AtCSLA7, a Cellulose Synthase-Like Putative Glycosyltransferase, Is Important for Pollen Tube Growth and Embryogenesis in Arabidopsis

Florence Goubet, Audrey Misrahi, Soon Ki Park, Zhinong Zhang, David Twell, and Paul Dupree*

Department of Biochemistry, University of Cambridge, Building O, Downing Site, Cambridge CB2 1QW, United Kingdom (F.G., A.M., Z.Z., P.D.); and Department of Biology, University of Leicester, University Road, Leicester LE1 7RH, United Kingdom (S.K.P., D.T.)

The cellulose synthase-like proteins are a large family of proteins in plants thought to be processive polysaccharide β-glycosyltransferases. We have characterized an Arabidopsis mutant with a transposon insertion in the gene encoding AtCSLA7 of the CSLA subfamily. Analysis of the transmission efficiency of the insertion indicated that AtCSLA7 is important for pollen tube growth. Moreover, the homozygous insertion was embryo lethal. A detailed analysis of seed developmental progression revealed that mutant embryos developed more slowly than wild-type siblings. The mutant embryos also showed abnormal cell patterning and they arrested at a globular stage. The defective embryonic development was associated with reduced proliferation and failed cellularization of the endosperm. AtCSLA7 is widely expressed, and is likely to be required for synthesis of a cell wall polysaccharide found throughout the plant. Our results suggest that this polysaccharide is essential for cell wall structure or for signaling during plant embryo development.

Plant cell walls are composed mainly of the matrix pectic and hemicellulosic polysaccharides and cellulose (Brett and Waldron, 1996; Fry, 2000). The matrix polysaccharides are diverse and complex in structure, with α- or β-linked sugars in long backbones often decorated with short side chains. Xyloglucan, which has a β-1,4-glucan backbone, is the most abundant hemicellulose found in the primary cell wall of dicotyledonous plants, and is thought to cross-link cellulose microfibrils. Hemicellulosic polysaccharides with backbones of β-1,3-glucan (callose), β-1,4-mannan, or β-1,4-xylan are also abundant in certain cell types (Brett and Waldron, 1996; Fry, 2000). Classical arabinogalactan proteins (AGPs), consisting of up to 90% polysaccharide, are often also considered hemicellulosic polysaccharides (Fry, 2000). The arabinogalactan chains on AGPs consist of β-1,3-galactan with β-1,6-galactan branches that are further decorated, mostly with Ara (Fry, 2000; Majewska-Sawka and Nothnagel, 2000). These arabinogalactan chains can probably be found on a wide variety of cell wall proteins (Börner et al., 2002). In contrast to these polysaccharides with β-linked sugars in the backbones, the main backbone of pectin is α-1,4-polygalacturonan or, in rhamnogalacturonan I (RG-I), alternating α-1,4-rhamnosyl and α-1,2-galacturonosyl residues. The backbone of RG-I is modified by the addition of side chains of α-1,5-arabinan and β-1,4-galactan. Other polysaccharides such as mixed linkage β-glucans are found in certain species or tissues (Fry, 2000). Therefore, cell walls contain a rich array of different polymers, but the role and the extent of functional redundancy between these different polysaccharides are unknown.

For many years, researchers have attempted to purify transferases involved in the synthesis of cell wall polysaccharides to isolate the corresponding gene. However, just two have been successfully purified because of difficulties both in retaining activity after solubilization and assaying the transferases has limited the success of this approach. A galactomannan galactosyltransferase was purified from developing fenugreek (Trigonella foenum-graecum) cotyledons (Edwards et al., 1999) and a xyloglucan fucosyl transferase was purified from pea (Pisum sativum) seedlings (Perrin et al., 1999). These two transferases are predicted to have a single transmembrane domain with the transferase activity within the lumen of the Golgi apparatus.

In contrast, the plant cellulose synthase genes were first identified based on the high homology of the gene family with cellulose synthases (CELA) of bacteria (Pear et al., 1996). Second, studies of Arabidopsis mutants have been invaluable in identifying and characterizing the cellulose synthase genes. Recently, callose synthase of Arabidopsis (Hong et al., 2001), tobacco (Nicotiana tabacum; Doblin et al., 2001), and cotton (Gossypium hirsutum; Cui et al., 2001) have also been identified by a sequence homology-based approach. Both the cellulose and callose synthases are plasma membrane proteins with multiple mem-
brane-spanning domains. Although there are 12 cellulose synthase (CESA) genes in Arabidopsis (Richmond and Somerville, 2000; Saxena and Brown, 2000), characterization of the various Arabidopsis mutants has indicated that the genes are not redundant (Williamson et al., 2001). This could be because they form hetero-oligomeric complexes (Taylor et al., 2000), and because the genes are expressed at different growth and development stages.

The cellulose synthases are part of a large family of inverting processive β-glycosyltransferases. The family includes mammalian hyaluronan synthases and fungal chitin synthases, and belongs to the glycosyltransferase superfamily GT2 (Henrissat and Davies, 2000). In silico analysis suggests that a large number of relatively uncharacterized genes in plants, called the CELLULOSE SYNTHASE-LIKE (CSL) genes (Richmond and Somerville, 2000; Saxena and Brown, 2000; Hazen et al., 2002) encode glycosyltransferases in this family. These proteins have been divided into two and eight different subfamilies (depending on the plant), and they show varying degrees of sequence similarity to CESA proteins. It was initially speculated that each subfamily might be involved in synthesizing the backbones of the abundant polysaccharides, namely callose, α-1,4-polygalacturonan, RG-I, RG-II, xyloglucan, and xylan (Richmond and Somerville, 2000). However, the recently identified callose synthase is not a member of the CSL family (Doblin et al., 2001; Hong et al., 2001). Furthermore, the pectin backbones contain α-linkages, which are unlikely to be synthesized by a member of the GT2 glycosyltransferase family. Thus, the CSL subfamilies might synthesize the backbone of the remaining β-linked polysaccharides such as β-1,4-galactan, xylan, mannan, xyloglucan, and the β-1,3- and β-1,6-galactan of AGPs. In this hypothesis, type II transferases would be used to synthesize the α-linked backbones of arabinan and pectin and the short side chains on the β-linked polysaccharide backbones.

Studies of two genes in the CSLD subfamily, the subfamily most similar to the CESA genes, have been published recently (Favery et al., 2001; Wang et al., 2001). Two Arabidopsis mutants (kojak and csl3) have been produced by T-DNA or dissociation element (Ds) insertions into AtCSLD3. The mutants have fewer root hairs than the wild type (WT). By genetic analysis, it appears that the CSLD3 gene acts early in the process of root hair outgrowth (Favery et al., 2001; Wang et al., 2001). Although this protein is expressed in all parts of the plant (Wang et al., 2001), it is only in the root hair that a phenotype has been observed in the mutant. In contrast, NaCSDL1 of tobacco is only expressed in anther and in vitro-grown pollen tubes, and it has been predicted that this might be a cellulose synthase in pollen (Doblin et al., 2001).

In the work described here, we identified a mutation in AtCSLA7 of the CSLA subfamily of putative processive β-glycosyltransferases. The gene is ubiquitously expressed, and is important for pollen tube growth and essential for embryogenesis, suggesting a requirement for a specific β-linked polysaccharide in plant development.

RESULTS

Isolation of AtCSLA7 cDNA

As part of an ongoing program to screen for Arabidopsis insertion mutants in genes encoding polysaccharide synthases, we selected SGT4425 in the collection of Ds transposon insertion mutants with flanking sequences generated by Parinov et al. (1999). The sequence flanking the insertion in this line indicated that a Ds element had inserted in a gene encoding a putative glycosyltransferase. Before further analysis of this insertion mutant line, we confirmed by reverse transcriptase (RT)-PCR on RNA isolated from WT Arabidopsis callus that this gene was expressed. Preliminary sequence alignments suggested that the annotation of the gene AAD15455.1 by The Institute for Genomic Research was not correct because nucleotide sequence upstream of the proposed initiator Met appeared to encode amino acid sequence conserved in homologous genes. Using Netplantgene2 (Brunak et al., 1991; Hebsgaard et al., 1996), we identified a potential upstream exon, and confirmed the existence of this exon by amplification of a longer cDNA by RT-PCR. The cDNA amplified contains an in-frame upstream stop codon; therefore, we are confident that this sequence is full length. The intron/exon structure of the gene is shown in Figure 1A. The protein contains 556 amino acids (Fig. 1B), and has a predicted molecular mass of 63,795 D and a pI of 9.0. We predict that the protein has six transmembrane domains (Fig. 1B) with N and C termini in the cytosol (Fig. 1C).

Homology searches indicated that the encoded protein is a member of the processive β-glycosyltransferase superfamily (GT2) that includes plant and bacterial cellulose synthases (Henrissat and Davies, 2000). The CSL genes of Arabidopsis have been grouped into six subfamilies by Richmond and Somerville (2000). Using this nomenclature, the cDNA isolated corresponds to the gene AtCSLA7 in the CSLA subfamily containing 15 members in Arabidopsis. The “D,D,D,QXXRW” characteristic motifs of processive β-glycosyltransferases (Karnezis et al., 2000; Saxena and Brown, 2000; Williamson et al., 2001) are also found in AtCSLA7 (Fig. 1B, boxed). By alignment with CESA and CSL subfamily proteins, we found many residues conserved in all members (Fig. 1B, bold), or conserved within the CSLA subfamily (Fig. 1B, shadowed). Therefore, AtCSLA7 contains all the characteristics expected of a processive β-glycosyltransferase.
AtCSLA7 Expression

To investigate any organ-specific expression of AtCSLA7, RT-PCR was used to amplify the cDNA from RNA isolated from a range of plant tissues and organs. We found that the gene was expressed in all tissues examined, including old and young leaves, roots, callus, and pollen. Some examples are shown in Figure 2.

Identification of an Insertion Mutant

The transposon insertion line SGT4425 was analyzed for potential insertion in AtCSLA7. The position of the Ds insertion in exon 7 (Fig. 1A) was confirmed by direct PCR amplification of both ends of the Ds element and associated flanking genomic DNA. Analysis of over 300 plants from seven generations showed that all kanamycin-resistant (kanr) plants contained the insert at this site, showing tight linkage between kanamycin resistance and this insertion. We also screened these plants for any homozygous individuals that would not yield PCR amplification of the gene with a pair of gene-specific primers. However, all the kanr plants were heterozygous for the insertion. This indicated that the AtCSLA7 gene is an essential gene.

Transmission of the Insertion in AtCSLA7

If homozygous plants die, we would expect a segregation ratio of 2:1 kanr:kanamycin-sensitive (kan·) plants in the surviving progeny of plants heterozygous for the Ds insertion. However, plants from four generations consistently produced progeny that segregated approximately 1.3:1 kanr:kan· seedlings (Table I), indicating reduced transmission of the Ds insertion. This analysis also demonstrated tight linkage of the Ds insertion and the reduced transmission phenotype. The reduced genetic transmission of Ds in SGT4425 suggested a gametophytic role for AtCSLA7.

Male and female transmission of Ds was determined by performing reciprocal test crosses with WT Arabidopsis Landsberg erecta (Ler) and analyzing progeny on kanamycin selection plates. The data from five separate experiments are shown in Table II.
Male transmission was reduced to 29% of WT. In contrast, the female transmission efficiency (TE) was not affected. These data suggested an important gametophytic role for AtCSLA7 in pollen development or function.

Embryo Lethality of Homozygous Ds Insertion in AtCSLA7

Given the reduced, but significant, male transmission of the Ds insertion in SGT4425, homozygous progeny were predicted to occur at a frequency of 11%. However, as described above, no homozygous progeny were detected. Moreover, no evidence was obtained for a seedling lethal phenotype, suggesting that homozygotes might be embryo lethal. Examination of developing seed in mature green siliques of hemizygous SGT4425 mutants revealed that all siliques contained a proportion of aborted seeds (Fig. 3A). The proportion of aborted seeds was found to be 14.7% (total no. of seeds scored = 2,301) in plants from several different generations. Siliques of WT Ler did not show aborted seeds and none were observed when SGT4425 pollen was used to pollinate Ler pistils. When SGT4425 was used as the female parent in a cross to Ler, aborted seeds were observed infrequently (approximately 2%, n = 393). These data indicated that the homozygous Ds insertion in AtCSLA7 is a recessive seed lethal mutation.

Transformation of SGT24425 with a 4-kb genomic region including AtCSLA7 allowed recovery of plants homozygous for the Ds insertion in AtCSLA7. There was approximately doubled male TE and one-half the proportion of aborted seeds in plants hemizygous for the complementing DNA, indicating complementation of the phenotypes by AtCSLA7 (not shown).

WT and aborted seeds from mature SGT4425 green siliques were examined by differential interference contrast (DIC) microscopy. WT seeds contained cotyledonary stage embryos (Fig. 3B), but all aborted seeds (n = 76) contained small, undeveloped embryos with a distinct suspensor that were arrested at globular stage, or were elongated along the apical-basal axis (Fig. 3C). Mutant embryos were globular or elongate structures showing no evidence of cotyledon development. Cell proliferation was severely reduced such that the terminal phenotype of most mutant embryos was to arrest with 16 to 48 cells. Similarly, the endosperm remained uncellularized in aborted seeds and peripheral free nuclear endosperm was clearly visible (Fig. 3C).

To investigate further the developmental progression, embryos from a series of developing siliques of WT and hemizygous SGT4425 plants were categorized into stages of development from four to 16 cells to cotyledon. This revealed that embryo development was by and large synchronous in WT siliques, with sibling embryos spanning two successive stages (Fig. 4, A–D; Table III). In contrast, SGT4425 siliques contained embryos of a wider range of developmental stages. A proportion (13%–23%) of embryos with delayed development (four–16-cell stage) was apparent when the majority of WT embryos (Fig. 4D) were at heart stage (Table III). This difference was already apparent at globular stage with delayed embryos at the one- to 16-cell stage.

It was not possible to distinguish most WT and mutant embryos at one- to eight-cell stages. However, some abnormal eight-cell embryos were observed in which the axial and transverse division planes were rotated by 45° (Fig. 4E). Delayed globular embryos also showed abnormal division patterns that often involved an incomplete set of protoderm divisions (Fig. 4F). In siliques containing WT embryos at the late heart stage (Fig. 4D), mutant embryos were often elongated along the apical basal axis (Fig. 4H). In most mutant embryos, the protoderm layer was incomplete and aberrant cell division patterns were observed in the basal region of the embryo (Fig. 4, E–H). A common phenotype involved the formation of two additional cell tiers resulting from additional transverse divisions (Fig. 4, G and H). Thus, SGT4425 mutant embryos showed delayed development and abnormal cell patterning. No evidence was obtained for incomplete cell divisions. However, we cannot rule out subtle effects on cytokinesis not detectable with the DIC microscopy procedure used.

We investigated whether the effects of insertion in AtCSLA7 were restricted to the embryo or were also seen in endosperm development. The mean number of endosperm nuclear divisions was determined in whole-mount seeds by DIC microscopy. Seeds containing
taining mutant embryos at approximately the 16-cell stage contained 60.9 nuclei per seed, which was comparable with 52.9 in WT seeds containing embryos at the 16-cell stage (number of nuclei/H/11022 600). In terminally arrested seeds, endosperm nuclei showed a small increase to 76.4 nuclei per seed, whereas nuclei in seeds containing WT globular embryos continued to increase beyond 175 nuclei per seed, when the number of nuclei could be reliably counted. Thus, endosperm proliferation is not maintained in mutant SGT4425 seeds and is associated with failure of the endosperm to cellularize. The nuclei present in the endosperm of the arrested mutant seeds were relatively uniform and of comparable size with those in WT seeds at the coenocytic endosperm stage (see Fig. 3C). However, in some mutant seeds, larger nuclei were observed at the micropylar pole (Fig. 4G), suggesting continued cycles of endoreduplication after failed cellularization.

**AtCSLA7 Is Important for Pollen Tube Growth**

The reduced male transmission indicated a role for AtCSLA7 in pollen development or during pollen function. We used fluorescein diacetate, Alexander, and 4',6-diamino-phenylindole staining to test pollen for plasma membrane integrity, cytoplasmic density, and nuclear constitution, respectively. In all tests, we found that pollen development and viability appeared normal in SGT4425 (data not shown).

To investigate whether pollen tube growth was affected, the distribution of aborted seeds in siliques was examined in an in vivo competition experiment. If mutant pollen grew more slowly than WT, less transmission would be expected in ovules fertilized toward the base of the silique (Meinke, 1982). Siliques were divided into equal halves, and the proportion of aborted seeds counted in the basal and apical halves (Fig. 3A; Table IV). In selfed SGT4425, the proportion of aborted seeds in the apical half was close to the expected maximum of 25% (Table IV), supporting the notion that pollen development and germination were not significantly affected. However, in the basal half, there were significantly fewer aborted seeds (8.2%, a 3-fold decrease), indicating that further growth of the AtCSLA7 mutant pollen tubes was impaired.

**DISCUSSION**

We have isolated an insertional mutant of AtCSLA7, a gene predicted to encode a processive β-glycosyltransferase. The mutant shows embryo lethality and pollen tube growth is impaired. The results suggest that a cell wall polysaccharide synthesized by AtCSLA7 is essential for aspects of growth and development in Arabidopsis.

**AtCSLA7 Is a Member of the Superfamily of Processive β-Glycosyltransferases**

AtCSLA7 is a member of the large GT2 family of inverting processive β-glycosyltransferases that in-

---

**Table II. Segregation of kanr and kans seedlings in reciprocal test crosses of SGT4425 heterozygotes and WT (Ler) in five separate experiments**

<table>
<thead>
<tr>
<th>Experiment</th>
<th>SGT4425 × WT</th>
<th>WT × SGT4425</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>kan'</td>
<td>kan'</td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>1</td>
<td>78</td>
<td>82</td>
</tr>
<tr>
<td>2</td>
<td>90</td>
<td>91</td>
</tr>
<tr>
<td>3</td>
<td>79</td>
<td>79</td>
</tr>
<tr>
<td>4</td>
<td>127</td>
<td>132</td>
</tr>
<tr>
<td>5</td>
<td>263</td>
<td>250</td>
</tr>
<tr>
<td>Mean</td>
<td>637</td>
<td>634</td>
</tr>
</tbody>
</table>
includes hyaluronan and cellulose synthases (Henrissat and Davies, 2000; Saxena and Brown, 2000; Nobles et al., 2001). Like the other members of this family, AtCSLA7 is predicted to contain several transmembrane domains. In comparison with other members of the family, we predict a topology with six transmembrane domains (Fig. 1B). The large cytosolic loop would contain the conserved glycosyltransferase domain, often known as the D,D,D,QXXRW motif (Saxena and Brown, 2000; Nobles et al., 2001). The first and second D residues correspond to the DDS and DAD motifs, respectively (Fig. 1B), both conserved in all the CSLA members. These residues are thought to be important in binding the donor NDP-sugar and Mn$^{2+}$ (Wiggins and Munro, 1998; Karnezis et al., 2000). The third Asp and the QXXRW motif are found in the acceptor domain of CSLA7 and these residues are conserved in all the processive glycosyltransferases (Davies and Henrissat, 2002). By aligning AtCSLA7 with CELA, CESA, and CSL proteins from different organisms, we found that the Asp and the QXXRW motif lie within a widely conserved sequence: G(X)$_3$ED(X)$_{10}$G[W/Y/F](X)$_{23-25}$ QXXRW(X)$_2$G (Fig. 1B), suggesting that these residues are essential for the activity of the proteins. There are also further conserved regions in all members of the CSLA subfamily. Therefore, AtCSLA7 contains all the characteristics expected of the processive β-glycosyltransferases.

Mutants in the Processive β-Glycosyltransferase Family

The best studied Arabidopsis mutants in processive β-glycosyltransferases are those in the cellulose synthase CESA family (Saxena and Brown, 2000; Williamson et al., 2001). Interestingly, despite the existence of 12 CESA genes, often expressed in the same tissue, single-gene defects have been found to lead to clear phenotypic alterations. However, unlike the Atcsla7 mutant, none have yet been found to be essential. A model has been proposed in which the cellulose synthase subunits are active as a protein complex, and an absence of one subunit might inhibit the function of all the subunits of the complex (Taylor et al., 2000; Dhugga, 2001). There may be some overlap in expression and function of the different cellulose synthase complexes, such that some cellulose is synthesized in the absence of any one complex.

Very few mutants in any CSL gene have been described yet. Two groups have recently characterized a mutant in AtCSLD3 (Favery et al., 2001; Wang et al., 2001). The plants have weakened root hair walls because they burst as they begin to extend. Despite the expression of this gene in every tissue examined, the phenotype was observed only in the root hairs. This suggests that some redundancy may exist among the five AtCSLD genes. The only other previously discussed CSL mutant is rat4, which contains an insertional disruption in AtCSLA9 (described in a review by Richmond and Somerville, 2001). The rat4 mutant is resistant to Agrobacterium tumefaciens transformation. This bacterium binds to plant cell walls at an early stage of the infection (Nam et al., 1999). Interestingly, rat4 is dominant, suggesting the heterozygous mutant has insufficient of a certain cell wall component that is essential for A. tumefaciens infection. The recessive embryo lethality of the Atcsla7 mutation demonstrates that AtCSLA7 is not redundant to the other 14 family members, at least in early embryos. Thus, the AtCSLA7 protein might work in a complex with other CSLA family members, as has been suggested in the CESA family. Alternatively, it might synthesize a variant of a polysaccharide with a specific function, or be part of the only CSLA complex expressed in pollen and in seed development.

Figure 4. Embryo development in WT (A–D) and SGT4425 mutant (E–H) embryos present within siliques of hemizygous SGT4425 plants at different developmental stages. A and E, Sixteen-cell embryo proper stage; B and F, mid-globular stage; C and G, late globular transition stage; D and H, late heart stage of WT embryos. E, Abnormal eight-cell embryo proper with altered axial and transverse divisions. F, Abnormal early globular embryo. G and H, Abnormal embryos containing 28 to 46 cells showing abnormal transverse divisions and incomplete protoderm formation. Bars = 10 μm in A through C and E through H. Bar = 20 μm in D.
AtCSLA7 Is Required for Normal Pollen Tube Growth

Mutant Atcsla7 pollen developed normally, but its TE was reduced by 71% compared with the WT. This suggested a defect during prolagic (postpollination) development, which involves a number of distinct steps including adhesion, cell polarization and germination, pollen tube growth, guidance, and fertilization (Franklin-Tong, 1999; Wilhelmi and Preuss, 1999). The efficient fertilization of ovules positioned toward the apical end of the pistil suggests that early events are not affected and that mutant pollen tubes are correctly guided. Similarly, defects in fertilization can be excluded because failed ovules that could result from occupancy of the micropyle by mutant pollen tubes were not observed in Atcsla7 siliques. However, mutant pollen tubes clearly do not compete effectively with WT pollen tubes in the basal region of the pistil. This suggests a late defect in either in the rate of pollen tube growth, or termination of pollen tube extension resulting in pollen tubes being unable to reach the most basal ovules. The incomplete penetrance of the Atcsla7 mutation on pollen tube growth could support a role for other family members, or may suggest that glycans synthesized by AtCSL7 have a quantitative role in pollen tube extension.

The specialized tip growth mechanism of the pollen tube is associated with dynamic changes in cell wall structure and composition (Hepler et al., 2001). The pollen tube wall has an inner callosic layer and an outer fibrillar layer containing predominantly pectic polysaccharides, cellulose, xyloglucan, and arabinogalactan (Li et al., 1999). During pollen tube extension, calcium-mediated cross-linking of de-esterified pectins in the flanks of the apical pollen tube wall is thought to reinforce the pollen tube wall and focus cell expansion at the apex (Franklin-Tong, 1999). The defect in pollen tube growth in Atcsla7 could result from changes in cell wall properties, including its extensibility and/or stability as a result of the absence of a specific polysaccharide. Alternatively, AtCSLA7 could affect pollen tube growth though disruption of signaling events that are wall mediated. Such interactions between the stylar environment and the pollen tube are clearly significant in tube growth and guidance. For example, nonclassical

### Table III. Embryo development in WT and SGT 4425

Embryo developmental stages correspond to the cell no. or morphology of the embryo proper.

<table>
<thead>
<tr>
<th>Silique</th>
<th>No. of Seeds at Each Embryo Developmental Stage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1–4 Cells</td>
</tr>
<tr>
<td>WT (Ler)</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>46</td>
</tr>
<tr>
<td>2</td>
<td>30</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>–</td>
</tr>
<tr>
<td>5</td>
<td>–</td>
</tr>
<tr>
<td>6</td>
<td>–</td>
</tr>
<tr>
<td>7</td>
<td>–</td>
</tr>
<tr>
<td>8</td>
<td>–</td>
</tr>
<tr>
<td>9</td>
<td>–</td>
</tr>
<tr>
<td>SGT4425</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>35</td>
</tr>
<tr>
<td>2</td>
<td>17</td>
</tr>
<tr>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>9</td>
<td>–</td>
</tr>
<tr>
<td>10</td>
<td>–</td>
</tr>
</tbody>
</table>

### Table IV. In vivo pollen competition

The no. of aborted seeds was determined in the apical and basal halves of the siliques of selfed SGT4425 from different generations of plants.

<table>
<thead>
<tr>
<th>Seeds</th>
<th>Aborted</th>
<th>Total</th>
<th>Aborted</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Generation F1</td>
<td>39</td>
<td>156</td>
<td>11</td>
<td>147</td>
</tr>
<tr>
<td>Generation F2</td>
<td>115</td>
<td>522</td>
<td>45</td>
<td>505</td>
</tr>
<tr>
<td>% Average</td>
<td>23.5</td>
<td>–</td>
<td>8.2</td>
<td>–</td>
</tr>
</tbody>
</table>
AtCSLA7 Is Required for Embryo Development and Endosperm Proliferation

By studying the development of mutant and sibling WT seeds in individual siliques, we found that the rate of development of AtcslA7 mutant embryos was severely impaired, and that simultaneously the endosperm failed to proliferate. Although the embryos continued to increase in cell number throughout the normal developmental period, they finally arrested with terminal phenotypes that were morphologically pro-embryo or early globular. Patterning was generally normal until the octant stage, but early defects were observed in the orientation of the first or second axial divisions. The most common phenotype involved defects at the dermatogen stage, when octant embryos undergo eight asymmetric periclinal cell divisions to form the protoderm layer. The protoderm was frequently incomplete and abnormal transverse divisions in the basal region of the pro-embryo resulted in axially elongated globular embryos.

In Atcsla7, both embryonic cell patterning and cell proliferation are affected, yet in many mutants these phenotypes are not linked. In mutants that act early during embryogenesis to disturb embryo patterning such as ton/fass, keule, and knolle (Torres-Ruiz and Jurgens, 1994; Assaad et al., 1996; Lukowitz et al., 1996), abnormal embryos continue to develop. Similarly, the cell wall Hyp-rich glycoprotein RSH is essential for determination of division planes and cell shape in the embryo, but the cells continue to proliferate (Hall and Cannon, 2002). Second, a number of mutants including rps1-3 (Yadegari et al., 1994) and edd1 (Uwee et al., 1998) arrest with terminal globular phenotypes, yet these mutants show normal globular embryo patterning, including a complete protoderm layer. The defects in Atcsla7 of both cell proliferation and cell patterning might result from the metabolic dysfunction and chaotic failure of individual cells. However, given the prediction that AtCSLA7 is involved in cell wall synthesis, we favor a model where the phenotype arises from disturbed cell signaling that normally regulates cell proliferation and cell division patterning in the embryo. Although little is known about the role of cell wall components in signaling in embryos of higher plants, studies in *fucus* show that localized deposition of a sulfated polysaccharide is required to establish polarity of the egg cell, and this cell wall polysaccharide can determine cell fate (Belanger and Quatrano, 2000). The effects of the Atcsla7 mutation on seed development were not restricted to the embryo. Detailed analysis of the developing seeds revealed arrested proliferation of the endosperm nuclei without cellulization. This may reflect a shared requirement for AtCSLA7 in the embryo and the endosperm. Alternatively, AtCSLA7 may have a primary role in either, with defects in signaling between the two being responsible for the associated delay in embryo and endosperm development. Such signals could come from the embryo itself or the endosperm. The role of the endosperm in seed development is thought to involve the provision of both nutrients and signals to the developing embryo (Berger, 1999). In the medea fis, and fie mutants, endosperm development can proceed independently of embryo development (Vinke-noog et al., 2000). However, complete development of the endosperm does not occur, and it may depend on the presence of a normal embryo. Conversely, embryo development can occur in the absence of the endosperm during somatic embryogenesis (van Hengel et al., 1998, 2002). The nature of potential signals that control embryo development are unknown, although the involvement of oligosaccharides derived from chitin containing AGPs that are released by EP3 has been suggested (van Hengel et al., 1998, 2002; Berger, 1999).

The Function of AtCSLA7

The CSLs are likely to be β-glycosyltransferases that synthesize the backbones of cell wall polysaccharides. There are at least eight classes of β-glycan backbone-like chains in the dicot cell wall: cellulose, callose, mannans, xylans, the glucan of xyloglucan, the β-1,4-galactan of RG-I, and the β-1,3- and β-1,6-galactans of AGPs. Because cellulose synthase and callose synthases have been described, the six CSL families (CSLA–E, CSLG) identified in Arabidopsis on the basis of sequence similarity (Richmond and Somerville, 2000) could synthesize the six remaining classes. Two further families (F and H) have been identified in rice (*Oryza sativa*; Hazen et al., 2002), and one of these might synthesize the mixed-linkage glucan not found in dicots. It is also important to consider that one family as defined by sequence similarity could make more than one polysaccharide, and conversely, two families may synthesize the same polysaccharide. Indeed, it has been proposed that the CSLD family might be cellulose synthases (Doblin et al., 2001).

Which polysaccharide does AtCSLA7 synthesize? We clearly do not yet know. Cell wall polysaccharides have structural and signaling roles. We believe that the AtcslA7 embryo phenotype is more consistent with a signaling role, and is more severe than that because of cellulose deficiency (Gillmor et al., 2002). A signaling role would suggest that xyloglucan or AGPs are possible candidates. AtCSLA7 is ubiquitously expressed, and these polysaccharides
are present in most cell types. The structural xylans are thought to be essentially secondary wall polysaccharides, and, therefore, are likely to be essential only at a later developmental stage. The cyp11 mutant, unable to synthesize GDP-Man, is likely to be deficient in mannans, glycoproteins, and GPI-anchored cell wall proteins (Łukowicz et al., 2001). This mutant has a less severe embryo development phenotype than AtcsLA7; therefore, mannan synthesis is unlikely. In contrast, the β-1,4-glucan backbone of xyloglucan is a good candidate because the bacterial β-1,4-glucan (cellulose) synthases are more closely related to the CSLA subfamily than other plant glycosyltransferases. Alternatively, a clue may come from Atrat1, a mutant in AtCSLA9 (described in a review by Richmond and Somerville, 2001). This mutant is resistant to Agrobacterial infection, like the AGP mutant rat1 (Nam et al., 1999). Therefore, could the CsIA family be involved in AG glycan synthesis? AGPs contain both β-1,3- and β-1,6-galactan backbones that could be synthesized by processive glycosyltransferases. Intriguingly, AGPs have been implicated both in embryo development and pollen tube growth. Because arabinoxylans are more likely to be essential only at a later developmental stage. The mutant line was backcrossed to WT L. The mutant plants were transformed by the floral dipping protocol of Clough and Heslop-Harrison (1998). Fluorescein diacetate staining was carried out by incubation of freshly isolated pollen in 0.3 m mannitol solution containing 2 μg mL−1 fluorescein diacetate according to Heslop-Harrison and Heslop-Harrison (1970). Alexander staining of pollen was carried out on pollen grains fixed in ethanol:acetic acid (3:1 [v/v]) for 30 min. Fluorescein diacetate staining was carried out by incubation of freshly isolated pollen in 0.3 m mannitol solution containing 2 μg mL−1 fluorescein diacetate according to Heslop-Harrison and Heslop-Harrison (1970). Alexander staining of pollen was carried out on pollen released from flowers fixed in ethanol:acetic acid (3:1 [v/v]) for 30 min. After washing flowers in 50 mM Tris-HCl buffer (pH 6.8), released pollen grains were mixed directly with Alexander stain (Alexander, 1969).

For phenotypic characterization of mutant embryos, silique silique 9-diamino-phenylindole was carried out as previously described (Park et al., 1998). Pollen was treated with aniline blue solution to detect callose staining as previously described (Park and Twell, 2001). Fluorescein diacetate staining was carried out by incubation of freshly isolated pollen in 0.3 m mannitol solution containing 2 μg mL−1 fluorescein diacetate according to Heslop-Harrison and Heslop-Harrison (1970). Alexander staining of pollen was carried out on pollen released from flowers fixed in ethanol:acetic acid (3:1 [v/v]) for 30 min. After washing flowers in 50 mM Tris-HCl buffer (pH 6.8), released pollen grains were mixed directly with Alexander stain (Alexander, 1969).

For phenotypic characterization of mutant embryos, silique silique 9-diamino-phenylindole was carried out as previously described (Park et al., 1998). Pollen was treated with aniline blue solution to detect callose staining as previously described (Park and Twell, 2001). Fluorescein diacetate staining was carried out by incubation of freshly isolated pollen in 0.3 m mannitol solution containing 2 μg mL−1 fluorescein diacetate according to Heslop-Harrison and Heslop-Harrison (1970). Alexander staining of pollen was carried out on pollen released from flowers fixed in ethanol:acetic acid (3:1 [v/v]) for 30 min. After washing flowers in 50 mM Tris-HCl buffer (pH 6.8), released pollen grains were mixed directly with Alexander stain (Alexander, 1969).

For phenotypic characterization of mutant embryos, silique silique 9-diamino-phenylindole was carried out as previously described (Park et al., 1998). Pollen was treated with aniline blue solution to detect callose staining as previously described (Park and Twell, 2001). Fluorescein diacetate staining was carried out by incubation of freshly isolated pollen in 0.3 m mannitol solution containing 2 μg mL−1 fluorescein diacetate according to Heslop-Harrison and Heslop-Harrison (1970). Alexander staining of pollen was carried out on pollen released from flowers fixed in ethanol:acetic acid (3:1 [v/v]) for 30 min. After washing flowers in 50 mM Tris-HCl buffer (pH 6.8), released pollen grains were mixed directly with Alexander stain (Alexander, 1969).

For phenotypic characterization of mutant embryos, silique silique 9-diamino-phenylindole was carried out as previously described (Park et al., 1998). Pollen was treated with aniline blue solution to detect callose staining as previously described (Park and Twell, 2001). Fluorescein diacetate staining was carried out by incubation of freshly isolated pollen in 0.3 m mannitol solution containing 2 μg mL−1 fluorescein diacetate according to Heslop-Harrison and Heslop-Harrison (1970). Alexander staining of pollen was carried out on pollen released from flowers fixed in ethanol:acetic acid (3:1 [v/v]) for 30 min. After washing flowers in 50 mM Tris-HCl buffer (pH 6.8), released pollen grains were mixed directly with Alexander stain (Alexander, 1969).

For phenotypic characterization of mutant embryos, silique silique 9-diamino-phenylindole was carried out as previously described (Park et al., 1998). Pollen was treated with aniline blue solution to detect callose staining as previously described (Park and Twell, 2001). Fluorescein diacetate staining was carried out by incubation of freshly isolated pollen in 0.3 m mannitol solution containing 2 μg mL−1 fluorescein diacetate according to Heslop-Harrison and Heslop-Harrison (1970). Alexander staining of pollen was carried out on pollen released from flowers fixed in ethanol:acetic acid (3:1 [v/v]) for 30 min. After washing flowers in 50 mM Tris-HCl buffer (pH 6.8), released pollen grains were mixed directly with Alexander stain (Alexander, 1969).

For phenotypic characterization of mutant embryos, silique silique 9-diamino-phenylindole was carried out as previously described (Park et al., 1998). Pollen was treated with aniline blue solution to detect callose staining as previously described (Park and Twell, 2001). Fluorescein diacetate staining was carried out by incubation of freshly isolated pollen in 0.3 m mannitol solution containing 2 μg mL−1 fluorescein diacetate according to Heslop-Harrison and Heslop-Harrison (1970). Alexander staining of pollen was carried out on pollen released from flowers fixed in ethanol:acetic acid (3:1 [v/v]) for 30 min. After washing flowers in 50 mM Tris-HCl buffer (pH 6.8), released pollen grains were mixed directly with Alexander stain (Alexander, 1969).

For phenotypic characterization of mutant embryos, silique silique 9-diamino-phenylindole was carried out as previously described (Park et al., 1998). Pollen was treated with aniline blue solution to detect callose staining as previously described (Park and Twell, 2001). Fluorescein diacetate staining was carried out by incubation of freshly isolated pollen in 0.3 m mannitol solution containing 2 μg mL−1 fluorescein diacetate according to Heslop-Harrison and Heslop-Harrison (1970). Alexander staining of pollen was carried out on pollen released from flowers fixed in ethanol:acetic acid (3:1 [v/v]) for 30 min. After washing flowers in 50 mM Tris-HCl buffer (pH 6.8), released pollen grains were mixed directly with Alexander stain (Alexander, 1969).

For phenotypic characterization of mutant embryos, silique silique 9-diamino-phenylindole was carried out as previously described (Park et al., 1998). Pollen was treated with aniline blue solution to detect callose staining as previously described (Park and Twell, 2001). Fluorescein diacetate staining was carried out by incubation of freshly isolated pollen in 0.3 m mannitol solution containing 2 μg mL−1 fluorescein diacetate according to Heslop-Harrison and Heslop-Harrison (1970). Alexander staining of pollen was carried out on pollen released from flowers fixed in ethanol:acetic acid (3:1 [v/v]) for 30 min. After washing flowers in 50 mM Tris-HCl buffer (pH 6.8), released pollen grains were mixed directly with Alexander stain (Alexander, 1969).

For phenotypic characterization of mutant embryos, silique silique 9-diamino-phenylindole was carried out as previously described (Park et al., 1998). Pollen was treated with aniline blue solution to detect callose staining as previously described (Park and Twell, 2001). Fluorescein diacetate staining was carried out by incubation of freshly isolated pollen in 0.3 m mannitol solution containing 2 μg mL−1 fluorescein diacetate according to Heslop-Harrison and Heslop-Harrison (1970). Alexander staining of pollen was carried out on pollen released from flowers fixed in ethanol:acetic acid (3:1 [v/v]) for 30 min. After washing flowers in 50 mM Tris-HCl buffer (pH 6.8), released pollen grains were mixed directly with Alexander stain (Alexander, 1969).
Bent (1998). Seeds were sown on Murashige and Skoog agar plates containing kanamycin (35 μg mL−1) and hygromycin B (20 μg mL−1).

ACKNOWLEDGMENTS

We thank the Nottingham Arabidopsis Stock Centre (UK) for providing mutant seeds. We thank Thomas Martin (Department of Plant Biology, University of Cambridge, UK) for helping us with some plant crosses and our colleagues for their helpful discussions.

Received September 13, 2002; returned for revision October 25, 2002; accepted November 14, 2002.

LITERATURE CITED


Lukowitz W, Nickle TC, Meinke DW, Last RL, Conklin PL, Somerville CR (2001) Arabidopsis cys1 mutants are deficient in a mannose-1-phosphate guanylyltransferase and point to a requirement of N-linked glycosylation for cellulose biosynthesis. Proc Natl Acad Sci USA 98: 2262–2267


Wiggins CAR, Munro S (1998) Activity of the yeast MNN1 α-1,3-mannosyltransferase requires a motif conserved in many other families of glycosyltransferases. Proc Natl Acad Sci USA 95: 7945–7950