Specification of Epidermal Cell Fate in Plant Shoots

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Specification of Epidermal Cell Fate in Plant Shoots

Running title: Specification of epidermal cell fate

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Abstract
Land plants have evolved a single layer of epidermal cells, which are characterized by mostly anticlinal cell division patterns, formation of a waterproof coat called cuticle, and unique cell types such as stomatal guard cells and trichomes. The shoot epidermis plays important roles not only to protect plants from dehydration and pathogens but also to ensure their proper organogenesis and growth control. Extensive molecular genetic studies in Arabidopsis and maize have identified a number of genes that are required for epidermal cell differentiation. However, the mechanism that specifies shoot epidermal cell fate during plant organogenesis remains largely unknown. Particularly, little is known regarding positional information that should restrict epidermal cell fate to the outermost cell layer of the developing organs. Recent studies suggested that certain
members of the HD-ZIP class IV homeobox genes are possible master regulators of
shoot epidermal cell fate. Here, we summarize the roles of the regulatory genes that are
involved in epidermal cell fate specification and discuss the possible mechanisms that
limit the expression and/or activity of the master transcriptional regulators to the
outermost cell layer in plant shoots.

**Keywords:** epidermal cell differentiation, positional signal, HD-ZIP class IV
transcription factor, cuticle, endosperm, receptor-like kinase, calpain-like cysteine
protease, *Arabidopsis thaliana*

### 1. Introduction

The shoot epidermis is a single layer of surface cells that are morphologically
categorized by anticlinal cell division patterns. The outer surface of the shoot
epidermis is covered with a hydrophobic structure called a cuticle, which prevents water
loss, pathogen attacks, and post-genital fusion of organs (Yeats and Rose, 2013).
Besides basic pavement cells, leaf epidermis contains specialized cell types such as hair
cells (trichomes) and stomatal guard cells, which function to cope with dehydration and
pathogen attacks. In addition to its protective function, the epidermis plays roles in the
regulation of organ growth and shoot stem cell maintenance (Savaldi-Goldstein et al.,
2007; Knauer et al., 2013; Nobusawa et al., 2013). In addition, protodermal cells in
the shoot meristem and the embryo are necessary for the production and transport of the
phytohormone auxin, which drives embryonic axis formation and lateral organ
primordia initiation (Reinhardt et al., 2003; Kierzkowski et al., 2013; Robert et al.,
2013; Wabnik et al., 2013).

Determination of shoot epidermal fate relies on a “position” rather than a cell “lineage,”
as clonal analyses has shown that there is no strict lineage restriction in developing
leaves; cells can flexibly change their fates, and only the cells finally located at the
surface of the organ develop into epidermis (Stewart and Dermen, 1975). However,
 positional cues that determine shoot epidermal cell fate remain largely unknown. This
review describes recent advances in the studies of epidermal cell specification in the
shoots, focusing mainly on the regulation of key transcription factors.
2. HD-ZIP class IV transcription factors are key regulators for epidermal cell specification

*Arabidopsis thaliana* Meristem L1 Layer (ATML1) was identified as an epidermis-specific homeobox gene that belongs to the HD-ZIP class IV family (Lu et al., 1996). ATML1 expression is first detected in the embryos as early as the one-cell stage and its expression is restricted to the outermost cells around the 16-cell stage (dermatogen stage) after the embryos have undergone tangential cell divisions to generate outer protodermal cells and inner cells (Lu et al., 1996; Sessions et al., 1999; Takada and Jürgens, 2007; Figures 1A–E).

Mutations in ATML1 and its closest homolog Protodermal Factor2 (PDF2) caused severe phenotypes associated with defects in epidermal cell specification (Abe et al., 2003). Strong mutant alleles of atml1;pdf2 showed embryo-lethal phenotypes with irregular division patterns of the protoderm, whereas weak mutant alleles of atml1;pdf2 produced a few leaves that lack an epidermis (Abe et al., 2003; San-Bento et al., 2014; Table 1). ATML1 homologs have been isolated in several species and most of them are preferentially expressed in the epidermis (Ito et al., 2003; Nakamura et al., 2006; Javelle et al., 2011). Several of these genes are implicated in epidermis-related functions, not only for the initial specification of surface cell fate but also for the generation of distinct cell types within the epidermis (Rerie et al., 1994; Peterson et al., 2013; Roeder et al., 2012; Takada, 2013).

HD-ZIP class IV transcription factors may function as transcriptional activators or repressors (Ohashi et al., 2003; Yu et al., 2008; Javelle et al., 2010; Depège-Fargeix et al., 2011; Peterson et al., 2013). ATML1 and PDF2 were shown to bind in vitro to an 8-bp sequence called the L1 box (Abe et al., 2001; Abe et al., 2003). Considering that an L1 box is often found in the promoters of epidermis-specific genes including ATML1 and PDF2, ATML1 and PDF2 have been proposed to positively regulate the expression of epidermis-specific genes (Abe et al., 2001; Abe et al., 2003). In fact, expression of epidermis-related genes was decreased in atml1;pdf2 (Abe et al., 2003; Takada et al., 2013). Moreover, gain-of-function experiments suggest that ATML1 activates expression of several epidermis specific genes during the initiation of new epidermal cell fate, but may also function as a negative regulator in maintaining expression levels.
Notably, overexpression of ATML1 was sufficient to induce differentiation of epidermal cells such as stomata and trichomes in the inner tissues of leaves (Takada et al., 2013; Table 1). These results are consistent with the idea that ATML1 is a master transcriptional regulator for epidermal cell specification in shoots.

Importantly, expression of ATML1 and its putative orthologs depends on a “surface” position, irrespectively of epidermal cell identity or cell lineage, as indicated by the presence of ATML1 promoter activity at the surface mesophyll cells of atml1;pdf2 leaves (Takada et al., 2013). In addition, expression of RICE OUTERMOST CELL-SPECIFIC GENE1 (ROC1), a rice HD-ZIP class IV gene, was induced on the cut surface during callus regeneration (Ito et al., 2002). In the maize extra cell layers1 (xcl1) mutant, which develops multilayered epidermis by amplification of differentiated epidermal cells, expression of an HD-ZIP class IV gene was detected only in the outermost epidermal layer (Kessler et al., 2002). These reports suggest that identification of upstream regulators that determine the outermost cell-specific expression of ATML1 homologs would be an effective strategy for identifying positional signals that specify shoot epidermal cell fate.

3. Positional signals that specify epidermal cell fate

Deletion and mutational analyses of an ATML1 promoter revealed the involvement of several positive regulators in the protoderm-specific activation of ATML1 (Takada and Jürgens, 2007). MicroRNAs and phytohormone auxin, two major components that are known for morphogen-like activity, appeared not to be involved in the outermost cell-specific expression of ATML1 (Takada and Jürgens, 2007; Nodine and Bartel, 2010). Below, we discuss the candidate molecules or genes that may provide positional cues for shoot epidermal cell specification (Figure 1F).

3.1. Cuticle

Cuticle is a hydrophobic lipid layer formed on the surface of the shoot epidermis. In cuticle-deficient plants, trichome numbers, stomatal density, and regular anticlinal cell division of the epidermis are impaired, suggesting that cuticular components and/or their precursors are required for the patterning of epidermis (Yephremov et al., 1999;
Cuticle can be observed in the zygote and is maintained only in the outermost cells of the embryo even before a layer of protoderm is visible (Bruck and Walker, 1985). Therefore, the presence of cuticle or cuticle biogenesis may be instructive for epidermal cell specification and/or maintenance. In fact, expression of ROC1 was reduced in a rice mutant defective in the biosynthesis of very-long-chain fatty acids (VLCFAs), which serve as precursor of cuticular wax (Tsuda et al., 2013). Although it has been shown that ATML1 and other HD-ZIP class IV genes positively regulate expression of cuticle biosynthesis genes and facilitate cuticle deposition, these results suggest that cuticle also functions to maintain epidermal cell identity as a positive feedback mechanism (Javelle et al., 2010; Wu et al., 2011; Takada et al., 2013; Takada, 2013). VLCFAs or its derivatives produced in the epidermis have been recently suggested to function as non-cell autonomous signals that promote cell proliferation in internal tissues (Nobusawa et al., 2013). Therefore, some intermediates or byproducts formed during cuticle biosynthesis may play roles in pattern formation in plants.

3.2. Signaling from the Endosperm

In the angiosperms, the embryo is surrounded by endosperm tissues, which provide nutrients to the developing embryo. Recent studies show that signaling from the endosperm is necessary for epidermal cell differentiation during embryogenesis. abnormal leaf shape1 (ale1) and zhoupi (zou) mutants are defective in cuticle formation in organs generated during embryogenesis (Tanaka et al., 2001; Yang et al., 2008; Table 1). ZOU encodes an endosperm-specific transcriptional regulator that promotes the expression of ALE1 and other genes required for the breakdown of the endosperm (Yang et al., 2008, Table 1; Figure 1F). ALE1 encodes a subtilisin-like serine protease, and its expression in the endosperm is sufficient to rescue cuticle-deficient phenotypes of ale1 and zou, suggesting that ALE1 non-cell-autonomously promotes epidermal cell differentiation (Tanaka et al., 2001; Xing et al., 2013; Table 1, Figure 1F). Considering that subtilisin-like proteases are involved in the processing of peptide hormone precursors in animals, the simplest scenario would be that ALE1 produces ligands promoting epidermal cell specification in the endosperm and the outermost cells of the embryos that receive those ligands differentiate into the epidermis (Steiner, 1998; Tanaka et al., 2001). GASSHO1 (GSO1) and GSO2 are candidate
receptor-like kinases that receive signals produced by ALE1. GSO1 and GSO2 are preferentially expressed in the embryo, and gso1;gso2 shows severe cuticle-deficient phenotypes epistatic to those of ale1 (Tsuwamoto et al., 2008; Xing et al., 2013; Table 1; Figure 1F).

It is not certain whether ALE1 and GSO1/GSO2 are generally required for the initiation of epidermal cell fate or specifically required for the cuticle formation on the surface of the embryo. In fact, epidermal cell specification can occur in the absence of the endosperm (such as during organ regeneration from calli, somatic embryogenesis, and aerial organ initiation in post-embryonic development). Plant embryos may require ALE1 and GSO1/GSO2-mediated signaling for efficient deposition of cuticle on the surface of the protodermal cells that develop in close physical contact with surrounding endosperm cells.

3.3. CRINKLY4

crinkly4 (cr4) is a maize mutant with defects in the development of leaf epidermis and aleurone layer (Becraft et al., 1996). CR4 encodes a plant-specific receptor-like kinase, and mutations in a homolog of CR4 in Arabidopsis [ARABIDOPSIS THALIANA HOMOLOG OF CRINKLY4 (ACR4)] cause phenotypes defective in epidermal cell differentiation, lateral root initiation, and root initial cell maintenance (Gifford et al., 2003; Watanabe et al., 2004; De Smet et al., 2008; Stahl et al., 2009; Stahl et al., 2013; Table 1). Although acr4 shows a mild effect on epidermal cell differentiation, acr4;ale1 double mutants show severe phenotypes with reduced ATML1 expression in the embryo, suggesting that ACR4 acts in parallel with the “endosperm pathway” to positively regulate epidermal cell differentiation upstream of ATML1 (Tanaka et al., 2007; Table 1; Figure 1F).

However, expression of ACR4 is restricted to the epidermis of the embryos and shoots, and its epidermis-specific expression depends on an L1 box in the promoter, suggesting that ACR4 is a downstream target of HD-ZIP class IV transcription factors (Tanaka et al., 2002; Gifford et al., 2003; San-Bento et al., 2014; Table 1; Figure 1F). ACR4 expression consistently began later than ATML1 expression during embryogenesis (Tanaka et al., 2002; Gifford et al., 2003). Moreover, ATML1 and PDF2 were
associated with an *ACR4* promoter *in planta*, and *ACR4* expression was reduced in
*atml1;pdf2* (Abe et al., 2003; San-Bento et al., 2014).

ACR4 is localized to the basal and lateral membranes of the epidermis, suggesting that it is involved in intercellular communication between the same and different layers (Gifford et al., 2003; Watanabe et al., 2004; Gifford et al., 2005). *ACR4* and CR4 were shown to localize to plasmodesmata (PD), pores connecting plant cells, in the aleurone cells and the cotyledon epidermis (Tian et al., 2007; Stahl et al., 2013). Pore sizes of the PD connecting aleurone cells were wider than those connecting aleurone and underlying starchy endosperm cells (Tian et al., 2007). These observations suggest that CR4 is involved in the modulation of the size of PD pores and facilitates intercellular communication between the same layer, maintaining epidermal/aleurone cell fate. Considering together, these results suggest that *ACR4* is possibly more involved in the maintenance of epidermal cell fate downstream of *ATML1* than in the perception of positional signals for epidermal cell specification (Figure 1F).

### 3.4. Leucine-rich repeat receptor-like kinase in the embryo

RECEPTOR-LIKE PROTEIN KINASE1 (RPK1) and TOADSTOOL2 (TOAD2) are leucine-rich repeat receptor-like kinases redundantly required for epidermal cell differentiation in the embryo (Nodine et al., 2007). *rpk1;toad2* shows embryo-lethal phenotypes with disorganized cell division patterns particularly in the basal half of the embryo proper (Nodine et al., 2007; Table 1). *ATML1* mRNA was detected in the outer cell layer of *rpk1;toad2* at the dermatogen stage but disappeared after the early globular stage, suggesting that these receptor-like kinases are necessary for the maintenance but not for the initial specification of epidermal cell fate (Nodine et al., 2007; Table 1; Figure 1F). Because of the embryonic lethality, the roles of RPK1 and TOAD2 in shoot epidermal cell differentiation in the post-embryonic development are unknown.

### 3.5. DEFECTIVE KERNEL 1: a calpain-like protease

DEFECTIVE KERNEL 1 (DEK1) is a calpain like cysteine protease that is conserved among land plants (Lid et al., 2002; Liang et al., 2013). Downregulation of *DEK1* expression in several species causes phenotypes associated with defects in epidermal
differentiation such as reduced deposition of cuticle, disorganization of cell division planes, and ectopic differentiation of mesophyll plastids (chloroplasts) in the surface cells (Becraft et al., 2002; Ahn et al., 2004; Johnson et al., 2005; Tian et al., 2007; Table 1). Strong mutant alleles of dek1 cause embryo-lethal phenotypes in Arabidopsis, maize, and rice, and expression of ATML1 homologs disappears in these embryos, implying that DEK1 is necessary for the initiation of epidermal fate in the early embryo (Lid et al., 2002; Johnson et al., 2005; Lid et al., 2005; Hibara et al., 2009; Table 1; Figure 1F).

DEK1 mRNA is expressed ubiquitously, suggesting that its activity is regulated post-translationally (Wang et al., 2003; Johnson et al., 2005; Lid et al., 2005; Hibara et al., 2009). DEK1 is composed of an N-terminal membrane-spanning region and a C-terminal cytosolic region that includes the calpain cysteine protease (Lid et al., 2002; Tian et al., 2007). It has been hypothesized that upon binding of epidermis-promoting ligands to the N-terminal region, DEK1 is cleaved by its autocatalytic activity to release the C-terminal region, an active form of the calpain (Wang et al., 2003; Tian et al., 2007; Johnson et al., 2008). In animals, calpain-like proteases are involved in the activation/inactivation of several signaling molecules, suggesting that DEK1 transduces signals for epidermal specification (Storr et al., 2011). However, overexpression of active truncated forms of DEK1 in Arabidopsis was not sufficient to upregulate the expression of ATML1 (Johnson et al., 2008; Table 1). Moreover, modulation of DEK1 activity affected cell division and growth in internal tissues, suggesting that the action of DEK1 is not epidermis-specific (Johnson et al., 2008; Table 1). Johnson et al. (2008) proposed that DEK1 controls mainly cell division in developing leaves and that epidermal cells respond more sensitively to the amount of DEK1; reduction in cell division rate in dek1 may cause discontinuity and abortion of the epidermis (Johnson et al., 2008). In this scenario, DEK1 is possibly more involved in the maintenance rather than the initiation of the epidermal cell layer.

3.6. Post-transcriptional regulation of HD-ZIP class IV activity

Localization of some HD-ZIP class IV transcription factors was not limited to nuclei of heterologous cells (Zhang et al., 2010; Yang et al., 2011). The HD-ZIP class IV transcription factor GLABRA2 (GL2), a positive regulator of trichome formation, was
restricted to the nuclei only in trichome cells and not in internal cells, suggesting a
cell-type-specific regulation of nuclear transport (Szymanski et al., 1998). HD-ZIP
class IV transcription factors contain a putative lipid/sterol binding domain (START)
and a dimerization motif (ZLZ), implying a regulation of their activities by dimerization
and binding of lipid/sterol ligands (Schrick et al., 2004). In fact, sterol and VLCFA
biosynthesis-deficient mutants are defective in proper distribution of stomata and
trichomes, respectively (Yephremov et al., 1999; Qian et al., 2013). However, to date
the roles of START domains have not been investigated, except for an observation
showing that an N-terminal part of a START domain can function as a transcriptional
activation domain in yeast and maize suspension cells (Depège-Fargeix et al., 2011).

ATML1 was shown to heterodimerize with PDF2 in planta and it is possible that
dimerization with other HD-ZIP class IV proteins changes the activity of ATML1 in a
cell-type dependent manner, although ectopic expression of ATML1 alone was sufficient
to induce epidermal cell fate in inner tissues (Takada et al., 2013; San-Bento et al.,
2014). In related transcription factors, DNA-binding was inhibited by oxidation of
Cys residues in the ZLZ motif, suggesting that redox signals are also involved in the
regulation of ATML1 activity (Tron et al., 2002).

4. Repression of inner cell fate
Several lines of evidence show that acquisition of epidermal cell fate is associated with
a loss of mesophyll or internal cell fate. First, epidermis-deficient atm11;pdf2 and
DEK1 knockdown lines showed ectopic differentiation of mesophyll cells on the
surfaces of leaves and cotyledons, respectively (Abe et al., 2003; Johnson et al., 2005).
Second, rpk1;toad2 embryos exhibited ectopic subepidermal marker expression in the
outermost cell layer (Nodine et al., 2007; Table 1). Third, overexpression of ATML1
decreased differentiation of green mesophyll cells in leaves (Takada et al., 2013).
Moreover, cr4 and dek1, which are defective in “surface” aleurone layer differentiation
in the maize endosperm, cause ectopic differentiation of “inner” starchy endosperm
cells on the surface of the endosperm (Becraft et al., 1996; Becraft and Asuncion-Crabb,
2000). Therefore, repression of internal or “default” cell fate may be a general
requirement for surface cell differentiation in plants. It is not possible, however, to test
whether or not mesophyll cell differentiation represses epidermal cell fate because no
positive regulators of inner cell fate are available at present. Chloroplast development itself appears not to exert a negative effect on epidermal cell differentiation, considering that stomatal guard cells possess chloroplasts and no ectopic epidermal cell differentiation has been reported in the mesophyll tissues of albino plants (Stewart and Dermer, 1975).

Mesophyll cells possibly represent a primitive state of leaf cells, considering that ancestral aquatic algae are composed mainly of mesophyll-like cells. Land plants may have repressed mesophyll cell differentiation to evolve an epidermis on the surface. Evolutionary studies, including comparative genomics, may be useful for identifying molecular components that promote epidermal cell formation (Zalewski et al., 2013).

5. Future perspectives

Despite the extensive molecular genetic studies in model plants, positional signals that specify shoot epidermal cell fate remain unknown (Figure 1F). Most of the receptor-like kinases, characterized by their roles in shoot epidermal cell differentiation, are possibly involved in the maintenance than in the specification of epidermal cell fate. This appearance may be because of the difficulties in distinguishing between phenotypes associated with “specification” and those related to “maintenance” of epidermal cell fate in forward genetic screens.

The cuticle-bearing outermost cells should have distinct mechanical properties compared with inner cells. Moreover, cells located at the surface are unique, in that they are in constant contact with the environment. These unique properties could influence the differentiation of epidermal cells. Attempts to directly isolate epidermis-promoting biomolecules and to identify physical/environmental constraints influencing epidermal cell fate may shed new light on the issue.

Conflict of Interest Statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author contributions
ST wrote the main manuscript text and HI prepared Figure 1 and Table 1. All authors reviewed the manuscript.

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**Figure Legend**

**Figure 1.** Regulation of *ATML1* expression in the embryos and shoot apices. *ATML1* promoter activity visualized using a nuclear-localized GFP reporter in the developing embryos (A–C) and the shoot apices (D, E). Two-cell stage (A), early globular stage (B), and heart stage embryos (C) are shown. GFP expression is restricted to the outermost cell layer after the 16-cell stage. Epidermis-specific expression is observed in vegetative (D) and inflorescence (E) shoot apices. (F) Genetic interactions among genes involved in epidermal cell fate specification during *Arabidopsis thaliana* embryogenesis. Arrows indicate positive interactions. For details, please see the main text. Scale bars, 20 μm in A–C; 100 μm in D–E.
<table>
<thead>
<tr>
<th>Gene name/product</th>
<th>RNA and protein localization</th>
<th>Mutant phenotypes</th>
<th>Phenotypes caused by gene manipulation</th>
<th>References</th>
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<tr>
<td><strong>ARABIDOPSIS THALIANA</strong></td>
<td>Ubiquitous mRNA expression in early embryos; the outermost cell-specific expression from the early globular stage; epidermis-specific expression in the shoot; expression in the root epidermis (atrichoblasts) and root cap cells.</td>
<td>atml1 No effect on initiation of epidermal fate. atml1;pdf2 Cell bulging and periclinal/oblique division of the protoderm in the embryo (strong embryonic-lethal alleles); loss of a leaf epidermis (weak alleles). atml1;acr4 Similar to pdf2;acr4. atml1;gso1;gso2 Similar to pdf2;gso1;gso2.</td>
<td>&lt;ATML1 overexpression&gt; Ectopic differentiation of epidermis in inner tissues; upregulation of several epidermis-specific genes. &lt;Induction of ATML fused with a repressor sequence (SRDX) after germination&gt; Filamentous protrusions on the surface of cotyledons; increased permeability of the shoot epidermis; fusion of cotyledons and leaves; downregulation of several epidermis-specific genes.</td>
<td>Lu et al., 1996; Sessions et al., 1999; Abe et al., 2003; Takada and Jürgens 2007; Racolta et al., 2013; Takada et al., 2013; Takada, 2013; San-Bento et al., 2014.</td>
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<td><strong>PROTODERMAL FACTOR2 (PDF2)</strong></td>
<td>Ubiquitous mRNA expression in the quadrant-stage embryo; the outermost cell-specific expression from the early globular stage; epidermis-specific expression in the shoot.</td>
<td>pdf2 No effect on initiation of epidermal fate. pdf2;acr4 fusion of ovules pdf2;ale1 Irregular division patterns of epidermal cells in embryos; increased permeability of the epidermis in the seedling. pdf2;gso1;gso2 Embryonic arrest; disorganized epidermal cell layer in embryos.</td>
<td>&lt;Constitutive expression of PDF2&gt; No effect on epidermal specification &lt;Overexpression of PDF2 in the epidermis&gt; Reduced expression of ATML1, endogenous PDF2, and ACR4 mRNAs. &lt;Suppression of PDF2 RNA&gt; Decrease in ATML1 RNA; abnormal epidermal cell morphology in sepals and petals.</td>
<td>Abe et al., 2003; San-Bento et al., 2014.</td>
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<td><strong>ARABIDOPSIS THALIANA</strong></td>
<td>mRNA expression in the embryo proper from the 8-cell stage; restricted to the protoderm by the mid-heart stage; shoot epidermis, root epidermis, quiescent center, and root cap cells in the seedling. Protein localization in the basal and lateral plasma membranes and plasmodesmata.</td>
<td>acr4 Increased permeability of leaf epidermis; disorganization of ovule integuments; lack of cuticle in ovule integuments and sepal margins. acr4;ale1 Markedly increased permeability of shoot epidermis compared with each single mutant; decreased ATML1 expression in the apical region of the embryos; severely fused leaves. acr4;alu2 See ALE2 mutant phenotype. zuo;acr4 See ZOU mutant phenotype. atml1;acr4 pdf2;acr4 See PDF2 mutant phenotype. acr4;gso1;gso2 See GSO1/GSO2 mutant phenotype.</td>
<td>&lt;Deletions of ACR4 domains&gt; Expression of ACR4 with a deletion or a mutation in a TUMOR NECROSIS FACTOR RECEPTOR (TNFR)-like domain, a C-terminal region, or a kinase domain can rescue acr4 when expressed under an ACR4 native promoter, whereas a deletion in a CRINKLY repeat or a transmembrane domain cannot rescue acr4.</td>
<td>Tanaka et al., 2002; Gifford et al., 2003; Watanabe et al., 2004; Gifford et al., 2005; Tanaka et al., 2007; Stahl et al., 2013.</td>
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<td>ABNORMAL LEAF SHAPE 1 (ALE1)</td>
<td>mRNA expression in the endosperm surrounding the embryo; low levels of expression in early embryos.</td>
<td>ale1 Reduced cuticle formation in the embryo and cotyledons; deformed pavement cell shape; wrinkled juvenile leaves and cotyledons; fusion of leaves and cotyledons. ale1;ale2 Severely reduced cuticle in the shoot; disorganized outermost cell layer in the shoot meristem; swollen and spherical protodermal cell morphology in the embryo and seedling; decrease in ATML1 mRNA in the apical part of the embryo. acr4;ale1 See ACR4 mutant phenotype ale1;gso1;gso2 See GSO1/GSO2 mutant phenotype</td>
<td>Expression of ALE1 under an endosperm-specific promoter rescues ale1 and partially rescues zou. Tanaka et al., 2001; Watanabe et al., 2003; Tanaka et al., 2007; Xing et al., 2013; San-Bento et al., 2014.</td>
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<td>ABNORMAL LEAF SHAPE 2 (ALE2)</td>
<td>Ubiquitous mRNA expression in the embryo proper in early embryos; strong expression in the cotyledon primordia at the heart stage.</td>
<td>ale2 Increased permeability of leaf epidermis; swollen protodermal cell morphology in the embryo; disorganized cell division orientation and malformed cell shape in the integument and endothelium; fusion of leaves. acr4;ale2 Similar to ale2; ale2 is semi-dominant in the acr4 mutant background. ale1;ale2 see ALE1 mutant phenotype. zou;ale2 See ZOU mutant phenotype.</td>
<td>Tanaka et al., 2007.</td>
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<td>ARABIDOPSIS THALIANA DEFECTIVE KERNEL1 (AtDEK1)</td>
<td>RNA expression in the embryo proper throughout embryogenesis; low level of expression in the endosperm; strong expression in all cell layers in the shoot meristem; low level of expression in other tissues of seedlings.</td>
<td>atdek1 Embryo-lethal phenotype; abnormal cell division planes and loss of obvious protodermal cell layer in the embryo; loss of ATML1 and ACR4 expression in the embryo. &lt;DEK1 RNAi&gt; Fusion of cotyledons; formation of mesophyll-like cells in the outermost cell layer. &lt;DEK1 RNAi in acr4&gt; Increased proportion of severe RNAi phenotypes. &lt;Overexpression of a calpain domain&gt; Sufficient to rescue the embryo-lethal phenotype of atdek1; increased proliferation of mesophyll and epidermal cells; variable epidermal cell sizes. &lt;Overexpression of a calpain domain (co-suppression)&gt; Decreased proliferation of mesophyll and epidermal cells; variable epidermal cell sizes. &lt;Expression of a calpain domain under a DEK1 promoter&gt; atdek1 seed phenotypes are slightly rescued. &lt;Overexpression of a membrane-spanning region&gt; Similar to DEK1 knockdown phenotypes (a dominant negative effect). Lid et al., 2005; Johnson et al., 2005; Tian et al., 2007; Johnson et al., 2008.</td>
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<td>ZHOUPI (ZOU)</td>
<td>mRNA expression in the endosperm surrounding the embryo; expression is reduced after the torpedo stage. Protein localization in the nuclei.</td>
<td>zou No apparent cuticle proper formation in the embryo; discontinuity of the cuticle in the cotyledon; large tears in the cotyledon epidermis; ALE1 mRNA expression is almost absent. zou;ale1 Similar to zou. zou;acr4 More severe defects in cotyledon surface formation than either single mutant. zou;ale2 More severe defects in cotyledon surface formation than either single mutant; fusion of cotyledons. zou;gso1;gso2 Adult plants resemble gso1/gso2; seed phenotypes are similar to those of zou. &lt;Ectopic expression of ZOU&gt; Ectopic expression of ZOU in leaves of zou-1D is not sufficient to induce ALE1 expression. Yang et al., 2008; Xing et al., 2013.</td>
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<td><strong>GASSHO1/2 (GSO1/GSO2)</strong></td>
<td>Leucine-rich repeat receptor-like kinase</td>
<td><strong>gso1</strong></td>
<td>Wild-type phenotype.</td>
<td><strong>gso1;gso2</strong></td>
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<td><strong>GSO1</strong> Ubiquitous promoter activity throughout embryogenesis; cotyledons, hypocotyl, roots, and floral organs after germination.</td>
<td><strong>GSO2</strong> Promoter activity in the embryo proper; cotyledons, hypocotyl, and floral organs after germination.</td>
<td><strong>zou;gso1;gso2</strong></td>
<td>See ZOU mutant phenotype.</td>
<td><strong>acr4;gso1;gso2</strong></td>
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<td></td>
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<td><strong>atml1;gso1;gso2</strong></td>
<td>Similar to <strong>gso1;gso2</strong> phenotype.</td>
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<td><strong>pdf2;gso1;gso2</strong></td>
<td>See PDF2 mutant phenotype.</td>
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<td><strong>rpk1</strong></td>
<td>Expression in the suspensor at the 8 cell stage; the basal half of the embryo proper and the suspensor after the 16-cell stage.</td>
<td><strong>rpk1;toad2</strong></td>
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<td><strong>toad2</strong></td>
<td>Expression in the central domain protoderm at the early globular stage; similar expression pattern as RPK1 at the late globular stage.</td>
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<td><strong>protein localization</strong> in the plasma membrane.</td>
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<td><strong>rpk1;toad2</strong></td>
<td>Embryo-lethal phenotype; irregular cell division planes in the hypophysis (frequently) and protodermal cells (occasionally); enlarged protodermal cells; loss of ATML1 mRNA expression after the globular stage; ectopic expression of inner cell markers in the protoderm.</td>
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<td><strong>CRINKLY4 (CR4)</strong></td>
<td>Receptor-like kinase</td>
<td><strong>cr4</strong></td>
<td>Crinkly leaves; fusion of leaves; patchy loss of aleurone cells; disorganized proliferation and irregular division planes of leaf epidermal cells; multiple epidermal cell layers; large epidermal cells.</td>
<td><strong>cr4;dek1</strong> (strong alleles)</td>
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<td><strong>protein localization</strong> in the plasma membrane, endosomes, and plasmodesmata; colocalized with DEK1 and SAL1.</td>
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<td><strong>DEFECTIVE KERNEL1 (DEK1)</strong></td>
<td>Calpain-like cysteine proteinase</td>
<td><strong>dek1</strong></td>
<td>Lack of the aleurone layer; embro-lethal (strong alleles); crinkly leaves; bulliform-like epidermal cells; multiple epidermal cell layers.</td>
<td><strong>cr4;dek1</strong> (strong alleles)</td>
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<td>mRNA expression in most tissues.</td>
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<td>Protein localization in the plasma membrane and endosomes; colocalized with CR4 and SAL1.</td>
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<tr>
<td><strong>SUPERNUMERARY ALEURONE LAYERS</strong> (SAL1)</td>
<td>Ubiquitous mRNA expression in most tissues.</td>
<td>Multiple aleurone layers; embro-lethal (strong alleles); crinkly leaves; enlarged and irregular shape of epidermal cells similar to those of weak <em>cr4</em>; normal subcellular localization of CR4 and DEK1.</td>
<td>Shen et al., 2003; Tian et al., 2007.</td>
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<td><strong>Class E vacuolar sorting protein</strong></td>
<td>Protein localization in endosomes; colocalized with CR4 and DEK1.</td>
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</table>

| **EXTRA CELL LAYERS** (XCL1) | Xcl1 | Semi-dominant phenotype; formation of an extra aleurone layer; epidermis-like extra cell layers in leaves; square pavement cells; irregular division planes in shoots and aleurone layers. | Kesseler et al., 2002. |

|  | Sector analysis | Extra cell layers are generated by oblique and periclinal divisions of epidermal cells late in leaf development. | |
|  | Dosage analysis | Decreased dosages of the wild-type gene increase anticlinal cell division in leaves; increased dosages of the wild-type gene cause formation of extra aleurone layers and enhance the mutant phenotype. | |