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Endosperm Developments

Endosperm, which is viewed by many botanists as a fundamental component in the evolutionary success of angiosperms (Stebbins, 1974), represents an important source of food, feed, and industrial raw materials for mankind. In spite of this, the origin of the endosperm and the processes involved in its development have remained enigmatic.

Shortly after the discovery of double fertilization around the turn of the century, two views on the origin of endosperm were advanced, both of which still persist today. In one hypothesis, it is postulated that the endosperm is derived from an altruistic twin embryo. In the other, endosperm is proposed to result from the extended development of the megagametophyte, which is thought to be promoted when the central cell is fertilized by the second male gamete (reviewed in Friedman, 1994).

Revisiting this debate in gnetophytes of the genus *Ephedra*, which seem to represent an evolutionary link between gymnosperms and angiosperms, Friedman (1994) discovered surprisingly that double fertilization in these plants leads to the formation of twinned embryos. These data can be taken to support the first hypothesis because they imply that endosperm evolved from one of the twin embryos. Nevertheless, further work is needed to provide a definitive answer to the question of endosperm origin.

Endosperm and its development also attracted the attention of early plant anatomists, who published their first accounts more than a century ago (see, e.g., Johannsen, 1884). Although we have learned a great deal about endosperm since these initial descriptions were generated, the pathway of endosperm development and, in particular, events associated with the transition from a coenocytic to a cellularized endosperm are not fully understood.

Recently, however, immunohistochemical methods have been used in combination with confocal microscopy to show that the events occurring during the cellularization process of nuclear endosperm are highly conserved in barley, wheat, rice (Brown et al., 1994, 1996a; Olsen et al., 1995), and maize (R.C. Brown, B.E. Lemmon and O.-A. Olsen, unpublished data). These studies, and many others, have helped to identify several distinct events that occur during early endosperm development, beginning with the polarization of the megagametophyte.

In maize, the megagametophyte is of the *Polygonum* type. It is indeed a highly polarized structure, containing the egg cell and the two synergids at the micropylar end, the central cell in the middle, and antipodals near the chalazal end (Drews et al., 1998). In the central cell, polarity is evidenced by the position of the nuclei and by the position of the bulk of the cytoplasm and organelles, such as starch grains, which accumulate near the micropylar end of the embryo sac. Although the underlying signals driving megagametophyte differentiation are largely unknown, genetic data demonstrate that sporophytic as well as female gametophytic gene products are involved in this process (see Drews et al., 1998). Moreover, it has been determined that cytoplasmic domains involved in megagametophyte cellularization are established via radiating perinuclear microtubules (Russell, 1993).

Shortly after karyogamy, the primary endosperm nucleus of maize undergoes three rapid nuclear divisions. These divisions occur at fixed planes and are followed by migration of the resulting eight nuclei to the periphery of the proximal central cell. Next, during a period of rapid mitotic divisions, the original eight daughter nuclei and their daughters migrate to distinct domains of the distal cytoplasm that surrounds the central cell vacuole, each lineage giving rise to a one-eighth sector of the mature endosperm (for a review, see McClintock, 1978). In barley, two distinct domains appear to be established in the young endosperm at this time (Engell, 1989). One is a small sector close to the embryo in which normal cell divisions continue. By contrast, in the second domain, which is termed the coenocytic endosperm, the formation of functional phragmoplasts is suppressed.

In barley, and most likely in other cereals as well, a 2-day mitotic hiatus occurs after the daughter nuclei have completed their migration to the distal cytoplasm. During this interval, radial microtubular systems mark out nucleocytoplasmic domains (NCDs) around each nucleus and cell wall formation is initiated in the interzone between opposing microtubular arrays (Brown et al., 1994). These first anticlinal walls then extend centripetally, guided by cytoplasmic phragmoplasts.

By the end of this stage, the peripheral endosperm nuclei are encased in tubelike wall structures termed alveoli. Similar structures are also seen in the endosperm of dicots such as *Brassica napus* (van Lammeren et al., 1996). Moreover, it has been pointed out that the process of endosperm alveolation is remarkably similar to events that occur during development of the female gametophyte in gymnosperms (reviewed by Singh, 1978), suggesting that the alveolation process itself is quite ancient.

After reorganization and polarization of the cytoplasm within the endosperm alveoli (Brown et al., 1996b), mitosis is synchronously resumed, with each nucleus dividing periclinally. For the first

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time during nuclear endosperm development, phragmoplast and cell plate formation occur, giving rise to a single peripheral cell layer and an inner syncytium that contains the daughter nuclei. This is a formative division in endosperm development, in that the peripheral daughter cells become aleurone initials, whereas the inner daughter cells give rise to starchy endosperm cells. In addition to different cell contents, the mechanisms by which subsequent cell division planes are selected differ in the two cell types. In the aleurone, anticlinal or periclinal division planes are initiated by preprophase bands (PPBs) and phragmoplasts. By contrast, starchy endosperm cells lack PPBs and divide in random planes.

It is clear from the outline of endosperm development presented above that several questions must be addressed in detail before the regulatory mechanisms that underlie this process can be elucidated. For instance, how is polarity established and maintained in the central cell? After fertilization, how is phragmoplast formation prevented in the initial phases of endosperm development? What fixes the division planes in the early nuclear divisions, and how is nuclear migration controlled in the endosperm coenocyte? In the coenocyte, how are mitotic arrest and the subsequent synchronous periclinal mitoses controlled? What initiates phragmoplast formation in this formative division, and how is the periclinal orientation controlled in the absence of a PPB? Finally, what specifies the separate fates of the aleurone initials and starchy endosperm daughter cells?

Although genetic analyses will undoubtedly continue to make important contributions to our understanding of endosperm development, many of the earliest steps are difficult to observe in intact plants. Moreover, using whole plants to evaluate whether specific signaling molecules that have been proposed to affect endosperm development actually do so may be quite challenging. For these reasons, it would be extremely useful to be able to manipulate endosperm development in vitro.

On pages 511–524 of this issue, Kranz et al. report that they have successfully isolated and fused maize central cells with sperm cells in vitro. Moreover, they show that many of the steps that occur subsequently during endosperm development in vivo appear to be faithfully replicated in vitro, making this system a useful model for experimental manipulation of endosperm development.

The authors begin by showing that although the isolated central cell protoplasts are spherical in shape, they retain clear signs of their polarity. For example, their nuclei, which are surrounded by starch granules, are positioned toward the periphery of the cell within the main bulk of the cytoplasm. Kranz et al. go on to demonstrate that after karyogamy, the resulting primary endosperm nucleus divides in the absence of cell wall formation and that subsequent cellularization events start from the periphery of the cell and proceed centripetally. After 5 days in culture, the in vitro endosperm develops into a bipartite structure consisting of one part with small cells and a second part with fewer but larger cells.

The work presented by Kranz et al. complements earlier studies in which Lörz and his colleagues demonstrated in vitro fertilization of the egg cell and the subsequent regeneration, via direct primary embryogenesis, of fertile plants (Kranz and Lörz, 1993). In contrast to their studies of in vitro zygotic development, Kranz et al. show here that neither callus nor shoot growth initiates from the fertilized central cells in culture. This finding indicates that developmental restrictions are imposed on the central cell in vitro, as they are in vivo, and supports the view that the in vitro system is eminently suitable for further investigation of the events that control and define endosperm development.

Perhaps the most exciting prospect associated with the ability to manipulate endosperm development in vitro is that it will now be possible to directly address questions regarding the nature and extent of developmental cues present in the central cell at the time it becomes separated from the ovule. It will also be posible to identify events occurring during early endosperm development that require maternal signals. Nevertheless, because karyogamy with a nucleus from the male gamete is clearly a prerequisite for the initiation of endosperm development in vitro, it must be assumed that the continuation of this process involves zygotic as well as maternal gene products.

The in vitro endosperm system may also shed light on the origin of the developmental cues that suppress the formation of phragmoplasts between the daughters of the primary endosperm nucleus. As is the case in vivo, initial cellularization in vitro seems to occur via NCD formation and alveolation which, if confirmed, would strongly suggest that these events are controlled primarily by the central cell cytoplasm. Moreover, this control appears to originate within the young gametophyte, which itself is capable of forming NCDs.

In the future, further detailed studies of endosperm development in culture will show whether the mitotic hiatus, the synchronized reinitiation of mitosis, and periclinal phragmoplast formation also occur in vitro. If they do, then one could surmise that the central cell provides an environment in which the endosperm nuclei are capable of initiating the genetic program that drives the early phase of endosperm development in vivo. Furthermore, future investigation of the structural details of in vitro endosperm development will demonstrate whether aleurone cell differentiation occurs in this system. If so, it should be possible to use the in vitro endosperm system to help unravel the mechanisms involved in specifying the different fates of the daughter cells of the endosperm formative division.

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Some clues as to the signals that may precipitate similar developmental decisions can be derived from animal systems. In many cases, fate specification occurs via signal transduction pathways that are initiated when an external ligand is perceived by the extracellular domain of a receptor kinase. This interaction sets off a cascade of events that result in the activation of specific gene programs. One such example is the Toll receptor in Drosophila which, after it binds the Spätzle (SPZ) ligand, triggers the activation of the Dorsal transcription factor. Dorsal goes on to activate the transcription of zygotic cardinal genes involved in the specification of embryonic ventral structures (Nusslein-Volhard, 1996).

Support for the possibility that aleurone cell specification may be triggered via a similar pathway comes from the recent cloning of the *crinkly4* (*cr4*) gene, which encodes a receptor-like protein kinase (Becraft et al., 1996). In homozygous *cr4* mutant endosperm, aleurone cells are occasionally missing. This leads to the formation of white patches on the surface of kernels that correspond to regions in which starchy endosperm cells have differentiated ectopically at the periphery of the endosperm. One interpretation of these observations is that the protein receptor-like kinase encoded by the *cr4* gene may be involved in the specification of aleurone cell fate. This process may be initiated when a peptide ligand originating in the maternal tissues binds to the CR4 kinase (Olsen et al., 1998).

There is emerging evidence that peptidelike hormones and protein receptor kinases may function in signal transduction pathways in plants as well as in animals (Braun and Walker, 1996; Clark, 1996; Baker et al., 1997), strengthening the case for a CR4–mediated signal transduction pathway in aleurone cell differentiation. Interestingly, the in vitro fertilization system developed by Kranz et al. offers an opportunity to test this hypothesis; if aleurone cell differentiation does occur in vitro, we can conclude that the proposed peptide ligand that activates the putative CR4 receptor is not supplied by the maternal tissue at the time aleurone cell differentiation begins.

Several additional lines of investigation may be opened up by the maize in vitro endosperm system that Kranz et al. have developed. First, injecting compounds that perturb cytoskeletal structure and/or function will hopefully help to identify the specific cytoskeletal components that are involved in central cell polarization, nuclear migration, NCD formation, and alveolation.

Second, the extent of polarization in the in vitro endosperm may be investigated using probes representing molecular markers for domains of gene expression in the endosperm. One marker for the syncytial endosperm is the *END1* gene, which is expressed in the nuclei that form over the nucellar projection in barley (a domain that corresponds to the pedicel in maize; Doan et al., 1996). Another probe that could be used to assess the extent of polarized gene expression in the in vitro endosperm is *ZmEsr*, which is expressed in the so-called embryo surrounding region of the maize endosperm (Opsahl-Ferstad et al., 1997). Additional probes available for assessing the differentiation status of in vitro endosperm include cDNAs for zeins and ADP-glucose pyrophosphorylase, the latter of which is a key enzyme in endosperm starch synthesis.

Third, as a result of current and future genome and EST sequencing initiatives, an increasing plethora of probes representing molecules that are involved in signal transduction or downstream events in the endosperm will become available. The in vitro system developed by Kranz et al. represents an attractive target for injection studies with such molecules, which are likely to be transiently expressed during endosperm development. In addition, appropriately expressed green fluorescent protein fusions and associated confocal microscopy studies of cultured endosperm cells are bound to become potent tools in the near future.

However, the most important advances in our understanding of endosperm development are likely to come from continued mutant analysis. Large populations of maize plants with active *Mutator* transposons are already available, and these collections make possible a systematic search for true endosperm developmental (i.e., homeotic) mutants and subsequent cloning of the mutant genes (Bensen et al., 1995). Furthermore, with the recent recognition that processes occurring during nuclear endosperm development are highly conserved in monocots and dicots (van Lammeren et al., 1996), the identification and analysis of Arabidopsis mutants may also be expected to contribute to the isolation of genes involved in endosperm development, particularly the early stages. With these cloned genes in hand, in vitro systems for investigating endosperm fertilization and growth, such as that described by Kranz et al., will become all the more valuable.

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LETTER TO THE EDITOR

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A Plant Genome Initiative

Over the last several months, we have attended various meetings and small conferences concerning the need for a coordinated and comprehensive plant genome initiative (PGI). From these meetings, and from our extensive discussions with plant and animal scientists with interests in genetics, agriculture, and science policy, we have been able to discern a general consensus among biologists both that a comprehensive program is desperately needed and that certain components of this program are indispensible.

Of course, with such a broad set of discussants, there were some lively debates on a number of technical points and some significant disagreements. Nevertheless, it is clear that many scientists feel that a comprehensive PGI will be essential if we are to maintain the agricultural competitiveness of the United States and to develop new sources of agricultural productivity at a time when demand for increased food production worldwide threatens to exceed traditional approaches to crop improvement. The fact that maize alone generates $80 billion annually in farm-gate value that is converted into over $400 billion through value-added activities is just one indication of the importance of agriculture to the economy of the United States.

To a degree, the United States Government apparently recognizes the need for increased support in the area of plant genomics and is prepared to back that recognition up with new funding. Indeed, since we prepared our first drafts of this letter, the National Science Foundation (NSF) has announced a plant genome