

Aleurone Cell Development

Philip W. Bercraft

Genetics, Development & Cell Biology/Agronomy, Iowa State University,
Ames, IA 50011, USA
bercraft@iastate.edu

Abstract The periphery of the endosperm of many plant species forms an epidermis-like layer called the aleurone. During germination, the aleurone performs an important digestive function, secreting hydrolases to break down the starch and proteins stored in the starchy endosperm cells. Several features of cereal aleurone cells make them an attractive system for studying fundamental questions of cell fate and differentiation. The system is conceptually simple, with a single fate choice between starchy endosperm or aleurone cell types. The surface location makes the cells accessible for study, and they can be readily isolated by peeling from developing grains. Because of these experimental advantages and its importance to crop utilization, aleurone development has been most intensively studied in cereals. This chapter describes a picture of aleurone cell fate specification and development as a dynamic system displaying unique modifications to the cell cycle and cytoskeletal arrays, and surprising plasticity in cell fate decisions.

1

Introduction

In cereals, and some dicots with persistent endosperms, the aleurone layer has important functions in the accumulation of storage compounds during seed development, and in the mobilization of storage compounds during germination. In other seeds, such as *Arabidopsis*, that have a transitory endosperm, an aleurone layer persists in the mature seed (Brown et al. 1999) and functions in seed dormancy and germination (Bethke et al. 2007).

The storage function of aleurone cells in cereals involves the accumulation of high levels of phytic acid, which chelates several minerals. In barley grains, the aleurone is the major storage site for phosphate, magnesium, potassium, and calcium, accumulating over 70% (97% for magnesium) of the endosperm stores of these minerals (Stewart et al. 1988). During germination, the aleurone performs an important digestive function, secreting hydrolases to break down the starch and proteins stored in the starchy endosperm cells, which undergo programmed cell death during endosperm development (Young and Gallie 2000). The germination response is under hormonal control; gibberellic acid (GA) produced by the germinating embryo induces amylase gene expression and secretion, while abscisic acid (ABA) acts antagonistically to suppress these activities. Barley aleurone has served as an important model system for studying the mechanisms of GA and ABA action.

Until recently it was a matter of debate whether the peripheral cell layer of the *Arabidopsis* endosperm should actually be considered an aleurone, but a recent report shows clearly that the structure and function of *Arabidopsis* aleurone cells are very similar to cereals (Bethke et al. 2007). Secretory activity during germination apparently causes cell wall weakening to facilitate seedling emergence from the seed. This secretory activity was induced by GA and suppressed by ABA, just as in cereals. Furthermore, the aleurone layer was shown to be required for seed dormancy, a previously unknown function.

In addition to the importance of aleurone to the plant, aleurone impacts human well-being in several ways. Aleurone has reported dietary and health benefits; aleurone flour dramatically decreased the incidence of colon adeno-

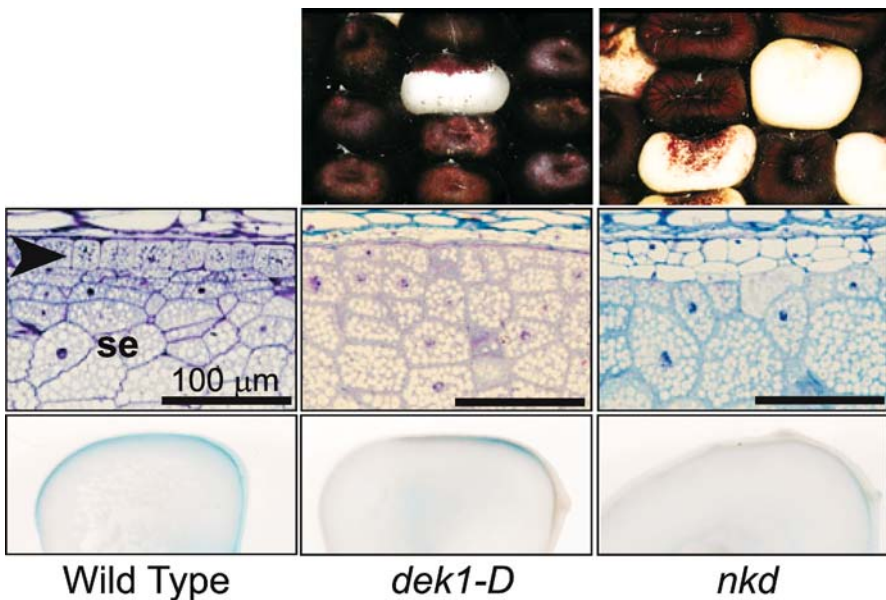


Fig. 1 Examples of mutations affecting maize aleurone development. Wild-type maize contains a single layer of aleurone cells (*arrow*), which accumulates anthocyanin in particular genotypes. Starchy endosperm cells (*se*) are recognizable by the large unstained starch grains. Aleurone markers, such as the *Vp1::GUS* reporter shown here (*blue*), express around the entire periphery of the endosperm (Cao et al. 2007). *dek1* is an example of a mutation affecting cell fate. Instead of aleurone, the peripheral layer of endosperm cells adapts a starchy endosperm cell fate. The *dek1-D* allele causes partial loss of aleurone, as seen by the partial loss of anthocyanin pigmentation and *Vp1::GUS* expression. In *nkd** mutants, the cell fate decisions appear normal because the peripheral cells are clearly distinct from starchy endosperm. However, they do not acquire the characteristics of normal aleurone cells, including the dense cytoplasm, anthocyanin accumulation, and marker gene expression, indicating that this mutant blocks the differentiation process

mas in rats, while raising red blood cell folate levels and decreasing plasma homocysteine levels in humans (McIntosh et al. 2001; Fenech et al. 2005). Aleurone is linked to environmental concerns; because phytate is not readily digested by livestock, most of the phosphate present in cereal grain-based animal feeds is excreted, posing a serious water pollution problem if runoff from manure is not controlled (Adeola 1999). Finally, aleurone has a major impact on leisure activities; amylases produced during germination convert starch to fermentable sugars, giving aleurone an all-important role in the malting process.

Several features of cereal aleurone cells make them an attractive system for studying fundamental questions of cell fate and differentiation. The system is conceptually simple, with a single fate choice between starchy endosperm or aleurone cell types. The surface location makes the cells accessible for study, and they can be readily isolated by peeling from developing grains. A number of aleurone markers are available, including several genes that have been used to regulate the aleurone-specific expression of GUS or GFP reporters (Gavazzi et al. 1997; Costa et al. 2003; Opsahl-Sorteberg et al. 2004; Wisniewski and Rogowsky 2004; Gruis et al. 2006; Cao et al. 2007). In maize, particular genotypes specifically accumulate anthocyanin pigment in the aleurone cells (see the chapter by Cone, in this volume), which has been used as a convenient marker for performing genetic screens for mutants that affect aleurone development (Gavazzi et al. 1997; Becraft and Asuncion-Crabb 2000). Figure 1 shows examples of anthocyanin pigmentation and expression of the *Vp1::GUS* reporter in wild-type and mutant maize endosperm. Because of these experimental advantages and its importance to crop utilization, aleurone development has been most intensively studied in cereals. The picture that emerges is of a dynamic system displaying unique modifications to the cell cycle and cytoskeletal arrays, and surprising plasticity in cell fate decisions.

2

Overview of Aleurone Ontogeny

Cereals show nuclear endosperm development, where the first several rounds of mitosis occur without cytokinesis to produce a coenocytic endosperm (see the chapter by Brown and Lemmon, in this volume). After a period of free-nuclear division, the nuclei come to occupy a thin layer of cortical cytoplasm surrounding a large central vacuole. Cellularization commences through the process of free cell wall formation whereby phragmoplasts form between both daughter and non-daughter nuclei. These cytoplasmic phragmoplasts direct cell wall deposition as walls grow inward to form a honeycomb-like assembly (see the chapter by Otegui, in this volume). Nuclei are located in separate alveoli, which are open on the inner face to the common syncytial cytoplasm.

Then a periclinal division occurs, accompanied by cytokinesis, to produce a cellular peripheral layer and an alveolar internal layer. This process reiterates in the alveolar layer until the volume of the endosperm is completely cellularized (Kiesselbach 1949; Brown et al. 1994, 1996; reviewed by Olsen 2001, 2004a).

After cellularization, cell division initially occurs throughout the endosperm but becomes localized to a cambium-like region at the periphery. Periclinal divisions produce distinct cell files that radiate from the center of the endosperm (Randolph 1936; Kiesselbach 1949; Morrison et al. 1975; McClintock 1978). As growth continues, cell divisions in the surface layer become strongly biased to the anticlinal plane to allow surface growth to keep pace with the expanding endosperm volume. At this point the aleurone cells are cuboidal in shape, thin-walled, and highly vacuolate. Most lines of maize and wheat contain a single layer of aleurone cells, while rice contains one to three and barley contains three layers.

As differentiation ensues, the cell walls become thick and highly autofluorescent, and the cytoplasm takes on a dense granular appearance (Morrison et al. 1975). Aleurone cells are rich in endoplasmic reticulum, mitochondria, and a variety of membrane bound vesicles (Jones 1969). Lytic vacuoles and protein storage vacuoles are both present (Swanson et al. 1998), and two types of inclusion bodies can be found within the same vacuole (Jakobsen et al. 1971). Globoid bodies, also known as aleurone grains, contain a crystalline matrix of phytin and protein surrounded by lipid droplets, while the second type of inclusion is a protein-carbohydrate body (Jones 1969; Jakobsen et al. 1971; Morrison et al. 1975; Kyle and Styles 1977; Swanson et al. 1998).

During seed maturation, the starchy endosperm cells undergo programmed cell death (Young and Gallie 2000), while the aleurone cells and embryo express a specific maturation program that allows them to survive seed desiccation (reviewed by Vicente-Carbajosa and Carbonero 2005). Maturation is positively regulated by ABA and involves the accumulation of late embryo abundant (LEA) proteins that function as dehydrins to protect cell membranes and proteins in desiccated seeds (Goyal et al. 2005). In particular genotypes of maize, anthocyanins specifically accumulate in the aleurone layer during the maturation phase (reviewed by Dooner et al. 1991).

The final stage in the life of an aleurone cell occurs after seed imbibition when the embryo sends a GA signal that induces the germination function of the aleurone. Hydrolase genes such as α -amylase are induced and their products secreted into the starchy endosperm for the remobilization of stored carbohydrates and amino acids to supply the growing seedling. This is a terminal process in that the aleurone cells expend all their resources and undergo non-apoptotic programmed cell death (Bethke et al. 1999; chapter by Nguyen et al., in this volume).

3 Positional Specification of Aleurone Cell Fate

Cells at the endosperm periphery must somehow interpret their position and assume aleurone cell fate. The first periclinal division is noteworthy because this establishes a peripheral cell layer, which constitutes the initial cells that will ultimately produce the aleurone layer. The propensity of anticlinal divisions led to early models which stated that the aleurone formed a separate lineage from the starchy endosperm (Randolph 1936; Kiesselbach 1949; Coe 1978; Levy and Walbot 1990; Walbot 1994). However, periclinal divisions can be observed in aleurone cells (Morrison et al. 1975) and genetically marked sectors showed that even late in development the aleurone contributed cells internally to the starchy endosperm (Becraft and Asuncion-Crabb 2000). Thus the peripheral cells serve as initials for both the aleurone and starchy endosperm.

Immediately following the first periclinal division the peripheral cells assume a distinct behavior from internal cells. Like most plant cells, the peripheral endosperm cells produce a pre-prophase band (PPB) of microtubules that forms prior to mitosis and predicts the plane of mitosis and cell plate formation (Brown et al. 1994, 1996). Internal cells have the unusual property of lacking a PPB. A phenomenon likely to be related to this is the difference in cell division behavior between peripheral and internal cells; the peripheral cells divide in anticlinal or periclinal planes, while the internal cells divide in random planes (Randolph 1936; Kiesselbach 1949). Thus it would appear that at the time of cellularization, peripheral determinants confer properties to these cells that distinguish them from internal cells.

Despite the early appearance of characters that distinguish aleurone cells from starchy endosperm cells, the identity of peripheral endosperm cells remains surprisingly plastic through late stages of seed development. As shown in Fig. 1, the aleurone is missing in *dek1* mutants and peripheral cells assume starchy endosperm identity (Sheridan 1982; Becraft et al. 2002; Lid et al. 2002). It follows that the *dek1* mutant cells are unable to perceive or respond to the positional cues that normally specify aleurone identity. Induction of *dek1* mutant sectors late in development caused aleurone cells to lose their identity and transdifferentiate into starchy endosperm (Becraft and Asuncion-Crabb 2000). Conversely, reversion of an unstable transposon-induced *dek1* allele allowed starchy endosperm cells in the peripheral layer to transdifferentiate to aleurone cells. In both cases, sectors as small as a single cell could be observed indicating that cell fate remained plastic up through the last cell division in endosperm development. Further evidence of plasticity is seen when maize kernels occasionally become conjoined. At the surfaces that form the junction, the aleurone layers transdifferentiate to starchy endosperm cells (Geisler-Lee and Gallie 2005). The upshot of these studies is that peripheral endosperm cells constantly monitor their position, and that

positional cues are required to specify and maintain aleurone cell identity throughout endosperm development.

What is the source of the positional cues? Current evidence suggests that the key determinant for aleurone identity is surface position on the endosperm per se, rather than juxtaposition to nucellus or other maternal tissues. A maize mutant produces an endosperm comprised of spherical masses of cells and each of these spheroids forms its own aleurone layer, even though it is not in direct contact with maternal tissues (Olsen 2004b). Conversely, when two maize kernels become conjoined, cells that had occupied a surface position become internalized and consequently lose their aleurone identity (Geisler-Lee and Gallie 2005). Most compelling is that endosperms grown in culture can establish and maintain an aleurone layer despite the absence of an embryo or any maternal tissues (Gruis et al. 2006). Thus the isolated endosperm appears competent to confer aleurone identity to cells occupying the surface position. An exception is the disorganized endosperm of the *globby* mutant of maize, where isolated aleurone cells can be found internally (Costa et al. 2003).

4 Genes and Molecules

Our understanding of aleurone development at the molecular level is still in the early stages. Genetic studies suggest that a hierarchical system regulates the acquisition of aleurone cell fate and the subsequent differentiation of aleurone characteristics (see Fig. 2). Several maize mutants, including *cr4* and *dek1*, block the formation of aleurone; peripheral cells that would normally form aleurone develop instead as starchy endosperm (Becraft et al. 1996, 2002; Becraft and Asuncion-Crabb 2000; Lid et al. 2002; Wisniewski and Rogowsky 2004). Thus the normal gene products are required for aleurone cell fate specification. Figure 1 shows an example of a *dek1* mutant in maize.

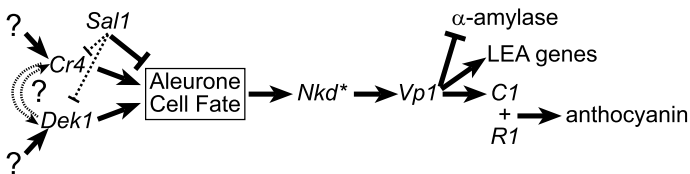


Fig. 2 Model genetic pathway for the regulation of maize aleurone cell development. Aleurone cell fate acquisition is positively regulated by CR4 and DEK1, and inhibited by SAL1. Hypothetical regulatory interactions among CR4, DEK1, and SAL1 are shown with *dotted lines*. As yet unknown factors function upstream of CR4 and/or DEK1. After aleurone cell fate specification, genes such as *Nkd** control cell differentiation, including the expression of *Vp1*. *Vp1*, in the presence of ABA, promotes aleurone maturation, including the accumulation of anthocyanin pigments in the appropriate genotypes

Mutation of *dek1* also blocks aleurone formation in *Arabidopsis*, indicating a conserved function (Lid et al. 2005). The *sall* mutant has the opposite effect; the fate of peripheral starchy endosperm cells is switched to aleurone, producing a multilayered aleurone (Shen et al. 2003).

Other mutants establish a peripheral layer of cells that is distinct from the starchy endosperm but which lacks some of the characteristics of normal aleurone (Gavazzi et al. 1997; Becraft and Asuncion-Crabb 2000; Lid et al. 2004). These are interpreted as functioning in the aleurone differentiation process, downstream of the cell fate genes. Interestingly, many of the genes that affect the aleurone also affect epidermal development on the plant (Becraft et al. 1996, 1999, 2002; Jin et al. 2000; Kessler et al. 2002; Lid et al. 2004, 2005; Watanabe et al. 2004; Johnson et al. 2005), suggesting that a related mechanism controls the development of both tissues. Hence, it appears appropriate to consider the aleurone as the epidermis of the endosperm.

Several of the mutants that disrupt maize aleurone differentiation, such as *cr4* and the weak *dek1-D* allele, cause mosaic phenotypes (see Fig. 1). The mosaicism shows a predictable pattern with a strong tendency for disruption of aleurone development at the crown region and abgerminal side of the kernel, while the germinal face tends to develop an intact aleurone layer. This is hypothesized to reflect a patterning mechanism that organizes endosperm development (Becraft and Asuncion-Crabb 2000). Interestingly, ectopic cytokinin production under the regulation of a senescence inducible promoter phenocopies these mutants to produce kernels with mosaic aleurones (Geisler-Lee and Gallie 2005). This suggests that a hormone gradient could be involved in establishing endosperm pattern and aleurone fate, or at least that the normal establishment or interpretation of pattern can be disrupted by inappropriate hormone levels.

In addition to the specific genes mentioned, global genome level factors are also important for endosperm development in general, and aleurone development in particular. Aleurone cells in endosperms with a 2 : 2, instead of the normal 2 : 1, balance of maternal and paternal genome contributions lack the large number of spherosomes normally found in aleurone cells (Charlton et al. 1995). It is not known whether this results from imprinting at a specific locus or is a symptom of more general effects of genomic imbalance.

Several of the genes involved in aleurone cell fate specification have been isolated and appear to encode signal transduction components. The *cr4* gene encodes a receptor-like kinase with a tumor necrosis factor receptor (TNFR)-like motif in the extracellular domain (Becraft et al. 1996). This suggests that CR4 could function in the perception of the positional cues that specify aleurone identity. *Dek1* encodes a large protein with 21 predicted transmembrane regions, an extracellular loop, and a cytoplasmic domain containing a calpain protease (Lid et al. 2002; Wang et al. 2003). This configuration also allows the possibility that DEK1 could function as a cell surface receptor.

Genetic studies suggest that *Cr4* and *Dek1* participate in the same biological process, possibly as components of a signal transduction system (Beecraft et al. 2002). At this point, the functional relationship between CR4 and DEK1 is unclear at the molecular level. In *Arabidopsis*, active forms of the CR4 ortholog, ACR4, are rapidly proteolyzed in endosomes (Gifford et al. 2005). Thus it is possible that CR4 is a substrate of the DEK1 protease. It is also possible that proteolysis of CR4 represents a processing step necessary for signal transduction. Alternatively, it is possible that CR4 regulates DEK1 activity by phosphorylation of either DEK1 itself or of a DEK1 substrate. The activity of animal calpains can be regulated by the phosphorylation of either the calpain protein itself or of its substrate (Nicolas et al. 2002; Shiraha et al. 2002). Yet another possibility is that both proteins regulate separate signaling events that converge further downstream.

The *Sal1* gene encodes a vacuolar sorting protein related to human CHMP1 (Shen et al. 2003). CHMP1 is involved in the endocytic recycling of receptors from the plasma membrane (Howard et al. 2001). This suggests the intriguing possibility that SAL1 could regulate CR4 and/or DEK1 by internalizing them, either for degradation or as part of the signaling mechanism. The opposite phenotypes of *sal1* versus *cr4* or *dek1* mutants suggests that SAL1 would probably function as a negative regulator of CR4 or DEK1 signaling, making the former possibility more likely. In addition, the aberrant vesicle traffic observed in *cr4* mutants (Jin et al. 2000) suggests the possibility that CR4 might also function to regulate SAL1 activity. This mutual regulation could establish a balance between CR4 and SAL1 activity, which would ultimately determine the number of aleurone cell layers formed.

The maturation phase of aleurone development is regulated in the aleurone in much the same way as it is regulated in the embryo (see Vicente-Carbajosa and Carbonero 2005 for review). ABA is the key hormone that promotes maturation, and maize mutants in ABA biosynthetic genes cause a viviparous phenotype involving precocious germination of the embryo and a concomitant activation of digestive functions in the aleurone. ABA induces the expression of LEA genes and other maturation associated genes in the aleurone (Miyoshi et al. 2002; Furtado and Henry 2005; Bethke et al. 2006). *Viviparous1* encodes a B3 domain transcription factor that is a central regulator of the maturation process. In maize, VP1 also activates anthocyanin production by promoting expression of the C1 gene (Hattori et al. 1992; chapter by Cone, in this volume). As such, VP1 is the most upstream known factor in the transcriptional regulation of anthocyanin synthesis in the aleurone. *vp1* mutants lack anthocyanin in the aleurone, and express α -amylase genes associated with germination (Hoecker et al. 1995). The *Vp1* gene is expressed in the embryo and aleurone (Cao et al. 2007), but how *Vp1* expression is spatially regulated in the endosperm is not known. In addition, the bZIP transcription factor HvABI5 is required for ABA induction of maturation associated genes in barley aleurone (Casaretto and Ho 2003).

5 Conclusions

The aleurone layer is critical to plant survival and several different functions for the aleurone have evolved in different taxa. The most familiar is in cereal grains where aleurone has both storage and digestive functions. The aleurone layer provides a powerful system for studying basic questions of cell fate specification and differentiation. Additionally, it might be possible to manipulate the aleurone layer to engineer altered seed properties, such as increased malting rate or enhanced mineral or lipid content. Several key regulators of aleurone development have been identified. Further work to understand how these factors interact, how they are regulated, and the signal transduction systems they control, will provide insights into the biology of this fascinating system and may provide the knowledge needed to realize the potential for improved seed quality.

References

- Adeola O (1999) Nutrient management procedures to enhance environmental conditions: an introduction. *J Anim Sci* 77:427–429
- Becraft PW, Asuncion-Crabb YT (2000) Positional cues specify and maintain aleurone cell fate during maize endosperm development. *Development* 127:4039–4048
- Becraft PW, Stinard PS, McCarty DR (1996) CRINKLY4: a TNFR-like receptor kinase involved in maize epidermal differentiation. *Science* 273:1406–1409
- Becraft PW, Chandok MR, Jin P, Guo T, Asuncion-Crabb Y, Zhang Y (1999) The function of the maize CRINKLY4 receptor-like kinase in a growth factor like signaling system. Symposium of Plant Signal Transduction, New Delhi, India
- Becraft PW, Li K, Dey N, Asuncion-Crabb YT (2002) The maize *dek1* gene functions in embryonic pattern formation and in cell fate specification. *Development* 129:5217–5225
- Bethke PC, Lonsdale JE, Fath A, Jones RL (1999) Hormonally regulated programmed cell death in barley aleurone cells. *Plant Cell* 11:1033–1046
- Bethke PC, Hwang Y-S, Zhu T, Jones RL (2006) Global patterns of gene expression in the aleurone of wild-type and dwarf1 mutant rice. *Plant Physiol* 140:484–498
- Bethke PC, Libourel IGL, Aoyama N, Chung Y-Y, Still DW, Jones RL (2007) The *Arabidopsis thaliana* aleurone layer responds to nitric oxide, gibberellin, and abscisic acid and is sufficient and necessary for seed dormancy. *Plant Physiol* 143:1173–1188
- Brown RC, Lemmon BE (2007) The Developmental Biology of Cereal Endosperm (in this volume). Springer, Heidelberg
- Brown RC, Lemmon BE, Olsen O-A (1994) Endosperm development in barley: microtubule involvement in the morphogenetic pathway. *Plant Cell* 6:1241–1252
- Brown RC, Lemmon BE, Olsen O-A (1996) Development of the endosperm in rice (*Oryza sativa* L.): cellularization. *J Plant Res* 109:301–313
- Brown RC, Lemmon BE, Nguyen H, Olsen O-A (1999) Development of endosperm in *Arabidopsis thaliana*. *Sex Plant Reprod* 12:32–42
- Cao X, Costa LM, Biderre-Petit C, Kbhaya B, Dey N, Perez P, McCarty DR, Gutierrez-Marcos JF, Becraft PW (2007) Abscisic acid and stress signals induce Viviparous1 (Vp1) expression in seed and vegetative tissues of maize. *Plant Physiol* p 106.091454

- Casaretto J, Ho TD (2003) The transcription factors HvABI5 and HvVP1 are required for the abscisic acid induction of gene expression in barley aleurone cells. *Plant Cell* 15:271–284
- Charlton WL, Keen CL, Merriman C, Lynch P, Greenland AJ, Dickinson HJ (1995) Endosperm development in *Zea mays*; implication of gametic imprinting and paternal excess in regulation of transfer layer development. *Development* 121:3089–3097
- Coe EH Jr (1978) The aleurone tissue of maize as a genetic tool. In: Walden DB (ed) *Maize breeding and genetics*. Wiley, New York, pp 447–459
- Cone KC (2007) Anthocyanin biosynthesis (in this volume). Springer, Heidelberg
- Costa LM, Gutierrez-Marcos JE, Brutnell TP, Greenland AJ, Dickinson HG (2003) The *globby1-1* (*glo1-1*) mutation disrupts nuclear and cell division in the developing maize seed causing alterations in endosperm cell fate and tissue differentiation. *Development* 130:5009–5017
- Dooner HK, Robbins TP, Jorgensen RA (1991) Genetic and developmental control of anthocyanin biosynthesis. *Annu Rev Genet* 25:173–199
- Fenech M, Noakes M, Clifton P, Topping D (2005) Aleurone flour increases red-cell folate and lowers plasma homocyst(e)ine substantially in man. *Br J Nutr* 93:353–360
- Furtado A, Henry RJ (2005) The wheat *Em* promoter drives reporter gene expression in embryo and aleurone tissue of transgenic barley and rice. *Plant Biotechnol J* 3:421–434
- Gavazzi G, Dolfini S, Allegra D, Castiglioni P, Todesco G, Hoxha M (1997) *Dap* (defective aleurone pigmentation) mutations affect maize aleurone development. *Mol Gen Genet* 256:223–230
- Geisler-Lee J, Gallie DR (2005) Aleurone cell identity is suppressed following connation in maize kernels. *Plant Physiol* p 105.064295
- Gifford ML, Robertson FC, Soares DC, Ingram GC (2005) ARABIDOPSIS CRINKLY4 function, internalization, and turnover are dependent on the extracellular crinkly repeat domain. *Plant Cell* 17:1154–1166
- Goyal K, Walton LJ, Tunnacliffe A (2005) LEA proteins prevent protein aggregation due to water stress. *Biochem J* 388:151–157
- Gruis D, Guo H, Selinger D, Tian Q, Olsen O-A (2006) Surface position, not signaling from surrounding maternal tissues, specifies aleurone epidermal cell fate in maize. *Plant Physiol* 141:898–909
- Hattori T, Vasil V, Rosenkrans L, Hannah LC, McCarty DR, Vasil IK (1992) The *Viviparous-1* gene and abscisic acid activate the *C1* regulatory gene for anthocyanin biosynthesis during seed maturation in maize. *Genes Dev* 6:609–618
- Hoecker U, Vasil IK, McCarty DR (1995) Integrated control of seed maturation and germination programs by activator and repressor functions of *Viviparous-1* of maize. *Genes Dev* 9:2459–2469
- Howard TL, Stauffer DR, Degnin CR, Hollenberg SM (2001) CHMP1 functions as a member of a newly defined family of vesicle trafficking proteins. *J Cell Sci* 114:2395–2404
- Jakobsen JV, Knox RB, Pylotus NA (1971) The structure and composition of aleurone grains in the barley aleurone layer. *Planta* 101:189–209
- Jin P, Guo T, Becraft PW (2000) The maize CR4 receptor-like kinase mediates a growth factor-like differentiation response. *Genesis* 27:104–116
- Johnson KL, Degnan KA, Ross Walker J, Ingram GC (2005) *AtDEK1* is essential for specification of embryonic epidermal cell fate. *Plant J* 44:114–127
- Jones RL (1969) The fine structure of barley aleurone cells. *Planta* 85:359–375
- Kessler S, Seiki S, Sinha N (2002) *Xcl1* causes delayed oblique periclinal cell divisions in developing maize leaves, leading to cellular differentiation by lineage instead of position. *Development* 129:1859–1869

- Kiesselbach TA (1949) The structure and reproduction of corn. Nebraska Agric Exp Stn Res Bull 161:1–96
- Kyle DJ, Styles ED (1977) Development of aleurone and sub-aleurone layers in maize. *Planta* 137:185–193
- Levy AA, Walbot V (1990) Regulation of the timing of transposable element excision during maize development. *Science* 248:1534–1537
- Lid SE, Gruis D, Jung R, Lorentzen JA, Ananiev E, Chamberlin M, Niu X, Meeley R, Nichols S, Olsen O-A (2002) The *defective kernel 1 (dek1)* gene required for aleurone cell development in the endosperm of maize grains encodes a membrane protein of the calpain gene superfamily. *Proc Natl Acad Sci USA* 99:5460–5465
- Lid SE, Al RH, Krekling T, Meeley RB, Ranch J, Opsahl-Ferstad HG, Olsen O-A (2004) The maize disorganized aleurone layer 1 and 2 (*dil1*, *dil2*) mutants lack control of the mitotic division plane in the aleurone layer of developing endosperm. *Planta* 218:370–378
- Lid SE, Olsen L, Nestestog R, Aukerman M, Brown RC, Lemmon B, Mucha M, Opsahl-Sorteberg H-G, Olsen O-A (2005) Mutation in the *Arabidopsis thaliana* *DEK1* calpain gene perturbs endosperm and embryo development while over-expression affects organ development globally. *Planta* 221:339
- McClintock B (1978) Development of the maize endosperm as revealed by clones. In: Subtelny S, Sussex IM (eds) *The clonal basis of development*. Academic, New York, pp 217–237
- McIntosh GH, Royle PJ, Pointing G (2001) Wheat aleurone flour increases cecal beta-glucuronidase activity and butyrate concentration and reduces colon adenoma burden in azoxymethane-treated rats. *J Nutr* 131:127–131
- Miyoshi K, Kagaya Y, Ogawa Y, Nagato Y, Hattori T (2002) Temporal and spatial expression pattern of the OSVP1 and OSEM genes during seed development in rice. *Plant Cell Physiol* 43:307–313
- Morrison IN, Kuo J, O'Brien TP (1975) Histochemistry and fine structure of developing wheat aleurone cells. *Planta* 123:105–116
- Nguyen HN, Sabelli PA, Larkins BA (2007) Endoreduplication and programmed cell death in the cereal endosperm (in this volume). Springer, Heidelberg
- Nicolas G, Fournier CM, Galand C, Malbert-Colas L, Bournier O, Krowiarski Y, Bourgeois M, Camonis JH, Dhermy D, Grandchamp B, Lecomte MC (2002) Tyrosine phosphorylation regulates alpha II spectrin cleavage by calpain. *Mol Cell Biol* 22:3527–3536
- Olsen O-A (2001) Endosperm development: cellularization and cell fate specification. *Annu Rev Plant Physiol Plant Mol Biol* 52:233–267
- Olsen O-A (2004a) Nuclear endosperm development in cereals and *Arabidopsis thaliana*. *Plant Cell* 16:S214–227
- Olsen O-A (2004b) Dynamics of maize aleurone cell formation: the “surface” rule. *Maydica* 49:37–40
- Opsahl-Sorteberg HG, Divon HH, Nielsen PS, Kalla R, Hammond-Kosack M, Shimamoto K, Kohli A (2004) Identification of a 49-bp fragment of the HvLTP2 promoter directing aleurone cell specific expression. *Gene* 341:49–58
- Otegui M (2007) Endosperm cell walls: formation, composition, and functions (in this volume). Springer, Heidelberg
- Randolph LF (1936) Developmental morphology of the caryopsis in maize. *J Agric Res* 53:881–916
- Shen B, Li C, Min Z, Meeley RB, Tarczynski MC, Olsen O-A (2003) *sal1* determines the number of aleurone cell layers in maize endosperm and encodes a class E vacuolar sorting protein. *Proc Natl Acad Sci USA* 100:6552–6557

- Sheridan WF, Neuffer MG (1982) Maize developmental mutant embryos unable to form leaf primordia. *J Heredity* 73:319–329
- Shiraha H, Glading A, Chou J, Jia Z, Wells A (2002) Activation of m-calpain (calpain II) by epidermal growth factor is limited by protein kinase A phosphorylation of m-calpain. *Mol Cell Biol* 22:2716–2727
- Stewart A, Nield H, Lott JNA (1988) An investigation of the mineral content of barley grains and seedlings. *Plant Physiol* 86:93–97
- Swanson SJ, Bethke PC, Jones RL (1998) Barley aleurone cells contain two types of vacuoles. Characterization of lytic organelles by use of fluorescent probes. *Plant Cell* 10:685–698
- Vicente-Carbajosa J, Carbonero P (2005) Seed maturation: developing an intrusive phase to accomplish a quiescent state. *Int J Dev Biol* 49:645–651
- Walbot V (1994) Overview of key steps in aleurone development. In: Freeling M, Walbot V (eds) *The maize handbook*. Springer, New York, pp 78–80
- Wang C, Barry JK, Min Z, Tordsen G, Rao AG, Olsen O-A (2003) The calpain domain of the maize DEK1 protein contains the conserved catalytic triad and functions as a cysteine proteinase. *J Biol Chem* 278:34467–34474
- Watanabe M, Tanaka H, Watanabe D, Machida C, Machida Y (2004) The ACR4 receptor-like kinase is required for surface formation of epidermis-related tissues in *Arabidopsis thaliana*. *Plant J* 39:298–308
- Wisniewski JP, Rogowsky PM (2004) Vacuolar H⁺-translocating inorganic pyrophosphatase (Vpp1) marks partial aleurone cell fate in cereal endosperm development. *Plant Mol Biol* 56:325–337
- Young TE, Gallie DR (2000) Programmed cell death during endosperm development. *Plant Mol Biol* 44:283–301