Cereal Endosperm.

(A) Cell types of maize endosperm: starchy endosperm (se), aleurone layer (al), transfer cells (tc), and ESR cells. e, embryo.
(B) Cell types of the barley endosperm.
(C) to **(E)** Proposed developmental domains in the young cereal endosperm at the syncytial stage **(C)**, the alveolar stage **(D)**, and the complete cellular stage **(E)**. Color coding is the same as in **(A)** and **(B)**. cv, central vacuole.
(F) Peripheral section of wild-type maize endosperm with one layer of aleurone cells. The arrow points to the maternal cell layer adjacent to the aleurone cells. P, maternal tissues.
(G) *cr4* endosperm lacks aleurone cells in discrete areas.
(H) *dek1* endosperm with a complete lack of aleurone cells. The arrow points to the corresponding layer shown in **(F)**.
(I) *sal1* endosperm with up to seven layers of aleurone cells.

embryo and the endosperm during seed development, and providing a zone for communication between the embryo and the endosperm. In maize, ESR cells are characterized by their dense cytoplasmic contents (Schel et al., 1984; Kowles and Phillips, 1988) and by the expression of the *Esr1*, *Esr2*, *Esr3* (Opsahl-Ferstad et al., 1997), *ZmAE1* (*Zea mays androgenic embryo1*), and *ZmAE3* (Magnard et al., 2000) genes between 5 and 20 DAP. Transgenic maize lines expressing the GUS reporter under the control of *Esr* promoters confirm the ESR-preferred pattern of expression for these genes (Bonello et al., 2000). Esr proteins localize to ESR cell walls (Bonello et al., 2000, 2002). Esr3 belongs to a family of small hydrophilic proteins that share a conserved motif with Clavata3 (Clv3), a protein that has been reported to interacts with the receptor-like kinases Clv1 and Clv2 in Arabidopsis and that functions in regulating meristem size (Fletcher et al., 1999). The functional significance of this similarity is strengthened by the observation that it is limited to a highly conserved region of 15 amino acids in Clv3 that contains two point mutations that result in the *clv3* phenotype (Fletcher et al., 1999), although the proposed role for Clv3 as a ligand for *clv1* has not been proven (Nishihama et al., 2003). This region of 15 amino acid residues also is shared by >40 predicted proteins called Cle (Clv3/Esr-related) that are all small hydrophilic proteins with a signal peptide (Cock and McCormick, 2001). Future studies will show whether, and how, ESR proteins may be involved in ESR signaling. In spontaneously occurring embryoless endosperm, *Esr* expression is lacking, suggesting a dependence of *Esr*

expression on signaling from the embryo (Opsahl-Ferstad et al., 1997). The promoters of *Esr* genes should provide useful tools to investigate the underlying mechanism of transcriptional activation in these genes (Bonello et al., 2000).

The mechanism underlying cell fate specification of the ESR is unknown. Based on the observation in maize that cell walls appear to form in the endosperm coenocyte around the embryo during the coenocytic stage (R.C. Brown, B.E. Lemmon, and O.-A. Olsen, unpublished data), it is possible that the ESR forms through a mechanism that permits functional phragmoplasts to form near the embryo. Also in barley, cellularization occurs early in the immediate vicinity of the embryo (Engell, 1989). Further studies are needed to confirm whether or not these cells represent ESR precursor cells. The observation that the endosperm of embryoless mutant grains forms a normal-sized embryo cavity suggests that the endosperm has an intrinsic program for the formation of the ESR domain (Heckel et al., 1999).

Transfer Cells

Transfer cells develop in the basal endosperm over the main vascular tissue of the maternal plant (Figures 7A and 7B), where they facilitate solute transfer, mainly of amino acids, sucrose, and monosaccharides, across the plasmalemma between the symplastic (maternal plant) and apoplastic (endosperm) compartments (Thompson et al., 2001). In maize, the *miniature1* mutant has reduced grain size and lacks normal levels of type 2 cell wall invertase in transfer cells, strongly suggesting that invertase contributes to the establishment of a sucrose concentration gradient in the apoplastic gap between the pedicel and the endosperm by hydrolyzing sucrose to glucose and fructose (Miller and Chourey, 1992; Cheng et al., 1996). In maize, two to three layers of transfer cells have wall ingrowths in a gradient decreasing toward the interior of the endosperm (Schel et al., 1984; Gao et al., 1998; Thompson et al., 2001). Several groups of transcripts have been shown to be expressed preferentially in transfer cells, including maize *Betl1* (*Basal endosperm transfer cell layer1*) (Hueros et al., 1995), *Betl2*, *Betl3*, and *Betl4* (Hueros et al., 1999), *Bap1* (*Basal layer-type antifungal protein1*), *Bap2*, and *Bap3* (Serna et al., 2001). Many of these proteins are similar to antimicrobial proteins, suggesting a role in defense against invading pathogens. Betl1 is synthesized and located in basal endosperm cells, where it is tightly bound to the cell wall (Hueros et al., 1995), whereas Bap2 is secreted into the intercellular matrix of the basal endosperm and accumulates predominantly in the adjacent, thick-walled cell layer of the pedicel (Serna et al., 2001).

Genes expressed at early developmental stages in transfer cells are of special interest as developmental markers for investigating the mechanisms of transfer cell fate specification. In barley, *Endosperm1* (*End1*) is present in the basal transfer cell domain of the endosperm coenocyte, which gives rise to the cells that differentiate into transfer cells (Doan et al., 1996) (Figure 7C). The function of this transcript has yet to be determined. A similar pattern of expression is seen for the maize *Zea mays MYB-related protein-1* (*ZmMRP-1*) transcript, which encodes a single Myb-repeat protein (Gómez et al., 2002). Interestingly, *ZmMRP-1* expression precedes that of other Betl-specific genes and has been shown to activate *Betl* transcription in transient assays

(Gómez et al., 2002). Although the mechanisms for early transfer cell domain transcription are unknown, differential transcription of *End1* and *ZmMRP-1* in the nuclei of this region of the coenocyte is a plausible explanation (Gómez et al., 2002). Such differential transcription could be triggered by either maternal factors deposited in the basal region of the central cell before fertilization or maternal factors from the pedicel in developing grains. The observations that xylem transfer cells are induced by high CO_2 concentration in lettuce and that transfer cells are induced in *Vicia faba* (broad bean) cotyledons as a response to glucose and fructose, but not by sucrose (reviewed by Thompson et al., 2001), make maternal factors from the pedicel attractive candidates for transfer cell gene-specific activators. During the cellularization process, two to three basal cells in cell files derived from the transfer cell domain of the endosperm coenocyte assume transfer cell identity (Figure 7E). This is different from the aleurone layer, where the border between the single layer of aleurone cells and the starchy endosperm is sharply defined (see below for details), suggesting that different mechanisms are involved in specifying the two cell types. One mechanism that could explain the gradient of transfer cell morphology in the basal endosperm is a dilution of transcription factor(s) present in the transfer cell region of the endosperm coenocyte as the cell files form and grow toward the central vacuole. Interestingly, kernels of the *defective kernel1* (*dek1*) mutant lack aleurone cells but contain normal transfer cells (Lid et al., 2002), supporting the notion that aleurone and transfer cell fates are specified by different mechanisms. Recently, based on the phenotype of the *globby1-1* mutant in maize, Costa and co-workers (2003) proposed that specification of transfer cells is an irreversible event that occurs during syncytial development and that transfer cell fate is inherited in a cell lineage–dependent manner. In addition to mutants that are impaired in transfer cell development (Maitz et al., 2000), mutants that lack transfer cells would be invaluable in elucidating the mechanisms underlying transfer cell fate specification.

Starchy Endosperm

Starchy endosperm cells represent the largest body of cells in the endosperm (Figures 7A and 7B). Starchy endosperm cells accumulate starch and prolamin storage proteins encoded by transcripts that are expressed differentially in these cells. Starchy endosperm cells are derived from two sources. The first, and most important, is the inner cells of cell files that are present at the completion of endosperm cellularization (Figure 7E, red zone). Soon after the completion of the cellularization phase, cell division resumes in the inner cell files (Figure 6E). Similar to the first periclinal divisions in alveoli, preprophase bands (PPBs) are absent, but unlike the alveolar divisions, which are strictly periclinal, the division planes are oriented randomly and the cell file pattern is soon lost (Figure 6F). The second source of starchy endosperm cells is the inner daughter cells of aleurone cells that divide periclinally (Figure 7E, blue zone). These cells redifferentiate to become starchy endosperm cells and likely are the source of the so-called subaleurone cells found adjacent to the aleurone layer in the starchy endosperm in all cereals.

Several collections of chemically induced mutants have led to the isolation of mutants broadly referred to as *dek* (*defective kernel*) in both maize and barley (Neuffer and Sheridan, 1980; Felker et al., 1985, 1987; Bosnes et al., 1987; Scanlon et al., 1994b). More recently, collections of maize mutant genes have been created in which the Mutator (*Mu*) transposon facilitates the identification and cloning of the mutant genes (Bensen et al., 1995). In the majority of these mutants, all tissues form normally, but the degree of filling of the starchy endosperm is reduced severely (Lid et al., 2002). Two such maize mutant genes have been cloned, *dsc1* (*discolored1*) (Scanlon and Myers, 1998) and *emp2* (*empty pericarp2*) (Fu et al., 2002). The *Dsc1* mRNA is detected specifically in kernels at 5 to 7 DAP, but no function has been assigned to the cloned genomic region. *emp2* is an embryo-lethal *dek* mutant that encodes a predicted protein with high similarity to Heat-shock binding protein1 (Fu et al., 2002). In addition to a predicted role in the heat-shock response, the mutant phenotype suggests that Emp2 also performs an important function(s) in seed development that has yet to be identified.

Pending the isolation of mutants that specifically affect starchy endosperm cell fate specification, little progress has been made in understanding the underlying mechanism of cell fate specification. It is interesting that in maize mutants that lack aleurone cells, *crinkly4* (*cr4*) (Becraft et al., 1996) and *dek1* (Becraft and Asuncion-Crabb, 2000; Lid et al., 2002) starchy endosperm cells are formed in place of aleurone cells (Figures 7G and 7H). Thus, signaling leading to aleurone cell formation appears to override signaling leading to starchy endosperm cell formation. Two important aspects of starchy endosperm development that are not discussed here are endoreduplication (reviewed by Larkins et al., 2001) and programmed cell death (Young et al., 1997).

Aleurone Cells

The aleurone layer covers the perimeter of the endosperm with the exception of the transfer cell region (Figures 7A and 7B). The aleurone layer functions in seed germination by mobilizing starch and storage protein reserves in the starchy endosperm through the production of hydrolases, glucanases, and proteinases after hormone (gibberellic acid) stimulation from the embryo. Maize (Figures 7A and 7F) and wheat have one layer of aleurone cells, rice has one to several layers, and barley has three layers (Figure 7B) (Buttrose, 1963; Hoshikawa, 1993; Walbot, 1994). Barley aleurone cells are highly polyploid (Keown et al., 1977). In the mature grain of maize, the aleurone layer consists of an estimated 250,000 aleurone cells derived by an estimated 17 rounds of anticlinal divisions (Levy and Walbot, 1990; Walbot, 1994). Toward the end of seed maturation, a specialized developmental program confers desiccation tolerance to the aleurone cells, allowing them to survive the maturation process (Hoecker et al., 1995; Kao et al., 1996, and references therein). Molecular markers for aleurone cells include *Ltp2*, *B22E*, pZE40, *ole-1*, *ole-2*, *per-1*, and *chi33* in barley (Klemsdal et al., 1991; Madrid, 1991; Smith et al., 1992; Kalla et al., 1994; Leah et al., 1994; Stacy et al., 1999) and *C1* in maize (Neuffer et al., 1997). Aleurone cells become morphologically distinct in barley endosperm at 8 DAP (Bosnes et al., 1992), comparable to the other cereals (Morrison et al., 1975; Brown et al., 1999). GUS

expression driven by the barley *Ltp2* promoter in transgenic rice grains is detectable at 9 DAP, closely matching the morphological differentiation of aleurone cells (Kalla et al., 1994). How early does aleurone cell fate specification occur? The analysis of barley endosperm development described above suggests that aleurone cell fate specification occurs after the first periclinal division of the alveolar nuclei, with the outer sister nuclei assuming an aleurone cell fate (Figures 4C and 7E) (Brown et al., 1994). The basis for this conclusion is the observation that after the completion of the cellularization process, these aleurone cell initials (Figures 6G and 6H) display the full complement of cytoskeletal arrays, including hoop-like cortical arrays and PPBs (Figures 6G and 6H). By contrast, the inner daughter cells of this periclinal division (giving rise to starchy endosperm cells) divide without cortical arrays and PPBs (Figures 6E and 6F). Anticlinal divisions in the aleurone layer expand the surface area of the aleurone layer, whereas periclinal divisions contribute to the inner starchy endosperm cells (see above). After 20 DAP, maize aleurone cell mitotic divisions are predominantly anticlinal (Kiesselbach, 1949). Because of the role of PPBs in mitotic division plane control in somatic cells, it is tempting to interpret the presence of PPBs as the first structural manifestation of aleurone cell fate specification. Three mutants in maize support the existence of a genetic control mechanisms for division plane control in the aleurone: *xcl1* (*extra cell layer1*), in which the aleurone layer possesses one extra cell layer as a result of aberrant periclinal divisions (Kessler and Sinha, 2000); and *dal1* and *dal2* (*disorganized aleurone layer1* and *2*), which have relaxed control over aleurone division plane determination (Lid et al., 2004).

In wild-type grains, aleurone cells develop in close contact with nucellus cells, which are part of the maternal plant. Recently, during a microscopy screen of ~12,000 maize mutant lines from a collection of *Mu* transposon insertion lines (Lid et al., 2004), we identified several hundred lines in which the endosperm displayed developmental defects (Olsen, 2004). In many of these, the mutant endosperm contained crevasses or indentations from the surface penetrating into the starchy endosperm. In all such cases, the crevasses were lined with aleurone cells. In other lines, the endosperm consisted of small bodies of endosperm cells that did not have direct contact with the maternal tissues surrounding the endosperm. The surfaces of these small bodies always were covered by one layer of aleurone cells on top of an inner mass of starchy endosperm cells. From these studies, we conclude that the endosperm of maize is programmed to develop a layered structure with aleurone cells on external surfaces.

What is the molecular basis of aleurone cell fate specification and maintenance? In light of the ability of the endosperm to develop a layer of aleurone cells on the surface of a body of starchy endosperm cells, it is interesting that the three genes known to affect aleurone cell fate specification and development in maize, *Cr4* (Becraft et al., 1996) (Figure 7G), *Dek1* (Lid et al., 2002) (Figure 7H), and *Sal1* (*Supernumerary aleurone layers1*) (Shen et al., 2003) (Figure 7I), encode proteins with similarity to proteins implicated in cell-to-cell signaling in animals.

Cr4 encodes a protein receptor kinase–like molecule with similarity to tumor necrosis factor receptors, prototypes of a large family of cell surface receptors that are critical for lymphocyte

development and function in mammals (Chan et al., 2000). The similarity to tumor necrosis factor receptors is limited to three Cys-rich domains of the extracellular domain that form the ligand binding pocket for tumor necrosis factor.

Dek1 encodes a predicted 2159–amino acid protein with a membrane-targeting signal in its N terminus followed by 21 transmembrane regions interrupted by an extracellular loop region (Lid et al., 2002). The cytosolic C terminus encodes a calpain-like Cys proteinase domain (Lid et al., 2002). Recently, Wang et al. (2003) showed that the calpain-like domain of Dek1, which is structurally very similar to animal calpains, has Cys proteinase activity in vitro. Although stimulated by Ca^{2+}, the Dek1 calpain is active in the absence of Ca^{2+}, suggesting that the regulatory properties may be different from those of typical animal calpains (Wang et al., 2003). The *Dek1* transcript is expressed ubiquitously in maize (Lid et al., 2002). The dependence of aleurone cell identity on *Dek1* throughout grain development was investigated by revertant sector analysis (Becraft and Asuncion-Crabb, 2000). In this analysis, aleurone cells in heterozygous *dek1/+* endosperm that lost the wild-type allele as a result of *Ds*-induced chromosome breakage and loss of the resulting acentric fragment carrying the *Dek1* allele were reported to revert to the starchy endosperm cell fate, even late in grain development. Conversely, starchy endosperm cells in the periphery of homozygous mutant *dek1:Mu/dek1* grains that gained *Dek1* function as a result of *Mu* transposition from a *dek1:Mu* insertion allele (restoring *Dek1* wild-type function) gained the aleurone cell fate. These data suggest that neither aleurone nor starchy endosperm cell fate is a terminal state of differentiation and that whatever cues are necessary for the specification of aleurone identity are present throughout development.

The third gene that affects aleurone cell specification, *Sal1*, encodes a predicted 204–amino acid protein that is a homolog of the human *Charged vesicular body protein1/Chromatin modulating protein1* gene, a member of the conserved family of the class-E vacuolar protein–sorting genes implicated in membrane vesicle trafficking (Shen et al., 2003). This gene also is expressed ubiquitously in maize.

In addition to genes that have already been cloned, other mutant genes have been described that result in multiple layers of aleurone cells, including *xcl1*. Also, mutants in which aleurone cell differentiation is disrupted have been reported, such as *collapsed2*, *opaque12*, which has thin walled, flattened aleurone cells with numerous vacuoles, *paleface*, with unusually rounded cells and sporadically more than one cell layer (Becraft and Asuncion-Crabb, 2000), and *dappled* mutants, with abnormal aleurone cell morphology (Stinard and Robertson, 1987; Gavazzi et al., 1997). Mutants in the *etched* loci (Scanlon et al., 1994a) and the newly isolated mutants *dal1* and *dal2* (Lid et al., 2004) also affect aleurone cell development.

Although significant progress has been made in identifying genes implicated in aleurone cell development, the current level of insight into signal transduction mechanisms in plants makes it difficult to propose a model integrating the functions of *Cr4*, *Dek1*, and *Sal1*. Based on analogy with animal signal transduction mechanisms, we have proposed that aleurone cell identity is specified by a ligand (unknown) in the periphery of the endosperm that activates the Cr4 protein receptor kinase (Olsen et al., 1998). As suggested by the identity of the Sal1 and Dek1 proteins, endosome trafficking and a calpain-like Cys proteinase also play roles in aleurone signaling. The *sal1* loss-of-function mutant endosperm has multiple layers of aleurone cells, suggesting that Sal1 functions to limit aleurone cell identity to the outer cell layer in wild-type endosperm. Obviously, additional research is needed before a complete model of aleurone cell fate specification can be proposed.

ENDOSPERM CELL FATE SPECIFICATION AND DIFFERENTIATION IN ARABIDOPSIS

The cellularization process for Arabidopsis described above results in a completely cellular endosperm except for a small area in the CZE adjacent to the chalazal cyst (Figure 4F). In contrast to the persistent endosperm of the cereals, the cellular endosperm of Arabidopsis is depleted gradually as the embryo grows. It is generally assumed that the purpose of the nonpersistent endosperm is to support the developing and growing embryo and that the support function for the germinating embryo is taken over by the cotyledons. In mature seeds not yet released from the silique, a massive embryo fills the ovule and a single peripheral layer sometimes referred to as the aleurone layer persists in the mature ovule (Vaughn and Whitehouse, 1971; Chamberlain and Horner, 1990; Groot and Van Caeseele Lawrence, 1993) (Figure 4G). In Arabidopsis, these cells exhibit few storage products and thin cell walls (Keith et al., 1994), and their function remains unknown. In the chalazal chamber, nodules of multinucleate endosperm line the wall and a large coenocytic cyst of multinucleate cytoplasm is positioned in the tip of the chalazal chamber atop a specialized pad of maternal tissue known as the chalazal proliferating tissue (data not shown), which has been suggested to serve a role similar to the transfer cells in cereal endosperm (for more details, see Brown et al., 1999).

MONOCOT AND DICOT NUCLEAR ENDOSPERM EVOLVED INDEPENDENTLY BUT MAY HAVE RECRUITED THE SAME ANCIENT SUBDEVELOPMENTAL PROGRAM FOR NCD FORMATION AND ALVEOLATION

A comparison of the cellularization processes leading to the cellular endosperm in cereals and the PEN in Arabidopsis reveals striking similarities. Other aspects, including cell cycle regulation during the cellularization process and the identity of the different cell types, obviously are different. Therefore, it is unclear whether knowledge about differentiation mechanisms beyond the alveolation process applies to both types of endosperm. Current phylogenetic data suggest that monocots represent a monophyletic group that shares a common ancestor. In agreement with this finding, the monocot nuclear endosperm is highly conserved in all species investigated to date. As described above, the monocot and dicot endosperm are believed to have evolved independently. In spite of this, nuclear endosperm in these groups show striking similarities with respect to the cellularization process (Figures 4 and 5). Importantly, the process of cellularization by RMS formation also occurs during the cellularization process in a number of

other systems, including sporogenesis in lower plants, male and female gametophyte development in gymnosperms and angiosperms, and embryogenesis in gymnosperms (Brown and Lemmon, 2001). By contrast, the alveolation process as seen in nuclear endosperm is found only in megagametophyte development in gymnosperms and not in other existing angiosperm systems. One possible explanation for the highly conserved cellularization process of nuclear endosperm in cereals and Arabidopsis is that the nuclear endosperm evolved independently from a cellular endosperm by the same two steps in these two angiosperm lineages. First came a mutation(s) that suppressed phragmoplast formation in the mitotic divisions of the central cell nucleus after fertilization, creating the endosperm coenocyte. Further investigation is needed to determine whether or not the same mechanisms for phragmoplast suppression occur in both groups. Second came recruitment of the same RMS-alveolation "subprogram" in both cases. In this scenario, insight into the process of endosperm cellularization is valid for both monocots and dicots.

CONCLUDING REMARKS AND PROSPECTS

Insight into the mechanisms of nuclear endosperm development has advanced considerably during the last decade. Currently, advances in nuclear endosperm research come from two of the most powerful plant experimental systems available, maize and Arabidopsis. Because of the assumed independent origin of monocot and dicot nuclear endosperm, efforts to solve questions related to nuclear endosperm evolution, coenocyte development, cell cycle regulation, cell fate specification, and differentiation need to continue with equal strength in both cereals and Arabidopsis.

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