The ASK1 and ASK2 Genes Are Essential for Arabidopsis Early Development

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The requirement of CUL1 for Arabidopsis embryogenesis suggests that Skp1–CUL1–F-box protein (SCF) complexes play important roles during embryo development. Among the 21 Arabidopsis Skp1-like genes (ASKs), it is unknown which ASK gene(s) is essential for embryo development. In this study, we demonstrate a vital role for ASK1 and ASK2 in Arabidopsis embryogenesis and postembryonic development through analysis of the ask1 ask2 double mutant. Our detailed analysis indicates that the double mutations in both ASK1 and ASK2 affect cell division and cell expansion/elongation and cause a developmental delay during embryogenesis and lethality in seedling growth. The expression patterns of ASK1 and ASK2 were examined further and found to be consistent with their roles in embryogenesis and seedling development. We propose that mutations in ASK1 and ASK2 abolish all of the ASK1- and ASK2-based SCF and non-SCF complexes, resulting in alteration of gene expression and leading to defects in growth and development.

INTRODUCTION

Ubiquitin-mediated proteolysis by the proteasome is a critical regulatory mechanism that controls diverse biological processes, including cell cycle progression, cell differentiation, transcriptional regulation, signal transduction, and development (Hershko and Ciechanover, 1998; Deshaies, 1999; Hellmann and Estelle, 2002). The ubiquitylation of protein substrates is achieved through the sequential actions of a ubiquitin-activating enzyme (E1), a ubiquitin-conjugating enzyme (E2), and a ubiquitin-protein ligase (E3). The resulting ubiquitin-substrate conjugates are degraded subsequently by the 26S proteasome.

The SCF ubiquitin-protein ligase complexes represent a family of E3s and consist of Skp1, CUL1/Cdc53, a ring finger protein, Rbx1/Hrt/Roc1, and an F-box protein. CUL1 functions as a scaffold protein that interacts with Skp1 and Rbx1, which functions in recruiting of the E2 enzyme; Skp1 serves as an adaptor that links CUL1/Cdc53 and the F-box protein (Hershko and Ciechanover, 1998; Deshaies, 1999). The crystal structure of the SCF complex (Kim and Delaney, 2002) demonstrated to control flower development (UFO) (Ingram et al., 1999), jasmonate (COI1) (Xie et al., 1998), and gibberellin (SLY1) (McGinnis et al., 2003) as well as the defense response (SON1) (Kim and Delaney, 2002). Some F-box proteins are demonstrated to control flower development (UFO) (Ingram et al., 1997; Samach et al., 1999; Zhao et al., 2001), flowering time and the circadian clock (ZTL, PDF1, and LKP2) (Nelson et al., 2000; Somers et al., 2000), leaf senescence and lateral shoot branching (ORE9/MAX2) (Woo et al., 2001; Stirnberg et al., 2002), and photomorphogenesis (EID1) (Dieterle et al., 2001).

Dysfunction of the SCF core components was found to cause severe defects in diverse developmental processes. In yeast, mutation of Rbx1 leads to cell cycle arrest before DNA replication and results in the stabilization of the SCF substrates Sic1 and the G1 cyclin Cin2 (Kamura et al., 1999; Seol et al., 1999). In Arabidopsis, altered expression of Rbx1 causes severe defects in plant growth and development (Gray et al., 2002; Lehner et al., 2002; Schwechheimer et al., 2002; Xu et al., 2002). Mutations in the core component, CUL1/Cdc53, lead to severe developmental defects, including arrest at the G1/S-phase in budding yeast (Schwob et al., 1994) and embryo lethality in mouse (Dealy et al., 1999; Wang et al., 1999) and Arabidopsis (Shen et al., 2002).

Another core component, Skp1, is involved in diverse biological processes. It is a subunit of SCF complexes essential for and yeast, where the core components Skp1, CUL1/Cdc53, and Rbx1/Hrt/Roc1 serve in multiple SCF complexes involving different F-box proteins. The F-box proteins function as receptors to recruit various substrates and confer specificity on SCF complex function. In Arabidopsis, 694 potential F-box genes have been identified (Gagne et al., 2002). Genetic analysis has revealed the roles of many F-box genes, including responses to the plant hormones auxin (TIR1) (Ruegger et al., 1998; Gray et al., 1999), jasmonate (COI1) (Xie et al., 1998), and gibberellin (SLY1) (McGinnis et al., 2003) as well as the defense response (SON1) (Kim and Delaney, 2002). Some F-box proteins are demonstrated to control flower development (UFO) (Ingram et al., 1997; Samach et al., 1999; Zhao et al., 2001), flowering time and the circadian clock (ZTL, PDF1, and LKP2) (Nelson et al., 2000; Somers et al., 2000), leaf senescence and lateral shoot branching (ORE9/MAX2) (Woo et al., 2001; Stirnberg et al., 2002), and photomorphogenesis (EID1) (Dieterle et al., 2001).

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the initiation of DNA replication at the G1/S checkpoint in yeast (Bai et al., 1996). Skp1 also has been shown to be an important subunit of other non-SCF complexes, including the CBF3 kinetochore complex that is required for the completion of the mitotic cell cycle (Connelly and Hieter, 1996). The requirement of CUL1 for embryogenesis suggests that SCF complexes play important roles during embryonic development in mouse and Arabidopsis (Dealy et al., 1999; Wang et al., 1999; Shen et al., 2002). Therefore, one or more Skp1 homologs may be important to embryonic development. In the Arabidopsis genome, there are 21 predicted Skp1-like genes (ASKs) (Farras et al., 2001; Gagne et al., 2002; Risseeuw et al., 2003). However, it is not known which ASK gene(s) is involved in the regulation of embryonic development.

Genetic studies indicate that the ASK1 gene is essential for male meiosis: a Ds transposon insertion into the ASK1 gene results in male sterility (Yang et al., 1999), reduction of leaf and petal sizes, alteration of floral organ identity (Zhao et al., 1999), and defects in auxin response (Gray et al., 1999). There are no detectable defects in embryogenesis and early seedling development in the ask1 mutant, although some of the ask1 phenotypes are consistent with a reduction in cell division (Zhao et al., 1999, 2001), suggesting that either another single uncharacterized ASK gene instead of ASK1, or two (or more) ASKs that are functionally redundant, may play a major role(s) in embryonic development.

Among the ASK genes, ASK2 is the most similar to ASK1 in sequence (75.4% amino acid identity and 84.8% similarity) (Gagne et al., 2002); in addition, ASK1 and ASK2 exhibit very similar expression patterns during vegetative and reproductive development (Zhao et al., 2003). Furthermore, phylogenetic analysis indicates that ASK1 and ASK2 are the most conserved among all ASK genes with respect to the yeast and human SKP1 genes, suggesting that ASK1 and ASK2 most likely share the functions of the yeast SKP1 gene in regulating the cell cycle (Kong et al., 2004). These observations indicate that ASK1 and ASK2 may play redundant roles in controlling cell division and early development. However, the fact that the ask1 mutant has visible developmental defects indicates that ASK2 cannot fulfill at least some ASK1 functions. In addition, phylogenetic analysis indicates that the gene duplication that produced ASK1 and ASK2 likely occurred very early during angiosperm evolution, suggesting that ASK1 and ASK2 represent separate lineages that might have been maintained for distinct functions (Kong et al., 2004). Therefore, it is not clear whether ASK1 and ASK2 are functionally redundant; in particular, it is unknown whether they have redundant functions in embryonic development.

In this study, we describe a new null mutant allele of the ASK2 gene and show that the ask2 mutant has normal development throughout its life cycle. Furthermore, we demonstrate that ASK1 and ASK2 together are important for embryogenesis and essential for seedling development. The double mutations in both ASK1 and ASK2 led to a developmental delay at mid embryogenesis and resulted in seedling lethality, whereas these novel defects were not detected in either ask1 or ask2. The expression patterns of ASK1 and ASK2 are consistent with their roles in embryogenesis and seedling development. In addition, the fact that the defects in the ask1 ask2 double mutant are much less severe than those in the ask1 null mutant suggests that other ASK genes also may be important for early embryonic development.

**RESULTS**

**An ask2 Mutant Exhibits Normal Development and Growth**

Among the 21 ASK genes, only ASK1 function was revealed previously through analysis of a Ds insertional ask1 mutant (ask1-1). The ASK2 protein was found to associate with several F-box proteins, such as TIR1 (Gray et al., 1999), COI1 (Xu et al., 2002), UFO (Samach et al., 1999), and EID1 (Dieterle et al., 2001). To investigate ASK2 function, we screened for and identified a T-DNA insertion in the ASK2 gene (ask2-1) 318 bp downstream of the start codon ATG (Figure 1A). RNA gel blot analysis showed that the ASK2 transcript was not detected in the ask2 mutant, indicating that ask2-1 is an RNA null allele (Figure 1C).

The ask2 plants were morphologically similar to wild-type plants under normal growth conditions (Figures 1D and 1F) and developed normal roots, leaves, shoots, and flowers. Whole-mount examination of embryonic development in ask2 siliques (Figure 1E) showed that the ask2 embryos were indistinguishable from wild-type embryos: they all reached the late heart or early torpedo stages at 4 days after fertilization (DAF) and the bent-cotyledon stage at 6 DAF. In short, ask2 has no obvious morphological defects in embryonic, vegetative, and floral development.

**Generation and Initial Characterization of ask1 ask2**

ASK1 and ASK2 are highly similar in both sequence (Gagne et al., 2002) and expression patterns (Zhao et al., 2003); furthermore, ASK1 and ASK2 proteins can interact with the same F-box proteins (Gray et al., 1999; Samach et al., 1999; Dieterle et al., 2001; Xu et al., 2002). To test for possible functional redundancy between ASK1 and ASK2, we generated and analyzed ask1 ask2 double mutant plants (ask1-1/ask1-1 ask2-1/ask2-1).

Because the homozygous ask1 mutant is male sterile and the ask1-1 allele must be maintained by a heterozygous plant (Yang et al., 1999), we identified ASK1/ask1-1 ask2/ask2 plants (referred to as ASK1/ask1 ask2) among the F2 progeny derived from a cross between the ask2 and ask1 mutants using PCR with gene-specific primers (Figures 1A and 1B). The F3 progeny were generated from the ASK1/ask1 ask2 plant and screened by PCR for the ask1 ask2 double mutants. When germinated on plates, most F3 seeds grew into normal plants, whereas a small proportion of seeds failed to germinate or to develop properly after germination. The abnormal seedlings usually had two small cotyledons but lacked significant development of roots and leaves (Figure 1F). They ceased growth within 8 to 10 days after germination. These tiny deformed seedlings and embryos from the nongerminated seeds were found to be homozygous for both ask1 and ask2 alleles (Figure 1G).
Next, we examined the morphology of seeds produced from self-pollinated ASK1/ask1 ask2 plants. As expected, wild-type siliques contained normal seeds that were large and green, as shown in Figure 2B1. However, the siliques of ASK1/ask1 ask2 plants contained both normal seeds that were large and green and a small number of abnormal seeds that were brown and shriveled (Figure 2A1). The embryos from the abnormal seeds (Figure 2A, arrows) were homozygous for both ask1 and ask2 mutant alleles (data not shown).

**Embryo Development of ask1 ask2**

The abnormal seed and seedling phenotypes of as1 ask2 strongly suggest that the combination of the ask1 and ask2 mutations affects embryo development. Therefore, we used scanning electronic microscopy to examine embryos of mature seeds. The abnormal seeds of ASK1/ask1 ask2 plants were found to contain embryos at the heart and torpedo stages (Figure 2A3), whereas all of the wild-type seeds contained mature embryos (Figure 2B3).

To examine embryo development at various stages, we manually pollinated ASK1/ask1 ask2 plants with their own pollen grains and examined developing embryos at specific times after fertilization. In the siliques of wild-type, ask2 homozygous, and ASK1/ask1 heterozygous plants, the majority of embryos reached the transition or heart stage by 3 DAF (days after pollination), the heart or torpedo stage at 4 DAF, the bent-cotyledon stage at 6 DAF, and the mature stage from 8 DAF onward (Table 1). However, ASK1/ask1 ask2 plants grown under the same conditions showed significant variation in the stages of embryo development within a single silique, as observed from 3 to 10 DAF (Table 1, Figures 2C and 2D). We found that 148 of 199 embryos at 3 DAF reached the transition or heart stage, as in wild-type plants; however, the remaining 51 embryos (25.6%) showed retarded development at the globular stage (Table 1). In terms of cell numbers, this retarded development represents an ~30% slower growth rate of the sibling embryos (Jurgens and Mayer, 1994). As development progressed, the abnormal embryos exhibited more severely retarded development from 4 DAF (Table 1, Figures 2C and 2D). All embryos from wild-type, ask2 homozygous, and ASK1/ask1 heterozygous plants reached the maturation stage at 10 DAF (Table 1, Figures 2C and 2D), demonstrating that the ask1 or ask2 single mutation had no obvious...
effect on embryo development. By contrast, at 10 DAF, approximately one-quarter of the embryos of ASK1/ask1 ask2/ask2 plants (64 of 265) were abnormal and formed a shape resembling that seen in the late heart or early torpedo stage (Table 1, Figures 2C and 2D). After seeds were harvested, longitudinal embryo sections of mature seeds showed that abnormal seeds contained embryos before the bent-cotyledon stage (Figure 2C). The progression of embryogenesis in Arabidopsis is well documented (Jurgens and Mayer, 1994; Meinke, 1995). The body plan of major embryonic structures, including the shoot meristem, cotyledon, radicle (embryonic root), and hypocotyl, is established early in embryogenesis (Jurgens, 2001). An examination of the progression of embryo pattern formation revealed that the early pattern formation was similar between embryos of the ASK1/ask1 ask2/ask2 siliques before the transition stage (Figures 2C and 2D, 3 and 4 DAF). At the point at which differences in pattern formation between the double mutant and sibling embryos were discernible, progenitor cells had formed that give rise to all of the major tissues: the epidermal layer, ground tissue, vascular tissue, and shoot and root initials (Figures 2C and 2D). However, the abnormal embryos showed a greatly reduced number of cells in the cotyledon primordia from the transition or heart stage (Figure 2C, 6 DAF), whereas cell division in wild-type embryos was most rapid at this stage (Figure 2D, 4 DAF). Additionally, the cells in the central region that give rise to the hypocotyl showed fewer cell divisions, and the mutant embryos failed to enter the bent-cotyledon stage (Figures 2C, 10 DAF and seed, and 2D 6 DAF).

In summary, these results demonstrate that the ask1 ask2 double mutant embryos exhibited retarded development, particularly at the stage at which a high rate of cell division is required, suggesting that the double mutant embryos were unable to undergo rapid cell division. The ask1 ask2 embryos eventually stopped growing before the bent-cotyledon stage, although the basic pattern elements, including epidermal layer, ground tissue, vascular tissue, and shoot and root initials, were generated.

Postembryonic Development of ask1 ask2

To gain additional insights into the defects of the ask1 ask2 double mutant, we examined the postembryonic development of ask1 ask2 in more detail. Because ask1 ask2 seedlings were much smaller than those of wild-type, ask1, or ask2 plants (Fig-
ure 1F and data not shown), we used scanning electron microscopy to examine seedling morphology from 1 to 9 days after germination (DAG). In wild-type plants, the cotyledons expanded and splayed open (an epinastic process) at ~2 DAG (Figure 3A1); roots and leaves were well developed soon afterward (Figures 1F and 3A2 and 3A3). Similarly, ask1 or ask2 single mutant seedlings were morphologically normal within the first 10 DAG (Figure 1F and data not shown). However, the ask1 ask2 double mutant grew extremely slowly after germination and eventually died (Figures 3A4 to 3A7). Cotyledons from ask1 ask2 seedlings sometimes failed to expand or were very slow to expand and displayed epinasty only at 5 to 9 DAG (Figures 3A6 and 3A7 and data not shown). Likewise, the hypocotyl elongation of ask1 ask2 seedlings was very slow or absent; instead, the double mutant hypocotyl mostly showed slight radial swelling (Figures 3A4 to 3A7).

The internal anatomy of these plants was examined further using thin sections (Figure 3B). Wild-type, ask1, and ask2 seedlings were indistinguishable from each other at 2 DAG (Figure 3B1 and data not shown) or at 5 DAG (Figures 3B2 to 3B4). These seedlings at 5 DAG had initiated several leaf primordia from the shoot apical meristem (SAM). In addition, the petiole of the cotyledon and the hypocotyl clearly showed apical-basal elongation of cells (Figures 3B2 to 3B4). Analysis of ask1 ask2 seedlings (Figures 3B5 to 3B7), however, revealed that the cells in the double mutant did not expand and grow as in the control seedlings at 2 or 5 DAG and even after 9 days of growth. Nevertheless, the basic apical-basal and radial patterns were maintained in the double mutant. The germinated ask1 ask2 mutant showed significantly reduced cell size, and most of the cells failed to elongate compared with those in the wild type or the single mutants (Figures 3A and 3B).

The SAM of ask1 ask2 seedlings also was abnormal, being enlarged relative to the other structures of the shoot. Also, the dense cytoplasmic cells that are typical of the SAM and very young primordia were distributed unevenly or restricted mostly to the outermost cell layers. Rudimentary organ primordia occasionally initiated by 9 DAG (Figures 3A7 and 3B7). The cells of these primordia became enlarged and vacuolated just before the seedling ceased to grow at ~10 DAG (Figures 3A7 and 3B7). As shown in Figures 3B6 and 3B7, ask1 ask2 plants exhibited little apical-basal elongation of the root cells, and the roots ceased growth without any apparent initiation of lateral roots.

Light has an inhibitory effect on hypocotyl elongation. Because light-grown ask1 ask2 seedlings showed no obvious hypocotyl elongation, we examined whether the hypocotyl was able to elongate in the dark. As expected, dark-grown wild-type seedlings had dramatic elongation of the hypocotyl (Figures 3A9). However, dark-grown ask1 ask2 seedlings failed to exhibit hypocotyl elongation, even by 8 DAG (Figure 3A8), indicating that ask1 ask2 was severely defective in hypocotyl cell elongation and not responsive to dark treatment.

Thus, during postembryonic development, ask1 ask2 displayed severe defects in cell expansion/elongation and in the development of a root system or a shoot.

### Table 1. Developmental Stages of Embryogenesis

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Siliques from the wild type, ask2 single mutants, ASK1/ask1 heterozygous plants, and ASK1/ask1 ask2 plants were dissected, and whole-mount preparations of cleared seeds were examined. The number of embryos at each developmental stage (Jurgens and Mayer, 1994) was recorded. Data for the ask1 ask2 double mutant embryos are shown in boldface.
The Plant Cell

Figure 3. Postembryonic Phenotypes of ask1 ask2 Mutants.

(A) Scanning electron micrographs (1 to 7) of wild-type (wt) plants (1) to (3) and ask1 ask2 mutants (4) to (7). The wild-type seedlings had commenced growth of the hypocotyl and opening of cotyledons at 2 DAG (1), and the cotyledons had expanded fully and leaf initiation was visible at 5 DAG (2) and (3) [enlargement]. The ask1 ask2 mutants are shown at 1 DAG (4), 2 DAG (5), 5 DAG (6), and 9 DAG (7). By 9 DAG, the cotyledons opened and the SAM was enlarged and revealed functionality by the initiation of leaf primordia (arrows). The ask1 ask2 mutant treated in the dark at 22°C for 8 days showed no hypocotyl elongation (8), whereas the wild type treated in the dark for 5 days exhibited a rapid elongation of hypocotyl (9).

Bars = 100 μm (1 to 7) and 1 mm (8) and (9).

(B) Histological longitudinal sections of the wild type (1) and (2), ask2 (3), ask1 (4), and ask1 ask2 (5) to (7). The ask2 (3) and ask1 (4) single mutants were indistinguishable from the wild type as revealed by histological longitudinal sections of shoots at 2 DAG (1) and 5 DAG (2) to (4). The ask1 ask2 double mutant was emerging from the seed coat at 2 DAG (5). By 5 DAG (6), the SAM region of ask1 ask2 showed enlargement; however, the cells at the base of the cotyledons remained small and round. By 9 DAG (7), the lateral organ primordia of ask1 ask2 had begun to initiate (arrows), but the cells at the base of the cotyledons and the hypocotyl showed limited expansion. Bars = 100 μm.
Cell Divisional Defects in ask1 ask2

Detailed examination of embryo development suggests that the ask1 ask2 embryos failed to undergo rapid cell division, particularly at the transition or heart stage and onward (Table 1, Figures 2C and 2D). Here, we investigated whether ask1 ask2 is defective in cell division through examination of hypocotyl cell number and expression analysis of the mitosis-specific CycB1 using a β-glucuronidase (GUS) reporter (Colon-Carmona et al., 1999).

When the hypocotyls of seedlings at the postembryonic developmental stages from 0 to 2 DAG were used to determine cell numbers, we found that the hypocotyl of ask1 ask2 was approximately eight cells in length (7.6 ± 1.76), whereas the hypocotyls of the wild type, ask1, and ask2 were on average 17 cells long (Figure 4A and data not shown). These data clearly indicate that ask1 ask2 is defective in cell division.

The Arabidopsis mitotic cyclin CycB1;1 is an excellent marker for cells undergoing mitosis, because it is expressed at the G2/M transition and degraded during cell cycle progression from metaphase to anaphase by the anaphase-promoting complexes/cyclosome (APC/C) (Doerner et al., 1996; Colon-Carmona et al., 1999; Capron et al., 2003). To further investigate the defect of cell division in ask1 ask2, we made a genetic cross between ASK1/ask1 ask2 and the transgenic plant harboring the labile CycB1-GUS reporter construct, in which the GUS reporter was fused translationally to the genomic fragment containing the CycB1;1 promoter and the N-terminal coding sequence encoding the mitotic destruction box (Colon-Carmona et al., 1999). In 4-day-old (Figure 4B1) and 8-day-old wild-type seedlings (data not shown), CycB1-GUS expression was detected as staining of GUS activity in tissues with mitotic activity, including root tips and young leaf primordia, but not in expanding cotyledons (Colon-Carmona et al., 1999). In 4-day-old ask1 ask2 seedlings, CycB1-GUS activity was present consistently in the cotyledons (Figure 4B3), and it was present at an extremely high level in 8-DAG seedlings (Figure 4B4), indicating a failure of degradation in the CycB1-GUS protein. The high levels of CycB1-GUS accumulation in ask1 ask2 cotyledons, but not in wild-type or sibling seedlings, suggest that cell division may occur slowly or arrest at mitosis.

In contrast to the CycB1-GUS accumulation in ask1 ask2 cotyledons, CycB1-GUS was absent in the root tips of 8-day-old ask1 ask2 seedlings that has ceased growth (Figure 4B4), although expression was observed initially in the radicles of ask1 ask2 embryos (Figure 4B2) and then in the root tips of 4-day-old ask1 ask2 seedlings (Figure 4B3). The absence of CycB1-GUS activity in root tips of the double mutant suggests that cell division had terminated, but not during M-phase.

In summary, the reduction of cell number in the hypocotyl, the abnormal accumulation of CycB1-GUS in the cotyledon, and the absence of CycB1-GUS expression in the root tips clearly indicate that ask1 ask2 is defective in cell division.

Defect of Callus Induction in ask1 ask2 Roots

Having found that the ask1 ask2 double mutant was defective in cell division, we further investigated the extent of this aberra-
tion by monitoring callus induction. Initially, we excised root tissues from wild-type, ask1, ask2, and ask1 ask2 plants and then cultured them on a root callus–inducing medium. After 3 weeks, wild-type, ask1, and ask2 roots had formed a large amount of callus tissue; however, ask1 ask2 roots failed to form callus (Figure 5). This finding was indicative of arrested cell division in the roots, which is consistent with the observation that CycB1-GUS expression was absent in roots of 8-DAG seedlings (Figure 4B4). It also is possible that the defect of callus induction in ask1 ask2 roots resulted from a failure of the cells to respond to plant hormones.

Misexpression of BREVIPEDICELLUS in ask1 ask2

Arabidopsis BREVIPEDICELLUS (BP; also known as KNAT1) is a key regulator that defines important aspects of growth and cell differentiation and that may play important roles in the establishment and maintenance of the meristem (Lincoln et al., 1994; Ori et al., 2000; Barton, 2001; Byrne et al., 2002; Venglat et al., 2002); additionally, misexpression of BP or a knockout of BP disrupts normal growth and development (Chuck et al., 1996; Ori et al., 2000; Byrne et al., 2002; Venglat et al., 2002). Because the morphological analysis of ask1 ask2 revealed enlarged SAMs and severe defects in growth, we investigated whether mutations in both ASK1 and ASK2 alter BP expression using the BP promoter–driven GUS reporter (Ori et al., 2000).

We introduced the BP-GUS marker (Ori et al., 2000) into the ask1 ask2 background by crossing and then examined the expression pattern in ask1 ask2 compared with that in the wild type. As shown in Figure 6, a patch of BP-GUS expression in the wild type was restricted within the SAM at early stages (Figures 6A1 to 6A3) and expanded to the hypocotyl vasculature but was absent from the leaves (Figure 6A4 and data not shown) (Ori et al., 2000). In ask1 ask2, the BP-GUS reporter displayed a broader expression pattern in embryos and was expressed abnormally in seedlings (Figure 6B).

The expression patterns of the BP and CycB1 genes, as monitored with GUS reporters, suggest that the loss of ASK1 and ASK2 activity might alter the expression of genes that are important for plant development and growth. Consistent with this notion, our microarray analysis also indicated that the global gene expression profile was altered severely in ask1 ask2 (data not shown).

Cyclin D3 Level and Ubiquitin-Conjugated Protein Patterns in ask1 ask2

The mammalian D-type cyclins are targeted by SCF complexes for ubiquitin-dependent degradation (Yu et al., 1998; Russell et al., 1999; Maeda et al., 2001). Arabidopsis cyclin D3, which is similar to the mammalian D-type cyclins, was found to accumulate to high levels when the SCF component Rbx1 was silenced (Lechner et al., 2002). Here, we tested the levels of Arabidopsis cyclin D3 by immunoblot analysis using total protein extracts from the wild type and ask1 ask2. The ask1 ask2 double mutant significantly accumulated cyclin D3 (Figure 7, middle panel). Furthermore, we examined ubiquitin-conjugated protein patterns and found that ask1 ask2 exhibited a pattern of ubiquitin-conjugated proteins different from that in the wild type (Figure 7, top panel). These data indicate that the loss of ASK1 and ASK2 activity may alter the ubiquitin-mediated proteolysis of SCF substrates.

Complementation

To further verify that the severe defects in embryo and seedling development were caused by the combination of both ask1 and ask2 mutations, we performed a functional complementation experiment with ask1 ask2. We generated a construct (tASK1) containing a genomic ASK1 fragment encompassing its 5’ flanking area and coding region (Figure 8A). The transformants harboring the tASK1 transgene were confirmed by PCR for the presence of the tASK1 transgene and for the absence of the wild-type ASK1 and ASK2 alleles (Figure 8C). Three independent ask1 ask2 tASK1 homozygous lines were identified for further functional analysis.

These ask1 ask2 tASK1 homozygous plants were found to exhibit normal development and to be morphologically indistinguishable from wild-type and ask2 plants during seedling and other stages, as shown in Figures 8B and 8D (and data not shown). In particular, they were able to develop normal roots, leaves, shoots, flowers, and seeds. In addition, ask1 ask2 tASK1 plants were found to display normal embryogenesis similar to that of wild-type plants (Figure 8E and data not shown).

Similarly, we also made the ask1 ask2 mutant plants transgenic for tASK2 (data not shown). The ask1 ask2 tASK2 plants, like the ask1 mutant plants, developed roots and leaves and exhibited insensitivity to the synthetic auxin 2,4-D (data not shown).

Together with the observations that the ask1 single mutant displays no severe defect in embryo or early seedling development (Table 1, Figure 3) (Zhao et al., 1999, 2001), the complementation of the ask1 ask2 mutations with tASK1 and tASK2 demonstrates that the defects in early development, including a delay in embryo development and seedling lethality, were caused by the combination of the ask1 and ask2 mutations.
Previous studies have shown that both ASK1 and ASK2 are expressed in different organs, including seedlings, roots, stems, leaves, flowers, and siliques. Furthermore, it was shown that ASK1 is highly expressed in all cells of the developing embryo using in situ mRNA hybridization (Porat et al., 1998; Zhao et al., 2003). However, ASK2 expression during early embryo development has not been documented. Here, we confirm ASK1 expression in the embryo and demonstrate that ASK2 also is expressed in developing embryos using RNA in situ hybridization, as shown in Figure 9.

In agreement with previous observations, ASK1 mRNA was detected throughout the developing globular- and heart-stage embryos at high levels and also was detected in the endosperm (Figures 9A1 and 9A2). In addition, ASK1 was expressed in the embryo at late stages (Figure 9A3 and data not shown), with a higher expression in cotyledon and axis. Compared with ASK1, ASK2 had a very similar expression pattern, but at a lower level. ASK2 mRNA was detected throughout the

Figure 6. Expression of the BP-GUS Reporter.

(A) In wild-type embryos, the expression of BP-GUS was restricted to the apical meristem at the torpedo stage (1), the mature stage (2), just after germination (3), and 3 DAG. BP-GUS was expressed in the SAM area of seedlings and in hypocotyls (4) (Ori et al., 2000).

(B) The ask1 ask2 embryos showed broader patches of expression at the torpedo stage (1) and after germination (2). Ectopic expression also was observed in the cotyledons at 8 DAG ([3] to [6]), even when expression was absent from the SAM (6). C, cotyledon.

Expression of ASK2

Previous studies have shown that both ASK1 and ASK2 are expressed in different organs, including seedlings, roots, stems, leaves, flowers, and siliques. Furthermore, it was shown that ASK1 is highly expressed in all cells of the developing embryo using in situ mRNA hybridization (Porat et al., 1998; Zhao et al., 2003). However, ASK2 expression during early embryo development has not been documented. Here, we confirm ASK1 expression in the embryo and demonstrate that ASK2 also is expressed in developing embryos using RNA in situ hybridization, as shown in Figure 9.

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globular- to mature-stage embryo as well as in the endosperm (Figures 9B1 to 9B3 and data not shown).

In addition to investigating the RNA expression pattern, we also assessed the ASK2 expression pattern at the translational level through the analysis of transgenic plants expressing an ASK2-GUS in-frame fusion protein under the control of the ASK2 endogenous promoter (Figure 10A). Histochemical staining of seedlings revealed that ASK2-GUS was expressed throughout the whole plant during seedling development, as shown in Figures 10B1 to 10B3. ASK2-GUS was expressed strongly in root tips and vascular tissues (Figures 10B4 to 10B7) and also was expressed in various organs, including flowers (data not shown). ASK2-GUS was expressed at high levels in the embryo.

**Figure 7.** Cyclin D3 Level and Ubiquitin-Conjugated Protein Patterns.

Total protein from the wild type (WT) and the ask1 ask2 mutant was immunoblotted with the antibodies @-ubiquitin (top) and @-cyclin D3 (middle). Total protein on a duplicated identical gel was stained with Coomassie blue solution and used as a protein loading control (bottom). Free ubiquitin is indicated.
from the globular to the maturation stages (Figure 10C); weak staining was observed in the endosperm (Figure 10C). The expression pattern of the ASK2-GUS fusion protein was consistent with the results of RNA gel blot and RNA in situ hybridization. Therefore, the expression of ASK1 and ASK2 in embryos and seedlings was coincident with their vital roles in embryogenesis and seedling growth.

**DISCUSSION**

Homologs of the SCF core components (CUL1/Cdc53, Skp1, and Rbx1/Roc1/Hrt1) are found in a wide range of eukaryotes, including human, mouse, *Drosophila*, *C. elegans*, plants, and yeast. In Arabidopsis, molecular studies and genomic sequencing have identified the homologs of SCF core components: AtCUL1, Rbx1, and Skp1-like proteins (ASKs). Genetic and molecular studies indicate that mutations in these core components can affect Arabidopsis growth and development. Embryos of *atcul1* null mutants arrest at the single cell stage after fertilization (Shen et al., 2002), and point mutations in *AtCUL1* also severely affect embryo development (Hobbie et al., 2000; Hellmann et al., 2003). By contrast, a knockout mutant of ASK1 (Yang et al., 1999), 1 of 21 Skp1 homologs (ASKs) (Farras et al., 2001; Gagne et al., 2002; Risseeuw et al., 2003), has no obvious defects in embryogenesis, although ASK1 clearly is important for normal vegetative and floral development and for male meiosis (Yang et al., 1999; Zhao et al., 1999, 2001). Here, the ask2 null mutant was shown to display no detectable defects in embryo development.

The absence of severe embryonic defects in the *ask1* and *ask2* single mutants either indicates the possibility that another uncharacterized ASK gene instead of ASK1 and ASK2 might play a major role in embryo development or suggests the hypothesis that 2 (or more) of the 21 ASKs may have redundant functions during embryogenesis. Here, we have demonstrated that the combination of *ASK1* and *ASK2* activities is essential for embryogenesis and seedling growth. The *ask1 ask2* double mutant displayed severe defects in embryo development and seedling growth, whereas the functional redundancy of *ASK2* with *ASK1* led to the lack of such severe abnormalities in either single mutant. Consistent with their functions, both *ASK1* and *ASK2* are expressed in embryos and seedlings as well as in various organs of plants. The broad expression pattern suggests that they also may play a general role in development and plant growth in addition to their role in early development.

The severe defects in *ask1 ask2* indicate that ASK1 and ASK2 are vital for fundamental processes, thereby ensuring early development and growth in Arabidopsis. However, the *atcul1* null mutant showed an earlier arrest of embryo development than did *ask1 ask2* (Shen et al., 2002). Furthermore, SCF complexes likely are required for gametophytic development, because both male and female gametophytes are affected in the *atcul1* null mutant (Shen et al., 2002). By contrast, the fact that *ask1 ask2* embryos constituted ~25% of the embryos in the siliques of *ASK1/ask1 ask2/ask2* plants indicates that *ask1 ask2* male and female gametophytes can develop normally. These findings suggest the participation of other ASK proteins, in addition to ASK1 and ASK2, in the fundamental processes that regulate Arabidopsis development and growth. In fact, several F-box proteins were able to interact with ASK1, ASK2, ASK4, ASK11, and/or ASK13 in yeast two-hybrid assays (Gagne et al., 2002; Risseeuw et al., 2003), and several ASK genes, including ASK3 and ASK4, were expressed in seeds or during pollen development (Zhao et al., 2003), supporting the possibility that these ASK proteins, including ASK3, ASK4, ASK11, and ASK13, may contribute to the fundamental processes that regulate Arabidopsis development and growth.

The loss of both *ASK1* and *ASK2* activities was found to cause defects in cell division and cell expansion/elongation, as indicated by several *ask1 ask2* phenotypes, including severe retardation during embryogenesis (Table 1, Figure 2C), a signif-

![Figure 9](image-url)
Figure 10. ASK2 Expression Pattern in Seedlings and Embryos, Assayed by the ASK2-GUS Fusion.

(A) Scheme of the ASK2-GUS reporter construct (not to scale). Arrows indicate the orientations and positions of the primers. HYG, hygromycin resistance gene; LB, T-DNA left border; Nos, nopaline synthase terminator; RB, T-DNA right border.

(B) Histochemical staining (12 h) of seedlings shows ubiquitous expression of the ASK2 gene (1 to 3). Histochemical staining for only 1 h shows that ASK2 was expressed most strongly in the roots (4 to 6). Examination of the root tips showed that it is strongest in the region of dividing cells and the root vasculature (6 and 7).

(C) Histochemical staining (8 h) revealed that ASK2 was expressed in embryos at all developmental stages, as indicated at the globular stage (1), the heart stage (2), and the maturation stage (3).
icant reduction of cell size and defective cell elongation during postembryonic development (Figures 3B and 3C), a decrease of cell number in hypocotyls (Figure 4A), a higher accumulation of CycB1-GUS in cotyledons (Figure 4B), the absence of CycB1-GUS in root tips (Figure 4B), and the inability of roots to induce callus formation (Figure 6). Animal D-type cyclins are G1 cyclins, and the Arabidopsis Cyclin D3 is thought to be required for the G1/S transition (Sherr, 1995; Riou-Khamlichi et al., 1999). The Arabidopsis cyclin D3, which is similar to the mammalian D-type cyclins that are targeted by SCF complexes for ubiquitin-dependent degradation (Yu et al., 1998; Russell et al., 1999; Maeda et al., 2001), was found previously to accumulate to high levels when the SCF component Rbx1 was silenced (Lechner et al., 2002). Together with our results that the level of cyclin D3 also increased in ask1 ask2 (Figure 7), these data indicate that ASK1- and ASK2-based SCFs may regulate cell cycle progression by reducing cyclin D3 after the G1-S transition.

Yang et al. (1999) demonstrated previously that ASK1 is required for homolog separation during male meiosis. In this study, the observations of an unusually high accumulation of CycB1-GUS activity in ask1 ask2 cotyledons indicate compromised APC/C activity and mitotic arrest or retardation, suggesting a new function for ASK1 and ASK2 in mitosis. It is possible that ASK1 and ASK2 also may be required for mitotic chromosome segregation. The observation that both CUL1 and ASK1 are localized to the mitotic spindle (Farras et al., 2001; Shen et al., 2002) supports the hypothesis that an ASK1 (ASK2)-containing SCF complex may regulate the structure and/or function of the spindle to facilitate mitosis. In budding yeast, the CBF3 kinetochore complex, one of the Skp1-based non-SCF complexes, is bound to a centromere DNA element and is essential for relaying the checkpoint signal to the spindle checkpoint pathway that regulates APC/C activity (Kitagawa et al., 2003). It also has been found that Skp1 homologs can localize to centromeres in different plant species (ten Hoopen et al., 2000). Therefore, ASK1 and ASK2 may have functions that involve non-SCF complexes.

We have shown that the ask1 ask2 mutant is defective in the regulation of cell division and has an enlarged SAM. This is consistent with the finding that transgenic plants with reduced CUL1 expression also have enlarged SAMs (Hellmann et al., 2003). Therefore, ASK1- and ASK2-containing SCF complexes may play a role in regulating the balance of cell division in the SAM and the recruitment of cells to form lateral organs. In particular, a greater number of cells in ask1 ask2 seedlings may retain the properties of meristematic tissues. This notion is supported by the observed ectopic expression of a GUS reporter gene for BP, which normally is expressed in the SAM. It is known that ASK1 is involved in the auxin response (Gray et al., 1999, 2001). Because auxin regulates lateral organ initiation (Reinhardt et al., 2000), our finding that ask1 ask2 is severely defective in leaf initiation suggests that the effect of ASK1 and ASK2 on lateral organs may be mediated by auxin-dependent pathways.

In summary, previous studies have demonstrated that Skp1 is a fundamental component of SCF complexes as well as an essential part of non-SCF complexes (Bai et al., 1996; Connelly and Hieter, 1996; Ayad et al., 2003; Kitagawa et al., 2003). Null mutations in both ASK1 and ASK2 are expected to abolish all of the ASK1- and ASK2-based complexes and result in the disruption of signaling and regulatory pathways mediated by these SCF and non-SCF complexes. It is likely that the ask1 ask2 mutant exhibits severe alterations in the expression of genes essential for cell division, cell expansion and differentiation, embryogenesis, and plant growth and development, leading to defects in embryo development and lethality in seedling growth. Mass-spectrometric comparisons and global gene expression analyses of ask1 ask2 and wild-type plants probably will reveal these complexes and genes. Further characterization and functional analysis of these genes would provide new insights into the mechanisms by which the ASK-based complexes regulate plant growth and development.

METHODS

Plant Materials

The ask2-1 mutant allele of Arabidopsis thaliana was isolated from T-DNA–tagged pools produced at the Wisconsin knockout facility (http://www.biotech.wisc.edu/Arabidopsis). The ask1-1 mutant was described previously by Yang et al. (1999). The BP-GUS transgenic line (Orli et al., 2000) and the labile CycB1-GUS line (Colon-Carmona et al., 1999) were crossed to ASK1/ASK1-1 ask2-1/ask2-1 plants, and F2 plants segregating for all alleles were selected for further analysis. Seeds were surface-sterilized, chilled at 4°C for 3 days, and then germinated and grown on plant growth medium (Murashige and Skoog [1962] medium supplemented with 2% sucrose) under a 16-h-light (22 to 24°C)/8-h-dark (16 to 19°C) photoperiod. Soil-grown plants also were grown under the same photoperiod.

Plasmid Constructs and Arabidopsis Transformation

A 1780-bp genomic fragment containing the ASK1 gene was amplified by PCR using the forward primer AP1000 (5′-AAARTGATTGTTTGGTGTT- GAA-3′) at 1000 bp upstream of the ASK1 ATG start codon and the reverse primer 780A (5′-AATAAAGGAGAAAGAAACTG-3′) at 40 bp downstream of the stop codon. The fragment then was cloned into the pCAMBIA1300 vector with the GUS reporter gene removed, resulting in the ASK1 construct (Figures 1A and 5A).

To construct an ASK2-GUS translational fusion, a 1850-bp genomic fragment containing the ASK2 promoter region and coding sequence without the stop codon was amplified by PCR using the forward primer BP800 (5′-TCTCTGTCTCCCTCTCTC-3′) at 817 bp upstream of the ASK2 ATG start codon and the reverse primer 1026B (5′-GGGATTGTCG- CTTAAAAGCCC-3′) that was engineered with an Ncol site to replace the ASK2 stop codon and allow an in-frame fusion with GUS. This fragment was cloned into the pCAMBIA1300 vector at Smal-Ncol sites, resulting in the ASK2-GUS construct (Figures 1A and 7A).

These constructs were verified by sequencing and introduced into Arabidopsis plants by the “floral-dip” method of in planta Agrobacterium tumefaciens–mediated transformation.

Determination of Genotypes by PCR

Gene-specific and allele-specific primers were designed to amplify ASK1 (wild-type allele), ASK2, ask1-1 (mutant allele), ask2-1, or the ASK1 transgene (tASK1). The forward primer A1 (5′-ATGTTCTGGAGAAGATGTC-3′) and the reverse primer 780A were designed to amplify a 780-bp fragment containing the ASK1 wild-type allele. The forward
primer B1 (5’-ATGTGACCGTGAGAAAAATC-3’) and the reverse primer 1026B were designed to amplify a 1026-bp fragment containing the ASK2 wild-type allele. The ask1-1 mutant allele was amplified with primer 780A and the forward primer D237 (5’-CCGGTATATCCGTT-TTCG-3’) at the 3’ end of the Ds transposon that is inserted into ASK1 at position 237 bp relative to the ATG start codon (Yang et al., 1999). The ask2-1 mutant allele was amplified with the forward primer B1 and the reverse primer 320T (5’-CATTTTATAAAACCGCTGAGCATCTAC-3’) located at the T-DNA left border that was inserted into ASK2 at position 318 bp relative to the ASK2 translation start codon.

To genotype the plants transformed with tASK1, the A1 forward primer and the reverse primer NR (5’-GTAACATGATAGCACCGCCGC-3’) specific to the NOS terminator sequence were combined to amplify the 318 bp relative to the ATG translation start codon.

GUS Staining Assays

Histochemical staining for GUS activity was performed using the standard X-Gluc solution (100 mM sodium phosphate buffer, pH 7.0, 0.1% Triton X-100, 1 mg/mL 5-bromo-4-chloro-3-indolyl b-D-glucuronide [X-Gluc]) with the addition of either 2 or 10 mM potassium ferricyanide and potassium ferrocyanide. The samples were vacuum-infiltrated in the X-Gluc solution for 5 min and kept at 37°C for 1 to 12 h, depending on the intensity of staining desired. Samples then were cleared in 70% ethanol and photographed.

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REFERENCES


Colón-Carmona, A., You, R., Haimovitch-Gal, T., and Doerner, P.


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