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Endosperm Origin, Development, and Function

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INTRODUCTION

Endosperm has been studied from a variety of vantage points: evolution, role in seed development and germination, genetics, physiology, and biochemistry. This tissue represents a renewable, biodegradable source of materials; much effort has been directed to improve its use in feed and food making as well as its refinement to secondary products such as oils and plastics. Although there is a vast literature dealing with each of these topics, we still have a remarkably superficial understanding of most of them. There has been revitalized interest in understanding the endosperm in relation to seed-specific developmental processes. Information from these studies could provide a basis for developing more efficient approaches for plant improvement and use. Recent advances in molecular biology have created the possibility for detailed study of many of the genetic and molecular mechanisms involved in endosperm development. This research could conceivably lead to answers to many basic questions in developmental biology as well as to new tools that enhance practical uses of endosperm. In this review, it is not our intention to present a comprehensive overview of what is known about endosperm. Rather, we have chosen to summarize some of the research that has been done, making note of comprehensive reviews and pointing out important questions that remain open to scientific inquiry.

ORIGIN AND FUNCTION OF THE ENDOSPERM TISSUE

The origin of endosperm is intrinsically linked to double fertilization, a unique biological process in which one sperm nucleus fuses with the egg to produce the embryo, while a second sperm nucleus fuses with two polar nuclei to form the triploid endosperm. These events occur within the embryo sac, which is embedded in maternal tissues (see Dumas and Mogensen, 1993, this issue; Russell, 1993, this issue). The term "double fertilization" indicates that the primary endosperm nucleus is considered to be derived from a fertilization event, even though the triple fusion nucleus does not go through a process that results in another organism, as expected from true fertilization. Discussion of the evolutionary origin of endosperm was first presented by Sargant (1900), who hypothesized that double fertilization in the ancestors of angiosperms generated a second embryo, which evolved into a body with storage function (the endosperm). This transformation might have occurred as a result of the addition of a second female nucleus to the second fertilization event.

The recent discovery that a second fertilization product regularly produces embryos in the nonflowering seed plant *Ephedra trifurca*, one of the closest relatives to the angiosperms, provides support for the first part of Sargant's hypothesis (Friedman 1990a, 1992), even though the fate of the second sperm nucleus does not result in a developmentally distinct nutritive tissue. It is unknown whether endosperm tissue evolved prior to or after the addition of a third nucleus to form the second fertilization product. Endosperm may originally have been diploid, if the addition of a third nucleus to the second fertilization event occurred after the transformation of the supernumerary embryo into a nutritive tissue. However, caution must be exercised in this comparison because it is not known whether double fertilization in *Ephedra* is evolutionarily homologous to double fertilization in flowering plants (Friedman, 1990b).

Double fertilization may be important for preserving epigenetic differences that direct the development of the endosperm and embryo. Kermicle and Alleman (1990) hypothesized that gametic imprinting, acting in conjunction with unequal gene contribution from the parents, could lead to four effective gene dosage levels: "a gene may be turned off when received from both parents, turned on following pollen transmission (one effective dose), turned on only following ovule transmission (two effective doses), or turned on when received from both parents (three doses)." The authors stress that under such conditions, triploidy could be a mechanism of fine tuning effective gene dosage levels; if endosperm were diploid, imprinting would provide less advantage because only three effective dosage levels would be possible. Such a phenomenon may explain why the central cell does not differentiate and function as an endosperm, as it does in gymnosperms, and why the endosperm in sexually reproducing species usually does not differentiate directly from the sporophyte, as it does in autonomous apomitics (see Koltunow, 1993, this issue). Kermicle (1978) suggested that epihybridity, or epistatic combinations of epigenetic variation, could be generated within the endosperm of self- and cross-pollinated species by

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differences in the combination of derepressed genes. If so, a novel form of hybrid vigor may derive from epigenetic differentiation between polar and sperm nuclei.

Other intriguing and unresolved aspects of endosperm origin and function include its intermediary position between the old and the new sporophyte and the general need of a fertilization process to trigger its own development. The angiosperm ovule contains little or no reserve food at the time of fertilization, by contrast with nonflowering plants, in which a large female gametophyte provides nutrients for the developing embryo. Evolution of an embryo-nourishing tissue from a supernumerary embryo might have provided for the reduction of the female gametophyte in angiosperms (Friedman, 1992). What kind of advantage could have been achieved by such innovation, given that it requires the embryo to develop with a less readily available nutrient supply than that found in plants with a large female gametophyte? Could double fertilization be a way for the developing sporophyte to gain partial genetic control over the female gametophyte by gearing the endosperm genome to serve the embryo's needs? What kind of specialization made the acquisition of nutrients from the maternal tissue and the feeding of the embryo and seedling a more efficient process? Brink and Cooper (1947a, 1947b) speculated that double fertilization may have given endosperm the advantage of heterosis. These authors also suggested that its triploid nature may be viewed as a way to acquire genetic advantage in an environment of diploid cells. Interestingly, endoreduplication, or the general polyploidization of the endosperm genome, which frequently occurs during development (see below), could be viewed as a way to amplify both advantages.

PATTERNS OF ENDOSPERM DEVELOPMENT

Different plants have developed patterns of seed formation that lead to different levels of endosperm persistence during development (Murray, 1988). For example, in cereal species, endosperm is formed and retained as a reserve tissue in the mature seed. In several dicotyledoneous species, endosperm is formed but is substantially degraded by the time the embryo matures. In peas, which have nonpersistent endosperm, the tissue is apparently absorbed in the free-nuclear state before it ever develops cell walls (Marinos, 1970). In species of the family Orchidaceae, endosperm nuclear divisions are terminated early in seed development or do not occur at all.

Depending on the developmental pattern of cellularization, three main modes of early endosperm development can be recognized: nuclear, cellular, and helobial (reviewed by Brink and Cooper, 1947a; Vijayaraghavan and Prabhakar, 1984). Nuclear is the most common type, in which the primary endosperm nucleus undergoes several rounds of division without cytokinesis to generate a large number of free nuclei organized at the periphery of the central cell. Cytokinesis then initiates, progressing centripetally toward the large central vacuole until the endosperm becomes entirely cellular. This pattern is typical of the seeds of cereals such as maize, barley, and rice. In the cellular type of endosperm development, mitosis and cytokinesis occur after the first division of the primary endosperm nucleus and persist throughout the development of the endosperm. This pattern occurs in species of the genus *Lycopersicon* and several members of the families Crassulaceae, Bignoniaceae, and Labiatae. An intermediate and infrequently found mode of endosperm development is the helobial type. The primary endosperm cell generates two cells of unequal size after the first division. The larger cell divides, as in endosperms of the nuclear type, whereas the smaller cell remains undivided and uninucleate or undergoes a limited series of mitoses to form a multinucleate cell. Although a great deal of information has been generated on endosperm histo-differentiation (reviewed by Vijayaraghavan and Prabhakar, 1984; Olsen et al., 1992), the genetic and biochemical mechanisms involved in the early definition of endosperm differentiation patterns remain unknown.

Clonal analysis has been used successfully to trace phenotypic variations occurring during endosperm development. McClintock (1978) studied the timing of unstable mutations caused by the *Activator* (*Ac*) regulatory system in the *Waxy* (*Wx*) locus of maize, which controls the accumulation of amylose during endosperm development. The excision of the *Ac* element from the *Wx* locus during endosperm development results in sectors of amylose-containing cells, which can be visualized in mature kernels by staining with I_2/KI. From the arrangement of these sectors, the patterns of cell division during endosperm development can be deduced. This method of clonal analysis showed that initial cell divisions lead to the establishment of the right and left halves of the kernel, as Figures 1A and 1B illustrate. In most cases, sectors of cells originate at one internal location in the endosperm, and the shapes of the mutant sectors suggest that cells proliferate in a conical pattern from the interior to the exterior of the tissue. Relatively little information on endosperm cell lineages has been reported for other species, although studies on barley endosperm mutants suggest a mechanism similar to that of maize (Olsen et al., 1992).

Many existing variations on the common pattern of clonal development in maize described by McClintock (1978) remain unexplained. An example of such variation is found associated with the *R-nj* (Navajo crown) allele, which confers aleurone color in the crown of maize kernels but leaves the sides and the region near the embryo colorless (Coe et al., 1988). In addition, the behavior of endosperm modifier genes in maize leads to phenotypic modification of *opaque2* (*o2*) mutant endosperms in a fashion that is also clearly independent of the systematic development of clones (Figure 1C; Lopes and Larkins, 1991). These patterns must be based on variation in spatial and/or temporal distribution of a factor(s) that generates phenotypic differences between regions of the endosperm.

Many aspects of endosperm differentiation are not understood. Little is known about the developmental decisions leading to the proliferation of cell lineages or to the cellular

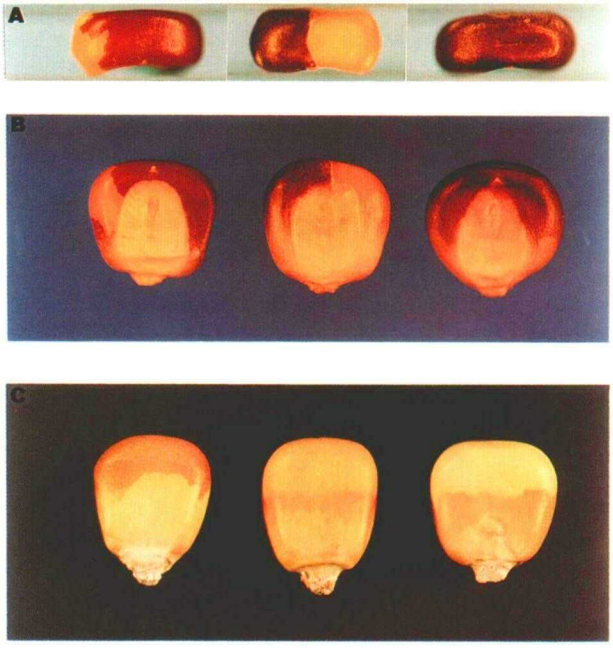

Figure 1. Endosperm Development in Maize.

(A) and **(B)** The same seed viewed from the crown **(A)** and from the embryo side **(B).** Transposon-induced variation in aleurone coloration illustrates the developmental relationships among endosperm cells. Cell lineages in the starchy endosperm and aleurone are directly related in terms of genetic constitution. Introduction of an autonomous *Spm* element in a maize stock with colorless aleurone leads to genetic instabilities at different stages of cell proliferation and appearance of aleurone coloration in defined regions of the endosperm. The seed at the left underwent an early excision event, leading to aleurone coloration in most of the endosperm. In the middle seed, the transposable element excised immediately after the first division of the primary endosperm nucleus. This division establishes the left and right halves of the kernel, and cell proliferation from the center to the surface therefore culminated in the formation of colorless and colored aleurone in the two halves of this seed. In the seed at the right, an early excision event, before the first division of the primary endosperm nucleus, led to a totally colored aleurone.

(C) Modified *opaque2* kernels showing vitreous (strong yellow color) endosperm in the top half (left), middle portion (center), and bottom half (right) of the endosperm. The formation of modified endosperm, which leads to alterations in the physical structure of the mutant seed, appears to act independently of the clonal mode of development illustrated in parts **(A)** and **(B).**

interactions that direct the wide range of patterns, shapes, and chemical composition of the endosperm. Molecules or events that trigger the biochemical switches controlling polarity, cell division, cell shape, polyploidization, and nutrient accumulation in endosperm tissue have not yet been identified in any species. Does cell proliferation in the endosperm simply result from the nutrient flow from maternal tissues, as suggested by Dure (1975), or does it result from more complex mechanisms? The recent achievements of molecular and cellular biology lead to the conclusion that many cellular interactions must be necessary to generate the remarkable invariance and complexity of the endosperm tissue.

RELATIONSHIPS BETWEEN ENDOSPERM, EMBRYO, AND MATERNAL TISSUES

The genetic balance between the endosperm, embryo, and maternal tissue has long been known to be a key requirement for normal seed development. In maize plants carrying the *indeterminate gametophyte* (*ig*) mutation, accessory polar nuclei are frequently present. Upon pollination, endosperms with ploidy levels ranging from diploid to octaploid, with only one chromosome set of paternal origin, are therefore generated. However, only the triploid endosperm with the standard ratio of one paternal to two maternal genomes develops fully (Lin, 1984). In addition, genetic imbalances generated by enforced self-fertilization, crossing species varying in ploidy level, and aneuploidy can lead to severe developmental defects in the endosperm and to seed abortion in many species (Brink and Cooper, 1947b).

The interactions between endosperm, embryo, and maternal tissues remain one of the most complex and unresolved aspects of seed development. What factors cause the zygote nucleus to develop into an embryo whereas the primary endosperm nucleus develops into a storage tissue? What kinds of biochemical exchanges occur between endosperm, embryo, and maternal tissues, and how do these exchanges affect the integrated development of these tissues? Is there a genetic program that coordinates the developmental processes that determine form, function, and integration of these tissues? Differences in the activity of maternally and paternally derived genomes in maize endosperm (Kermicle and Alleman, 1990) point to the possibility that many aspects of endosperm development, as well as the relationship between the endosperm and the embryo and maternal tissues, are defined by differentially imprinted genes. However, better understanding of the mechanism of gametic imprinting and its precise role in seed development is needed. Given our present knowledge of the complex developmental interactions taking place in most living organisms, the repertoire of morphogenetic events leading to the progressive development of endosperm and embryo within maternal tissues will challenge developmental biologists for years to come.

The isolation and characterization of mutations that disrupt normal development have been useful in addressing several aspects of endosperm development, including the complex interactions that take place among endosperm, embryo, and maternal tissues. Neuffer and Sheridan (1980) analyzed 855 maize ethylmethane sulfonate–induced *defective kernel* (*dek*) mutants representing ~285 different loci scattered throughout the genome. Most of these loci showed effects on both embryo and endosperm, and very few could be assigned to

potentially endosperm-specific genes. Similar results were reported for defective kernel (*sex*) mutants in barley (Bosnes et al., 1987). Surprisingly, both studies suggest that a large set of seed-specific genes is expressed in both endosperm and embryo, even though these tissues are remarkably different at the morphological and physiological levels. Such a conclusion could be viewed as further support for Friedman's (1990a, 1992) hypothesis of endosperm evolution from a supernumerary embryo (Olsen et al. 1992).

Genetic analysis suggests that maternal and endosperm tissues may regulate each other's development. In cereals, the base of the endosperm differentiates into several layers of transfer cells with extensive wall ingrowths. This specialized region functions in metabolite transport and provides an indirect connection with the maternal tissues. The processes of protein and starch biosynthesis are extremely dependent upon metabolic events taking place in this region. Nevertheless, little research has been directed on its biochemical and molecular characterization. The maize mutant *miniature-1* (*mn1*), first described by Lowe and Nelson (1946), illustrates the important role of the basal endosperm cells and their interaction with the nearby maternal tissue. Miller and Chourey (1992) showed that developing seeds of this mutant have drastically reduced invertase activity. Most of the sucrose arriving at the base of the endosperm from maternal cells is cleaved to glucose and fructose by invertases. These two hexoses then move to the other portions of the endosperm, where sucrose is partly resynthesized and used (reviewed by Shannon et al., 1986). In *mn1* mutants, loss of invertase activity at the base of the endosperm leads to the destruction of maternal cells transporting sucrose. Thus, the endosperm fails to accumulate starch and protein (Miller and Chourey, 1992).

On the other hand, analysis of maternal effects in *shrunken endosperm* (*seg*) barley mutants suggests that maternal transcripts could mediate endosperm development (Felker et al., 1985). This is especially interesting because maternally encoded gene products have been shown to play essential roles in early morphogenetic events leading to specification of the body axes in Drosophila larvae (Ingham, 1988). Understanding of the biological "cross-talk" that allows the endosperm genome to control the phenotype of a maternal tissue and vice versa promises to shed light on some of the complex interactions taking place between these tissues.

The differences in ploidy level and genetic constitution represented in the triad endosperm–embryo–mother plant leads to many questions concerning the integrated development of these tissues, especially in relation to nutrient flow. Nutrients flow from 2n maternal tissues to a 3n endosperm and a 2n embryo. The ratio of maternal to paternal constitution in these three tissues is 2:0, 2:1, and 1:1, respectively. It is unknown whether coordinated genome action regulates nutrient flow or whether each phase is under the independent control of a respective genome (Evenari, 1984). Vascularization stops before the point at which the maternal tissues and the developing endosperm connect. Does this indicate a boundary for control of the nutrient flow? In cereals, the apparent lack of symplastic connections between vegetative plant parts and the endosperm (Felker and Shannon, 1980; Thorne 1980, 1985) makes it difficult to find obvious physiological mechanisms involved in the flow of nutrients from maternal tissues to the growing seed. Although evidence exists for apoplastic nutrient transport from maternal tissues to the cells of cereal endosperm (Jenner, 1974; Shannon et al. 1986), the absence of similar evidence for most species, together with an as-yet poor understanding of gene activity in the regions involved in nutrient transport, make it difficult to identify key steps in the regulation of this complex process.

In addition to its better known function as a source of nitrogen, sulfur, minerals, and energy for the embryo during the germination process, endosperm is also generally considered to be a medium that supports embryogenesis. Little is known regarding the time at which the embryo begins to utilize the endosperm for nutrition. It is not clear whether the embryo passively receives nutrients mobilized by the endosperm or whether such mobilization is coordinated by the developing embryo itself or by a joint action of both tissues. Structural and physiological adaptations controlling such interactions and balancing embryo and endosperm growth must be needed, but again we have no clues about how these interactions are established and regulated. The fact that in several species mitotic activity begins earlier in the primary endosperm nucleus than in the zygote could indicate an early dependency of the embryo on endosperm development (Marinos, 1970; Raghavan, 1986). However, it is not yet possible to judge how this interaction is established and regulated.

The ephemeral nature of the endosperm tissue in many angiosperms is an intriguing but largely ignored aspect of seed development and evolution. The absence of endosperm in mature seeds of many species known to produce it at early stages of ovule development seems to indicate that the endosperm is consumed by the growing embryo (Raghavan, 1986). This process is extreme in most dicotyledonous species, which assimilate the endosperm during embryogenesis and produce embryonic leaves (cotyledons) as a means of nutrient storage. Marinos (1970) provided one of the few examples of cytological relationships between developing embryo and endosperm and the mechanisms involved in embryo nutrition. His study of developing pea seeds showed that the endosperm is absorbed in the free-nuclear state, before cell walls start to form. In addition, a cellulosic wall structure appears in the endospermic cytoplasm; Marinos suggested that this might be involved in the precise positioning of the embryo within the embryo sac and in the control of nutrient transfer to the embryo. Similarly, in sunflower (Newcomb and Steeves, 1971), cotton (Schulz and Jensen, 1977), and alfalfa (Sangduen et al., 1983), the inner wall of the embryo sac produces wall invaginations, which appear to be specialized transfer structures, into the endosperm.

The physiological mechanisms involved in the process of endosperm degradation during embryogenesis are unknown. Evenari (1984) asked questions in this regard that remain

largely unanswered: If the endosperm is digested by the embryo, what kind of signal triggers this process? Does the embryo digest the endosperm, or does it just absorb its contents after they are made available by some process of endosperm degeneration? On the evolutionary side, it is not obvious that any advantage would be provided by such remobilization. As pointed out by Dure (1975), "the detailed biochemistry of this nutrient flow, with its rise and fall of tissues, its integration and regulation, presents a unique and potentially rewarding system for developmental biologists."

Many variations in embryo and endosperm genetic constitution are generated by apomixis, a developmentally instructive deviation in sexual reproduction (see Koltunow, 1993, this issue). Apomixis consists of asexual seed formation, which leads to offspring with a maternal genotype, and it allows for the maintenance of alternation of generations without affecting the genotype. In autonomous apomicts, which are common in the families Asteraceae and Compositae, embryo and endosperm develop independently of pollination. In pseudogamous apomicts, fertilization of the polar nuclei by a sperm nucleus appears to be necessary for the initiation of endosperm development, even though the embryo is formed from an unfertilized egg cell. The existence of adventitious embryogenesis in many species, with no formation of embryo sac and associated endosperm (Ramachandran and Raghavan, 1992; Koltunow, 1993, this issue), indicates both that somatic cells have the capacity to express, under appropriate conditions, a developmental program that leads to egg cell and embryo formation (Goldberg et al., 1989) and that there is no absolute requirement for endosperm as an accessory tissue for normal embryogenesis.

ENDOREDUPLICATION OF THE ENDOSPERM GENOME

Several specialized features of genome organization and expression have been investigated in endosperm tissue. Although it has not been widely studied, a dramatic increase in nuclear DNA content is common during endosperm development. In some species, the endosperm tissue differentiates without endoreduplication (e.g., *Lilium* species), whereas in many others, the ploidy level reaches values above 300C (for review, see D'Amato, 1984). In maize endosperm, after the syncytial stage there is a dramatic increase in the size and DNA content of nuclei (reviewed by Kowles and Phillips, 1988). The most significant change occurs between 10 and 20 days after pollination, when the DNA content of these cells increases from 3C to as much as 690C. In the inbred line A188, most cells range between 10C and 50C, with a maximum of 90 to 100C. A similar, although somewhat smaller (e.g., 64C) increase in nuclear DNA content takes place in developing cotyledon cells of legumes (Millerd, 1975). In fact, genome amplification occurs frequently in metabolically active plant cells.

The purpose of the increase in DNA content of endosperm cells is not understood. Among the early hypotheses to explain the phenomenon was the possibility that it represents a mechanism for storing nucleotides for subsequent use by the developing seedling. It has also been suggested that this process may reflect amplification of genes encoding proteins and enzymes involved in the synthesis of reserve protein and carbohydrate. Despite the fact that DNA amplification and the rapid synthesis of storage metabolites appear to be linked (Tsai et al., 1970; Kowles et al., 1986), studies with gene probes demonstrated that storage protein genes and genes involved in starch biosynthesis are not specifically amplified. Rather, the entire genome is copied through repeated cycles of DNA replication (Ronison, 1983; Phillips et al., 1985). This may seem like an inefficient mechanism to increase the number of gene copies, unless it evolved to provide an additional function, such as nucleotide storage.

If genome endoreduplication increases the capacity for efficient synthesis of storage reserves, then it could have an important effect on seed development and dry matter accumulation (yield). However, these questions have not been investigated in many species. Maize *dek* mutants have been described that develop small or reduced amounts of endosperm. Kowles et al. (1992) found that endosperm nuclei in several of these mutants have significantly reduced DNA contents compared to their normal counterparts. However, the genetic lesions of these mutants are unknown, and it is difficult to assign cause and effect. Kowles and Phillips (1988) also performed experiments to describe the inheritance of endosperm nuclear DNA content. In the few crosses they examined, the inheritance of nuclear DNA content was complex, and there was no correlation between DNA content and heterosis. However, they concluded that additional experiments were warranted before it was safe to make conclusions regarding these relationships.

ENDOSPERM-SPECIFIC GENE EXPRESSION

In the course of the evolution of many plant species, many of the genes involved in endosperm functions (i.e., biosynthesis and degradation) became duplicated and acquired endosperm-specific transcriptional regulation. This was probably an early event that occurred as plants developed seedlike structures. These gene duplications were useful because they provided mechanisms whereby the same function could be differentially regulated. This was no doubt of great benefit to the plant, and it also later proved to be very useful to plant biologists because it allowed them to identify mutations in biosynthetic pathways in seed tissue that would otherwise be lethal to the plant. As a consequence of mutations in endosperm-specific genes, we know a great deal about the biosynthesis of starch, proteins, and anthocyanins. We are just beginning to learn about the transcriptional factors that interact with these genes,

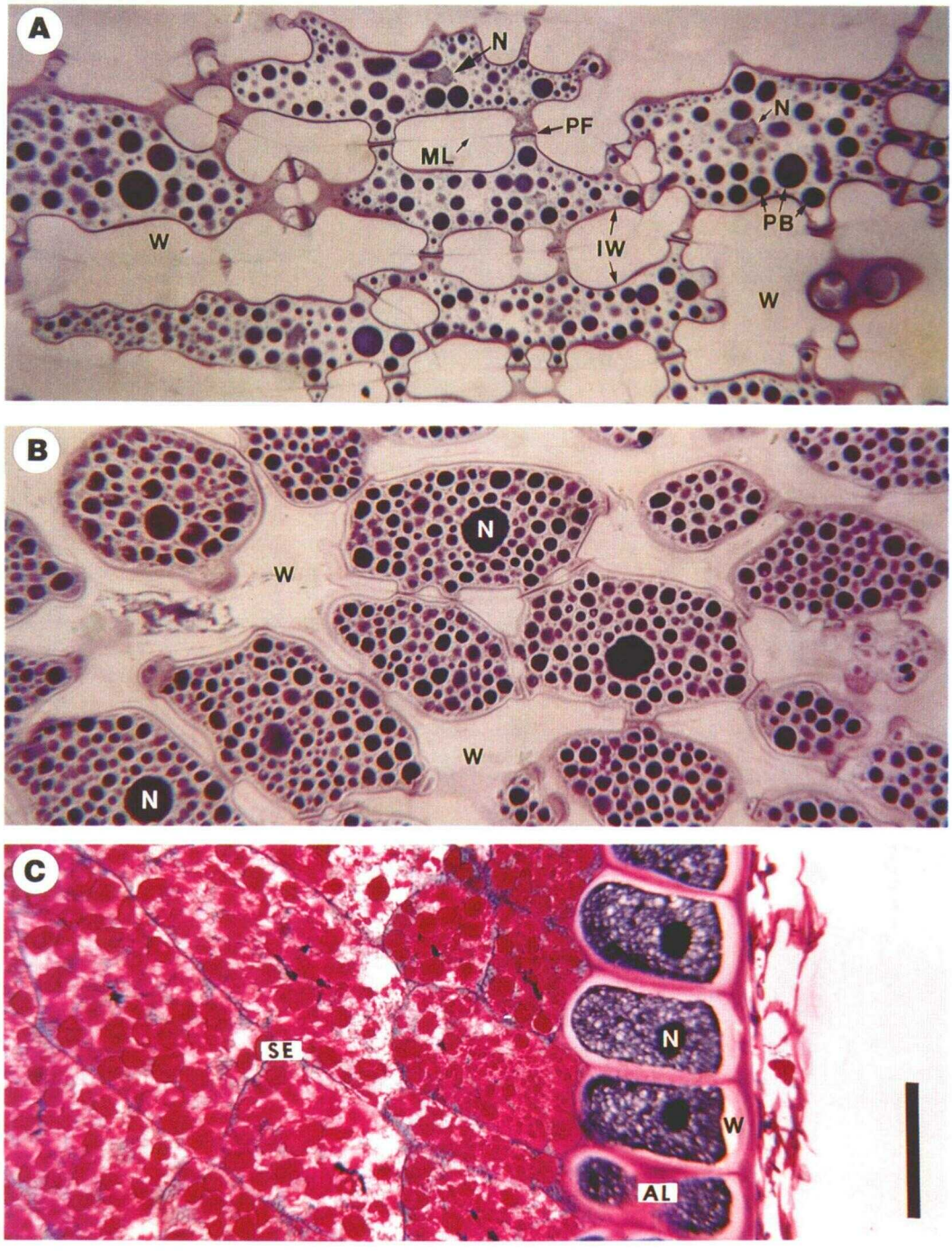

Figure 2. Light Microscope Images of Sectioned Endosperm.

(A) Endosperm from California fan palm, *Washingtonia filifera.* The endosperm consists of similar, living, nucleated cells. The reserves stored are, in order of relative abundance, carbohydrate in the form of mannan-rich cell walls (W), lipid, and protein. The cell walls are thickened except in the areas of primary pit fields (PF) and consist of three layers that differ in their staining characteristics: a middle lamella (ML), a thickened outer wall, and a thin, darkly staining inner wall (IW). Section was stained with toluidine blue.

(B) Endosperm from common onion, *Allium cepa.* The endosperm consists of similar, living nucleated cells with reserves similar to those of the previous species. Section was stained with toluidine blue.

(C) Endosperm from maize. The endosperm consists of two distinct types of cells: a single outer layer of living, thick-walled, nucleated aleurone layer (AL) cells and an inner region of "non-living," thin-walled, starch-filled cells of the starchy endosperm (SE). Starch grains are deep pink. Section was stained with periodic acid-Schiff reagent for carbohydrates and counterstained with amido black for proteins.

N, nucleus; PB, protein bodies. Bar = 50 μM.

leading ultimately to the coordinated regulation of gene expression and biosynthetic pathways.

Starch Biosynthesis and Accumulation

Reserve carbohydrate is critical for providing the embryo with a source of energy and carbon until it is photosynthetically competent. In many angiosperms, the endosperm is the primary site for storage of this type of carbohydrate. Mutants that do not accumulate storage carbohydrate germinate poorly, and in some cases this condition is lethal. In some species, reserve carbohydrates are mannans and xyloglucans deposited in endosperm cell walls, as Figure 2 illustrates (Meier and Reid, 1982). However, in most species the principal storage carbohydrate is starch, which is composed of two α-glucan polymers, amylose and amylopectin, that are packed as crystalline granules in amyloplasts, as shown in Figure 3.

Despite its importance, the mechanism of starch biosynthesis is not very well understood. The genes responsible for the key enzymatic reactions are just beginning to be characterized, and the intermediates in the pathway are a point of conjecture (for reviews, see Preiss et al., 1991; Singh, 1991; John, 1992; Hannah et al., 1993). Starch is synthesized from sucrose following its transport to the developing endosperm. The sucrose is converted to glucose-1-phosphate, which is incorporated into ADP-glucose, the key substrate for starch synthesis. Through the action of starch synthase and branching enzymes, the glucose from this high energy intermediate is polymerized, presumably via a primer, into amylose and amylopectin. The nature of the reactions that convert sucrose to ADP-glucose is not clear, and the precursor that traverses the membrane of the amyloplast has not been identified. Is it a triosephosphate, glucose-1-phosphate, or ADP-glucose? In addition, we do not know the nature of the primer used to polymerize glucose into amylose and amylopectin, the enzymatic mechanism that distinguishes between amylose and amylopectin, or how a constant ratio of the two polymers is maintained.

Finding answers to many of these questions is confounded by the difficulty of isolating intact amyloplasts. These are fragile organelles that are easily damaged during isolation by the enclosed starch grains. As a consequence, it has been difficult to draw conclusions regarding the compartmentalization of substrates and enzymes and the mechanism mediating substrate

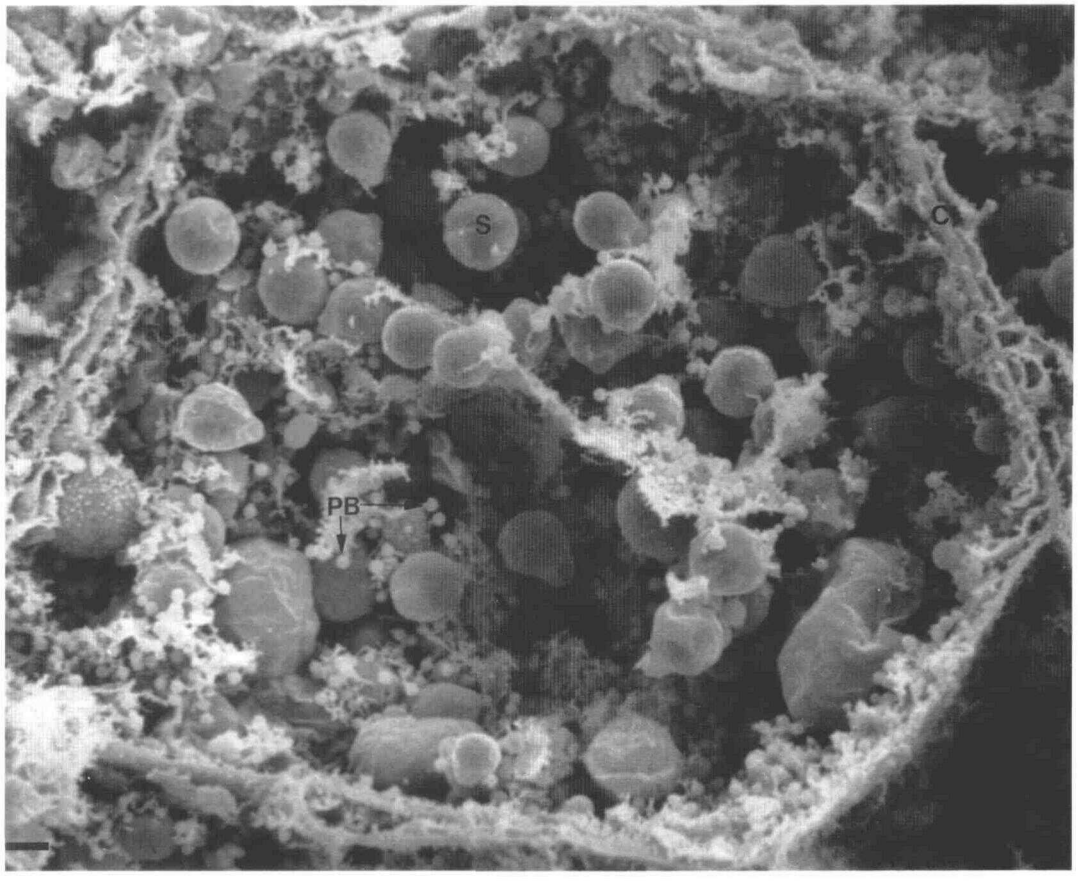

Figure 3. Scanning Electron Micrograph of a Developing Maize Endosperm Cell.

This micrograph shows starch granules (S), protein bodies (PB), and cell wall (C). Bar = 5 μM.

transport into the amyloplast. Much of what we understand about the enzymatic reactions of starch synthesis has come from biochemical and genetic studies of maize endosperm. A large number of mutations occur in maize (and a few occur in barley, rice, and sorghum) that cause defects at various steps in the pathway of starch biosynthesis (Nelson, 1980). Some mutations lead to a higher content of sucrose in the kernel and have led to the development of "sweet corn." What follows is a brief description of starch synthesis in maize endosperm, with notation of the mutations that have led to the identification of the key enzymatic steps.

The first reaction in starch biosynthesis occurs in the cytoplasm and involves the cleavage of sucrose by sucrose synthase (encoded by *Shrunken-1* [*Sh1*] and *Sus*) to yield UDP-glucose and fructose. Both of these hexoses are then converted to glucose-1-phosphate by UDP-glucose pyrophosphorylase and enzymes of the glycolytic pathway. Glucose-1-phosphate reacts with ATP via ADP-glucose pyrophosphorylase (whose subunits are encoded by the *Shrunken-2* [*Sh2*] and *Brittle-2* [*Bt2*] genes) to yield ADP-glucose. It is generally thought that glucose-1-phosphate or a precursor, dihydroxyacetone phosphate, is transported into the amyloplast and that ADP-glucose synthesis occurs in the amyloplast. However, some evidence suggests that ADP-glucose may itself be transported into the amyloplast (Akazawa, 1991). Also consistent with the idea that ADP-glucose is synthesized cytoplasmically is the observation that the Sh2 and Bt2 proteins show no evidence of transit peptides (Bae et al., 1990; Bhave et al., 1990), which might have been expected if they are transported into the amyloplast.

The *Bt1* locus encodes a protein of unknown function, although it structurally resembles an adenylate carrier protein. The phenotype of the *bt1* mutant is similar to that of *sh2* and *bt2*, which encode the subunits of ADP-glucose pyrophosphorylase. Thus, it is conceivable that the Bt1 protein controls transport of ADP-glucose into the amyloplast, although it has also been implicated in the synthesis of the primer for amylose and amylopectin synthesis (reviewed by Hannah et al., 1993).

Synthesis of amylose and amylopectin from ADP-glucose involves multiple forms of starch synthase (ADP-glucose glucosyl transferase) and branching enzymes. Two starch synthase enzymes can be distinguished, one soluble and one bound tightly to the starch grain. These may function separately because there appear to be distinct pathways for amylose and amylopectin synthesis. The *Wx* gene, which encodes starch granule–bound ADP-glucose glucosyl transferase (starch synthase I), is responsible for amylose synthesis. Starch from the *wx* mutant contains nearly normal amounts of amylopectin but no amylose. Mutants lacking the soluble starch synthase enzyme have not been found, so there is no direct evidence for its presumed role in amylopectin biosynthesis.

Starch-debranching enzymes appear to play an important role in the synthesis of both amylose and amylopectin. The *sugary* (*su1*) maize mutant accumulates phytoglycogen at the expense of amylose and amylopectin. Phytoglycogen resembles amylopectin but has approximately twice (10% versus 5%) the number of α-1,6-linkages. The *Su1* gene has not been cloned, but a starch-debranching enzyme that cleaves 1,6-linkages is greatly reduced in the *sugary* mutant. This suggests that phytoglycogen is the precursor for amylose and amylopectin. Additional evidence that phytoglycogen may be the precursor of amylose and amylopectin comes from studies of the *Amylose-extender* (*Ae1*) gene. This gene encodes a major starch-branching enzyme, which in the mutant form causes a decrease in amylopectin and a slight increase in amylose content. The *su1 ae1* double mutant contains less phytoglycogen than *su1* mutants, which suggests that amylopectin and phytoglycogen may be interconverted. Without the branching enzyme, there is reduced synthesis of the more highly branched phytoglycogen; consequently, loss of the debranching enzyme would have no obvious effect (Pan and Nelson, 1984; Hannah et al., 1993).

Mechanisms of gene regulation based on *trans*-acting regulatory proteins have been identified in pathways leading to storage protein synthesis (Schmidt et al., 1990), seed pigmentation (Ludwig et al., 1989), and seed dormancy (McCarty et al., 1991). However, there is no evidence that a similar mechanism of regulation exists for starch biosynthesis. The maize mutation *dull* (*du1*) affects the levels of starch synthase II and branching enzyme IIa in the endosperm (Preiss and Boyer, 1979). Because the activity of two enzymes is affected, *Du1* could be a regulatory locus (Shannon and Garwood, 1984). However, no specific genetic lesion has been associated with the mutation, making it impossible to verify this hypothesis. The absence of definitive evidence for the existence of transcriptional factors controlling starch biosynthesis is surprising, considering the number and variety of starch mutations identified so far. This may be an indication that nutrient flow is the key regulatory stimulus.

Sugars such as sucrose, glucose, fructose, and maltose induce patatin gene expression in potato (Wenzler et al., 1989; Park, 1992). Sucrose synthase and ADP-glucose pyrophosphorylase have been shown to be sucrose inducible in potato (Salanoubat and Belliard, 1989; Müller-Röber et al., 1990). Sucrose induction would be an advantageous mode for control of gene activity because it provides for direct response to the level of photosynthate arriving in the storage tissue. Sugars have been shown to act as modulators of source and sink interaction in maize (Foyer, 1988) as well as feedback regulators of photosynthetic gene expression (Sheen, 1990). However, it is not known whether the starch biosynthetic genes in endosperm respond to sugars in the same fashion. This and many other aspects of the regulation of starch biosynthesis await clarification, which should be forthcoming, considering the potential of genetic engineering for regulating this pathway and thus altering its products.

Storage Protein Synthesis and Deposition

Endosperm contains a variety of proteins. The most abundant of these are referred to as storage proteins, although this can be a misnomer. By definition, storage proteins accumulate for

the sole purpose of storing nitrogen and sulfur for the seedling when it germinates. Storage proteins typically have high amide contents and in some cases are rich in sulfur-containing amino acids. They are generally found within vacuoles or endoplasmic reticulum membranes as insoluble accretions. Many other proteins also accumulate to high levels within the endosperm. Among these are protease inhibitors, α-amylase inhibitors, lectins, thionins, ribosome-inactivating proteins (RIPs), and certain enzymes, e.g., sucrose synthase, urease, etc. This diverse group of proteins may act secondarily as a source of nitrogen and sulfur for the seedling during germination, but its primary role is to protect the seed from pathogens and predators and to provide biosynthetic functions.

In general, two major types of seed storage protein can be distinguished: the globulins and certain albumins, which occur in all the seed-forming species, and the prolamines, which are unique to cereals. The globulins are soluble in saline solutions of various concentrations, whereas the prolamines are typically very hydrophobic and are soluble only in alcoholic solutions or denaturing solvents. In the genera *Triticum* (wheat), *Hordeum* (barley), *Secale* (rye), *Zea* (maize), *Sorghum* (sorghum), and *Pennisetum* (millet), which constitute most of the economically important cereal species, the prolamines account for 50 to 60% of the total endosperm protein. Rice and oats are interesting exceptions because their prolamine fractions account for only 5 to 10% of the endosperm protein, and the bulk of the storage protein is made up of globulins. Globulins and prolamines are structurally very different, and they have distinctive mechanisms of synthesis and deposition (reviewed by Shotwell and Larkins, 1989).

Early studies of seed proteins revealed that soluble proteins with sedimentation coefficients in the range of 2S, 7S, and 11S are present in most angiosperms (Danielsson, 1949). These proteins, generally called globulins, are found in the endosperm, the embryo, or both. The 2S, 7S, and 11S proteins of dicots, particularly legumes, in which they are found in the cotyledons, have been very well characterized (reviewed by Higgins, 1984; Casey and Domoney, 1987; Shotwell and Larkins, 1989). Similar proteins have been described in cereal endosperms and embryos (Kriz, 1989), gymnosperm gametophytes (Leal and Misra, 1993), and fern spores (Rödin and Rask, 1990), so it appears that these globulin-type proteins occur widely in many seed-related tissues. The structures of some of these proteins are well defined (Lawrence et al., 1990; Ko et al., 1993), as are the steps in their synthesis, processing, transport, and assembly. A major area of current research is the characterization of peptides within these proteins that direct their transport to subcellular compartments (Nakamura and Matsuoka, 1993).

The genes encoding 7S and 11S globulins occur in small or moderate-sized multigene families, typically containing from 5 or 10 to as many as 20 members (Shotwell and Larkins, 1989). Sequence comparisons suggest that these genes evolved from a common ancestor (Argos et al., 1985; Borroto and Dure, 1987), although their structures are different. The genes encoding 11S globulins contain two or three introns, whereas the 7S globulin genes have as many as five. The role introns may have played in the divergence of these genes has not been investigated.

During the course of cereal evolution, the regulation of certain of the genes encoding storage proteins was apparently switched from the embryo to the endosperm. For example, cereal embryos contain a 7S but not an 11S globulin. In several cereals, notably oats and rice, the 11S globulins are major storage protein components of the endosperm tissue, but these proteins are found in only trace amounts in wheat endosperm. The nature of the evolutionary pressures that brought about the differences in tissue-specific expression of these genes is unclear. If we assume that endosperm evolved as a specialized storage tissue, then the change in deposition site may reflect increased efficiency of precursor assimilation and assembly or hydrolysis during germination.

Prolamines contain large amounts of proline and glutamine (hence, the name) and have few charged amino acids. Consequently, these proteins are insoluble in aqueous solvents. Although small amounts of proteins with these solubility characteristics can be recovered from many kinds of seed, only in cereal endosperms are they major components. Two basic types of prolamine protein can be distinguished: those found in wheat (gliadins and glutenins) and its relatives barley and rye, and those found in maize (zeins) and its relatives sorghum, millet, and coix. The prolamines in oats and rice more closely resemble those in wheat than in maize.

A common feature of prolamine proteins is the occurrence of multiple, tandem copies of short repeated peptides. These repeated peptides are flanked by unique regions that bear a strong resemblance to peptide regions found in protease inhibitors, α-amylase inhibitors, and the 2S storage globulins, and it has been postulated that genes encoding these soluble proteins were the precursors of the prolamine genes (Kreis et al., 1985; Kreis and Shewry, 1989). What was the nature of the evolutionary pressure that brought about the appearance of these tandemly repeated peptides? What is the significance of their high content of proline, glutamine, and glycine? Recent studies suggest that the repeated peptides play a role in the retention of these proteins within the rough endoplasmic reticulum (RER) (Altschuler et al., 1993), so they are perhaps important for protein–protein interactions and protein body assembly. These proteins are also important for dough formation for cooking and baking (Flavell et al., 1989; Colot, 1990). Doughs of maize, sorghum, and millet flours have very different elastic and cohesive properties, and their unique functional properties for making tortillas and many other foods are thought to be related to the properties of their storage proteins (Hoseney and Rogers, 1990).

Compared to the storage globulins, the structures of the prolamines have not been well characterized. Their analysis is much more difficult than that of globulins because of their insolubility in aqueous solvents. Consequently, few studies have addressed the conformation of prolamines (Argos et al., 1982; Tatham et al., 1985; Miles et al., 1992).

The genes encoding some prolamines, e.g., wheat gliadins and maize α-zeins, exist in very large multigene families of up to 100 members. However, others, e.g., wheat glutenins and

maize β-, γ-, and δ-zeins, are encoded by genes present in only one or two copies (reviewed by Shotwell and Larkins, 1989). As yet, there are no data regarding the significance of the absence of introns in prolamine genes, and there are no obvious explanations as to why some genes became amplified into such large families, whereas others did not.

Accumulation of storage proteins in discrete cellular bodies (Figure 3) is an adaptation that probably prevents their exposure to enzymes responsible for turnover of metabolic proteins. Other potential advantages of sequestering these proteins in membrane-bound organelles are the facilitated packaging provided by an environment of proteins with similar biochemical properties and interactive capabilities and the fact that the proteins are deposited in a relatively nonhydrated condition, which facilitates seed desiccation. In cereals such as maize and sorghum, the storage proteins are synthesized on the RER membranes and are deposited in an organized fashion within the lumen of this organelle (Lending et al., 1988; Lending and Larkins, 1989; Shull et al., 1992). In wheat (Parker and Hawes, 1982; Rubin et al., 1992), barley (Cameron-Mills and Von Wettstein, 1980), and oats (Lending et al., 1989), storage proteins occur within the vacuole. Rice storage proteins are found in both RER (prolamine) and the vacuole (globulin) (Krishnan et al., 1986). It is not known why different mechanisms are used for the transport and assembly of cereal storage proteins or why different compartments are used for deposition. Properties of their primary structure may help direct them to specific cellular organelles or specific locations within the protein body (Altschuler et al., 1993).

Maize mutations such as *floury-2*, *De-B30*, and *Mucuronate* appear to alter RER function and/or protein body assembly, resulting in severe alterations in storage protein deposition and endosperm development (Boston et al., 1991; Fontes et al., 1991; Marocco et al., 1991; Lending and Larkins, 1992). Mutations of this type affecting 7S and 11S globulins have not yet been found. The possibility of synthesizing storage proteins and expressing the genes encoding them in transgenic plants (Hoffman et al., 1987), yeast (Coraggio et al., 1988; Utsumi et al., 1991), and frog oocytes (Wallace et al., 1988) provides a way to dissect the molecular and cellular mechanisms by which these proteins are targeted and deposited in their correct cellular locations. These studies will eventually explain the unique biochemical features of these proteins.

Storage protein genes provide excellent models for studying the regulation of endosperm-specific gene expression. However, the mechanisms that regulate the expression of storage protein genes are only beginning to be uncovered. A major focus of current research is the identification of the *cis*-acting DNA sequences and *trans*-acting protein factors that control the developmental and spatial expression of storage protein genes in developing seeds (reviewed by Motto et al., 1989; Schmidt, 1993; Ueda and Messing, 1993). In maize, *O2* is the first regulatory gene shown to play an important role in controlling expression of storage protein genes. This gene has been cloned (Schmidt et al., 1987; Motto et al., 1988) and shown to encode a basic domain/leucine zipper transcriptional activator (Schmidt et al., 1990) that regulates a subset of zein storage protein genes (Schmidt et al., 1992) as well as a RIP gene (Bass et al., 1992). Another gene encoding a protein with a similar regulatory domain has been isolated and the protein it encodes has been shown to interact with *O2* in vitro (Pysh et al., 1993). Multiple pleiotropic effects caused by maize mutations at loci known to be regulatory, such as *O2*, and loci suspected to be regulatory, such as *O7*, indicate that transcriptional activator complexes are potentially involved in the regulation of genes encoding proteins with diverse functions.

Aleurone Pigmentation

The aleurone layer of maize endosperm has been an excellent system by which to study the genetic regulation of anthocyanin biosynthesis. The aleurone tissue of cereal grains is composed of one or more layers of cells that surround the starchy endosperm of the seed (Figure 2), except in the area adjacent to the embryo. Aleurone cells usually contain a large number of protein granules, oil bodies or spherosomes, and anthocyanin pigments. Anthocyanins are phenolic compounds (flavonoids) derived from the amino acids phenylalanine and tyrosine. These pigments are biochemically inactive, and their precise location within the aleurone cells remains to be established (Jayaram and Peterson, 1992).

Commercially grown maize usually has no anthocyanins in the aleurone, indicating that selection for colorless aleurone led to the fixation of mutations that prevent pigment synthesis. Instabilities at the mutant loci, which lead to total or partial reconstitution of the pathway and colored or variegated aleurone (Figure 1), were first recognized by Emerson (1914, 1917). Later, association of this phenomenon with transposable element action was established through the work of Barbara McClintock during the 1950s (reviewed by Fedoroff, 1983). By analyzing variegated mutants at loci required for anthocyanin pigment production in the aleurone, she established that transposable elements could reversibly alter the expression of genes at different chromosomal locations involved in this biosynthetic pathway.

Studies on transposable element biology and regulation have provided great insight into the regulatory control of the anthocyanin biosynthetic pathway in maize aleurone. As a consequence, it has become one of the most thoroughly investigated hierarchical systems of gene interaction and control. Variations in the alleles of the regulatory and structural loci involved in the pathway provide a great variety of pigmentation patterns and the possibility to study important aspects of interactive gene action at the molecular level (reviewed by Coe et al., 1988; Jayaram and Peterson, 1992).

OTHER ENDOSPERM COMPONENTS

There is great diversity among species in the nature of food reserves in the endosperm. In addition to starch and protein, many seeds store lipids, a variety of carbohydrates, and a diverse array of other organic and inorganic compounds.

However, the biosynthetic regulation of many of these storage components remains to be described.

Oilseeds are among the most ancient crops to be domesticated by man for both edible and nonedible applications. Oils have a much higher caloric value (38 kJ/g) than protein or carbohydrate (17 kJ/g) (John, 1992), but few species store large amounts of oil in the endosperm. Species storing oil in the endosperm, such as castor bean (*Ricinus communis*), oil palm (*Elaeis guineensis*), opium poppy (*Papaver somniferum*), and spurge (*Euphorbia* species) accumulate up to 50% of the seed dry weight in oils (Bewley and Black, 1978). These reserves are packaged into discrete organelles known as lipid bodies, spherosomes, or oleosomes, and they usually consist of triacylglicerols surrounded by a half-unit phospholipid membrane and few proteins (Huang, 1991; Bednarek and Raikhel, 1992).

Carotenoid synthesis and function in endosperm tissues have received only scant attention. These lipid-soluble compounds are found in the endosperm of maize and sorghum, where their biological function is unknown. Carotenoids in maize endosperm occur in two forms, carotenes and xanthophylls, and are responsible for the yellow color of most commercial cultivars (Goodwin, 1980). Of the cereal grains, only yellow maize endosperm contains significant amounts of β-carotene, a major source of provitamin A, which is essential in human diets (Buckner et al., 1990).

Many seeds store complex polysaccharides such as mannans and xyloglucans in the endosperm cell walls (Figure 2). The mature cell walls of palm (*Phoenix dactylifera*) endosperm are composed mainly of carbohydrate that is synthesized as a galactose-rich galactomannan and later polymerized into relatively pure mannan (Meier and Reid, 1982; DeMason et al., 1992). Ivory nut (*Phytelephas macrocarpa*) has mannans as the major storage carbohydrate (79% of the endosperm dry weight); the very hard endosperm of this species is due to the impregnation of the cell walls with this compound (Bewley and Black, 1978).

Macronutrients such as phosphorus, magnesium, potassium, and calcium are also sequestered within the endosperm, primarily as the organic phosphorus reserve phytin, which is the mixed potassium, magnesium, and calcium salt of *myo*-inositol hexa*kis*phosphoric acid (Greenwood, 1989). Accumulation of phytin in the endosperm of cereals occurs preferentially in discrete globoid bodies associated with proteins inside the aleurone grains (Bewley and Black, 1978; Irving et al., 1991). Although phytic acid constitutes only a minor fraction of the total seed weight, it represents 50 to 90% of the total phosphorus reserve in the mature seed. During germination, phytase catabolizes phytic acid, releasing phosphate and *myo*-inositol to the growing embryo (Greenwood, 1989).

STORAGE PRODUCTS AND ENDOSPERM DEVELOPMENT

Much contemporary thought on morphogenesis centers on gene regulation, even though the key influences or controls do not operate in isolation (Green, 1991) but as a series of complex temporal and spatial interactions that are context dependent (Goodwin, 1985). The products of regulatory genes, or those of the genes they regulate, most probably affect the dynamics of one or more physiological or interactive processes, leading to a cascade of inter- and intracellular reactions that affect differentiation and morphogenesis. For instance, actin and tubulin are necessary for morphogenetic movements to occur during animal development. Deficiencies leading to impairment in synthesis or function of these gene products would prevent or severely affect morphogenesis (Nijhout, 1990).

In this context, storage products may cooperate with other cellular components to provide the material basis for the development of endosperm tissue. Very little is known about the role endosperm components play in morphogenesis and differentiation, but evidence for a possible involvement comes from the phenotype of mutants missing certain storage components. Mutations affecting endosperm storage protein deposition and starch biosynthesis in several cereal species are known to cause developmental perturbations that lead to dramatic changes in the chemical composition and physical structure of the endosperm. In the diploid cereals barley, maize, and sorghum, such mutations are common and well studied (Nelson, 1980). Storage protein mutants often have a fragile and lusterless endosperm and are usually described as opaque or floury. Starch mutants usually have collapsed or shrunken seeds, with the severity of the phenotype generally correlated to the extent of reduction in starch accumulation. Both types of mutants germinate poorly and are more susceptible to insects and pathogens. Dombrink-Kurtzman and Bietz (1993) suggest that maize protein composition and distribution influence endosperm texture and physical properties in normal kernels. In addition, altered storage protein deposition patterns in maize *o2* mutants have been shown to be associated with profound modifications in endosperm development and physical structure (reviewed by Larkins et al., 1992). Although several mutations that affect storage product accumulation and endosperm morphogenesis exist in many other species, most of the research has been directed to understanding various aspects of yield and nutritional quality and not to questions pertaining to endosperm development and physical structure.

Although endosperm tissue is viable in many germinating seeds, in cereals it is thought to be "dead." The massive accumulation of storage compounds (Figure 3) and subsequent desiccation may lead to total impairment of physiological activities in mature cereal endosperm cells, with the exception of aleurone. This has been a somewhat controversial issue because there is no definitive experimental evidence that cereal endosperm is physiologically "dead" in mature seeds. The scutellum and aleurone in cereals differentiate as digestive tissues specialized in the secretion of enzymes that mobilize endosperm reserves during seed germination. Due to their autotrophic mode of nutrition, plants have only infrequently developed such types of tissue (Jones and Jacobsen, 1991). The development of this mechanism to mobilize seed reserves could be considered an indication of the physiological inactivity of the starchy endosperm in cereals at the time of germination.

On the other hand, endosperm of species that lack an aleurone must remain "alive" and capable of producing hydrolytic enzymes to mobilize reserves during germination. This seems to be the case in lettuce, in which structural evidence indicates that active metabolism takes place in the endosperm cells during early phases of germination (Jones, 1974). In date, the endosperm consists of living cells that undergo aerobic respiration upon imbibition, even though they are not capable of de novo enzyme synthesis (De Mason et al., 1983). In this case, the seed must use other, as yet unknown, mechanisms to mobilize reserves to the growing embryo.

SEED MATURATION, DORMANCY, AND RESERVE MOBILIZATION

Maturation is an essential step in seed development that leads to a decline in reserve synthesis and to desiccation and dormancy. Maturation in cereal seeds occurs late in seed development as abscisic acid (ABA) levels rise, preventing the immature embryo from germinating. ABA is also thought to promote embryo tolerance to desiccation (see Thomas, 1993, this issue). In maize and other cereals, the aleurone layer of the endosperm remains viable after the seed dries, and for this reason it too undergoes a maturation process (McCarty and Carson, 1991).

Several mutations (*viviparous, vp*) affect seed dormancy in maize (Coe et al., 1988). Most of these appear to have reduced levels of ABA and probably have defects in the hormone's biosynthetic pathway (Neill et al., 1986). An exception is *Vp1*, which instead has been shown to have a reduced sensitivity to ABA (Robichaud and Sussex, 1986). This mutant also fails to accumulate anthocyanins in the aleurone (Robertson, 1955). Transposon mutagenesis has been successfully used to tag and isolate the *Vp1* gene (McCarty et al., 1989). The gene product is a multifunctional regulatory protein that controls the expression of *C1*, a regulatory gene in the anthocyanin biosynthetic pathway, as well as the response of the embryo and aleurone tissues to ABA. Molecular analysis of *vp1* mutations has shown that these two functions are associated with distinct domains in the regulatory protein, indicating that a functionally complex molecule may be necessary for the regulation of the diverse steps involved in the maturation process (McCarty and Carson, 1991; McCarty et al., 1991).

Most of the research on the control of the enzymes involved in the mobilization of endosperm reserves during seed germination has been performed in cereals, in which the aleurone layer is one of a few examples of digestive tissues found in the plant kingdom (Jones and Jacobsen, 1991). Its presence makes germinating cereal seed a convenient system for studies of the molecular action of plant hormones and transport processes involved in seed germination and reserve mobilization. Shortly after the onset of germination, the aleurone cells respond to gibberellic acid produced by the embryo by synthesizing and secreting a battery of enzymes that hydrolyze the stored starch, proteins, cell wall polysaccharides, and nucleic acids. ABA, on the other hand, induces seed dormancy and prevents all the above effects of gibberellic acid (Ho, 1991).

The metabolic reversal that takes place in the endosperm of species that do not develop specialized digestive tissues is an intriguing aspect of seed development and reserve mobilization (Dure, 1975). For example, when germination commences in castor beans, physiological activity in the endosperm is resumed, with protein hydrolysis and gluconeogenesis mobilizing reserves for the embryo. Therefore, the same cells that synthesize large amounts of reserve materials completely reverse this process and rapidly hydrolyse the same materials during germination. The mechanisms that deactivate and activate genes to operate such a fundamental reversal remain largely unknown.

FUTURE PROSPECTS IN ENDOSPERM RESEARCH

Very little is known about the biochemical composition of the endosperm tissue of many species. Components such as storage and nonstorage proteins, phytin, oils, carotenoids, polysaccharides, free amino acids, and phenolics are known to exist in varying proportions in the endosperm. A better understanding of their cellular locations, functional roles, and effects on nutritional quality, seed storage, and germination will provide knowledge to improve seed quality and use. Improvement of nutritional quality in cereals has been hampered by lack of knowledge of endosperm proteins that contain nutritionally desirable amino acids. Development of cereal cultivars with high nutritional quality has traditionally been based on the use of mutations that have undesirable pleiotropic effects. The development of biochemical assays that allow efficient characterization of endosperm components among a large number of progeny promises to help breeders better explore existing genetic variation for development of improved cultivars.

The availability of a variety of genes specifically expressed in endosperm and the development of efficient reporter gene systems and transformation techniques have been useful for probing gene regulation and cell function. Research in this area will likely be important for many years to come because identification of endosperm-specific promoters is essential for developing engineered genes that can lead to accumulation of novel or quality-enhanced products in the endosperm. Molecular biology is already providing alternative and complementary approaches to conventional breeding techniques for plant improvement. Expression of high levels of nutritionally desirable proteins encoded by introduced genes will eventually lead to the development of seed crops with improved nutritional quality. Yield increases are greatly dependent on the possibility of increasing the efficiency of deposition of major endosperm reserves such as starch. For example, transgenic potatoes containing a mutant *Escherichia coli* gene

encoding ADP-glucose pyrophosphorylase that enhances a rate-limiting step in starch biosynthesis have a 30% increase in starch content in the tuber (Stark et al., 1992). This achievement provides hope that similar developments with engineered cereal endosperm will result in cultivars with higher yield potential.

Endosperm is unique in several aspects of its development, physiology, cell biology and biochemistry. Its apparent simplicity, reflected by the presence of a few differentiated cell types, has permitted detailed studies on histodifferentiation and the biosynthesis of certain stored metabolites. However, little attention has been devoted to understanding the genetic and developmental basis of endosperm differentiation and function. In the past, the apparent morphological simplicity of endosperm tissue led many to view it as a mass of storage cells or a simple appendage to the embryo. Some believe that the nutrient flow from maternal tissues could be the only factor guiding differentiation in this tissue. However, the characterization of developmental mutants in cereal endosperm is beginning to show that complex genetic controls are responsible for the remarkable constancy observed in the development of the endosperm tissue.

The study of interactions of endosperm, embryo, and maternal tissues is an exciting area of research that promises to provide many answers concerning the complex temporal and spatial interactions that affect the development of plant tissues. It is surprising that so little attention has been dedicated to the study of the developmental differences between species in which the endosperm is absorbed during seed development versus species in which this tissue is absorbed during germination. In addition, many questions remain to be answered regarding the regulation of endosperm digestion during germination of endospermic species that lack an aleurone.

The functional importance of double fertilization in angiosperms is one of the most puzzling questions in plant biology. Evidence that in maize, at least, the products of double fertilization are differentially imprinted points to the possibility that epigenetic differences in progenitor nuclei could be responsible for the steps leading to endosperm and embryo differentiation (Kermicle and Alleman, 1990). If so, differences in gene expression generated by transmission through the female versus the male may help explain other obscure aspects of plant development.

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