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The *CLAVATA1* Gene Encodes a Putative Receptor Kinase That Controls Shoot and Floral Meristem Size in Arabidopsis

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Summary

The shoot apical meristem is responsible for aboveground organ initiation in higher plants, accomplishing continuous organogenesis by maintaining a pool of undifferentiated cells and directing descendant cells toward organ formation. Normally, proliferation and differentiation are balanced, so that the structure and size of the shoot meristem is maintained. However, Arabidopsis plants homozygous for mutations at the *CLAVATA1* (*CLV1*) locus accumulate excess undifferentiated cells. We describe the molecular cloning and expression pattern of the *CLV1* gene. It encodes a putative receptor kinase, suggesting a role in signal transduction. The extracellular domain is composed of 21 tandem leucine-rich repeats that resemble leucine-rich repeats found in animal hormone receptors. We provide evidence that *CLV1* expression in the inflorescence is specifically associated with meristematic activity.

Introduction

In higher plants, organogenesis is not limited to embryonic development but occurs throughout the life of the plant. This is accomplished by initiating and maintaining meristems, groups of stem cells that have the ability to divide and replenish themselves as well as to produce progeny that can enter specific differentiation pathways. During embryogenesis, only a simple body plan is established, with a basal root meristem and a shoot meristem. After germination, the shoot meristem grows to produce leaves, axillary meristems, and floral meristems in succession. For proper formation of an adult plant, the shoot meristem must maintain a tight balance of initiating organs while retaining undifferentiated cells. As the shoot tip grows away from the undifferentiated cells as a result of mitosis, the newly distal cells enter a specific developmental pathway leading to eventual differentiation (Figure 1A). These features account for the continued maintenance of the shoot structure and for its function in organogenesis.

The angiosperm shoot meristem has traditionally been divided into two regions. The central zone (CZ) is characterized by slowly dividing cells and is surrounded by the peripheral zone (PZ), where rates of mitosis increase (Steeves and Sussex, 1989; Lyndon, 1990). Some evidence indicates that the PZ is where organ initiation occurs, but it remains unclear if the CZ corresponds to the undifferentiated cells of the shoot meristem (Steeves and Sussex, 1989). The shoot meristem in most dicotyledonous flowering plants, including Arabidopsis, is also composed of three cell layers (Figure 1C). The tunica consists of the L1 and L2 cell layers that remain clonally distinct, owing to anticlinal cell divisions, and that give rise to epidermis and mesophyll, respectively. The corpus, or L3, lies beneath the tunica layers and is characterized by more variable cell-division planes and cell size. The corpus produces pith and vascular tissue. The fact that the three cell layers are largely clonally isolated but are coordinated in their growth and organ initiation indicates that cell–cell signaling may be important in shoot meristem function (Meyerowitz, 1997).

In Arabidopsis, the shoot meristem produces floral meristems that give rise to the floral organs (sepals, petals, stamens, and carpels) in a whorled pattern (Smyth et al., 1990). The floral meristem is similar in structure to the shoot meristem but has a different fate. In addition to initiating different types of organs having a different phyllotactic pattern than the shoot meristem, the floral meristem is a determinate structure: in the flower, carpels are initated in the center of the flower meristem, terminating growth, while the shoot meristem maintains undifferentiated cells and can initiate an indeterminate number of flower anlagen. Thus, the balance of keeping undifferentiated cells while initiating organs is altered in flowers as compared to shoots.

Many mutants with defects in shoot and/or floral meristem development have been isolated in Arabidopsis. A few exhibit phenotypes specific to meristem development. Mutations in one such gene, *CLAVATA1* (*CLV1*), result in plants with enlarged shoot and floral meristems (Figure 1B; Leyser and Furner, 1992; Clark et al., 1993). In the shoot meristem, the *clv1* phenotype is apparent as early as in the mature embryo (Running et al., 1995). As the *clv1* mutant plants continue through vegetative and inflorescence development, their shoot meristems continue to enlarge by accumulation of undifferentiated cells. In contrast, wild-type meristems are maintained at nearly the same size throughout the plants' life (Leyser and Furner, 1992; Clark et al., 1993). This overproliferation can result in a *clv1* shoot meristem growing over 1000-fold larger (by volume) than wild type. A separate gene, *CLAVATA3* (*CLV3*), has mutant phenotypes identical to those of *clv1* mutants and appears to function in the same pathway as *CLV1* (Clark et al., 1995).

clv mutations (*clv1* or *clv3*) have a similar effect on the development of the floral meristem as on the development of the shoot meristem (Figure 1; Clark et al., 1993, 1995). The *clv* floral meristem can be twice as tall as the wild-type floral meristem at the earliest stage of organ initiation. Analysis of maturing flowers revealed that the *clv* floral meristem continues to proliferate and gives rise to a large mass of undifferentiated cells in the center of the flower, instead of terminating like the wild-type floral meristem. Thus, in both the shoot and the floral meristem, *clv* mutations affect the balance of cell proliferation versus cell differentiation.

‡These authors contributed equally to this work.

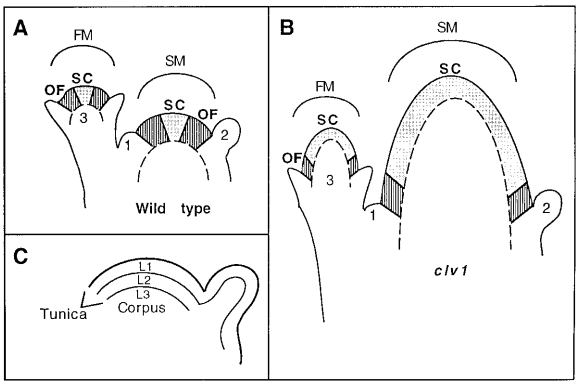

Figure 1. Diagram of Wild Type and *clv1* Shoot and Flower Meristems

Idealized longitudinal sections through inflorescences of wild type (A and C) and *clv1* (B). Undifferentiated stem cells (SC) and organ-forming regions (OF) of both the shoot meristem (SM) and flower meristem (FM) are shaded. In *clv1* mutants, this balance is disrupted, resulting in the accumulation of undifferentiated stem cells. 1, 2, and 3, stage 1, 2, and 3 flowers, respectively. (C) The clonally distinct L1, L2, and L3 cell layers of the tunica and corpus are indicated.

In this report, we describe the cloning and sequence of the *CLV1* gene. Its coding sequence suggests that CLV1 may function as a signal transduction component that acts in the communication of cell division and/or differentiation signals. We also present evidence that in the inflorescence meristem, *CLV1* mRNA is expressed specifically in a central region of the shoot and in early flower meristems, implying nonautonomous action in the surface cell layer(s) of the meristem.

Results

Cloning *CLV1*

We isolated the *CLV1* gene based on its map position. *CLV1* maps on chromosome 1, between the gibberellin biosynthesis gene *GA2* and the floral homeotic gene *AP1* (Koornneef et al., 1983). It was found that 1 of 124 recombination events between *ap1–1* and *clv1–1* lies between the RFLP clone m532 (Chang et al., 1988) and *clv1–1*, indicating that m532 maps approximately 0.1 cM (roughly 17 kb) proximal to *CLV1*. Using m532 as a probe, we isolated two overlapping cosmids (Olszewski et al., 1988), cosQ and cosT, which contain *CLV1*. CosQ contains the same polymorphism as m532. One of eighty-two recombinants between *clv1–1* and *ga2* lies in cosT, delimiting the *CLV1* gene to the region covered by the cosmids. CosT, but not cosQ, restored a wild-type phenotype when transformed into *clv1–4* mutant plants and thus contained the whole *CLV1* gene.

CosT was subcloned and sequenced (Figure 2), which revealed four genes, each with similarity to sequences in the database. These included a leucine-rich repeat (LRR) kinase (LRR kinase), the plant defensin gene *PDF1.1* (Penninckx et al., 1996), a gene we call *AtVPS-35* that is similar to a yeast vacuolar sorting protein (VPS-35; Paravicini et al., 1992), and *ARAC5*, a recently identified small GTP-binding protein (Figure 2). To define further the region that corresponds to *CLV1*, we mapped the nearest recombination breakpoint on the distal side of *CLV1*. This breakpoint mapped to a single fragment in the middle of the cosmid, ruling out the *ARAC5* and *AtVPS-35* genes. The remaining region contained the LRR kinase and *PDF1.1*.

We sequenced the *PDF1.1* gene from plants homozygous for three different *clv1* alleles (*clv1–1*, *clv1–5*, and *clv1–4*) and found no changes relative to the wild-type sequence. In sequencing the putative LRR kinase, we identified either a missense or a frame-shift mutation in each of the seven *clv1* alleles that we analyzed (see below and Table 1).

To confirm that the putative LRR kinase corresponds to the *CLV1* gene, we transformed *clv1–1* mutants with constructs containing various regions of genomic sequence spanning the *CLV1* gene. The CLV1 6.3 construct provided consistent, partial suppression of the *clv1–1* phenotype (Figure 3A). Transformation of the CLV1 5.7 construct provided no rescue of the mutant phenotype, whereas the CLV1 5.1 construct provided partial rescue of the *clv1–1* phenotype, suggesting that there may be a 3′ regulatory sequence or a negative regulatory element in the 5′ region in between the XbaI and the SacI sites (Figure 3B).

CLV1 Sequence

The nucleotide and deduced amino acid sequence of *CLV1* is shown in Figure 4. The transcriptional start site was determined by 5′ RACE. The poly(A) site of the transcript was determined by sequencing the 3′ end of a cDNA clone. A canonical poly(A) addition site (AATAAA; Joshi, 1987) was found 28 nt upstream of the poly(A) tail. Based on the cDNA 5′ RACE and 3′ poly(A) site, the *CLV1* transcript is predicted to be 3262 bases long. This is consistent with RNA blot data that shows an approximately 3.4 kb mRNA (data not shown). The *CLV1* genomic sequence contains a single 79 nt intron at a position that is conserved in the similar LRR kinase gene *TMK1* (Chang et al., 1992).

The amino terminus of the CLV1 protein sequence has a potential signal peptide that directs secretion. This is followed by a putative extracellular domain consisting of 21 complete LRRs (Figure 5A) with 15 N-linked glycosylation consensus sites (N-X-S/T). As with many LRR-containing proteins, the LRR region is flanked by pairs of conservatively spaced cysteines. The LRR domain is followed by a stop-transfer sequence, suggesting a

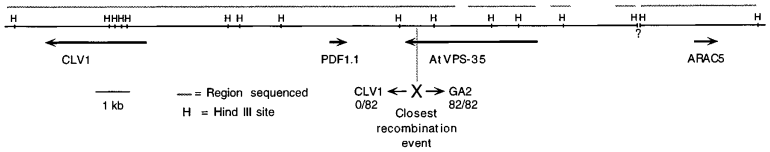

Figure 2. Physical Map of Cosmid T

The location of each ORF and the direction of transcription are indicated by the arrows. The accession number for *ARAC5* is U52350. H, HindIII sites.

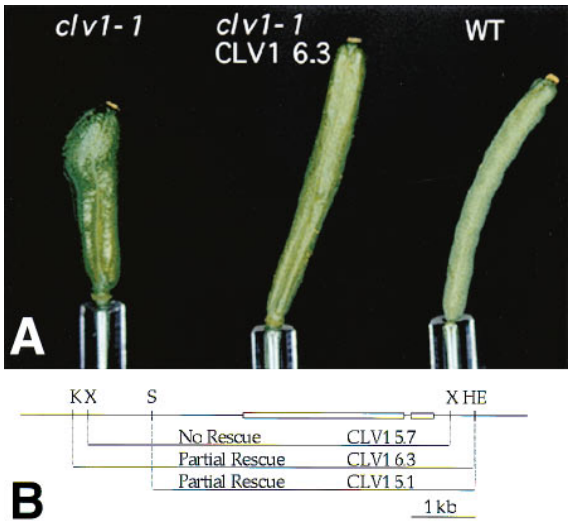

Figure 3. CLV1 6.3 Genomic Construct Provides Partial Suppression of the *clv1–1* Phenotype

(A) Comparison of typical gynoecia from a *clv1–1* plant, a *clv1–1* plant transformed with the CLV1 6.3 construct, and a wild-type plant.
(B) Diagram of the *CLV1* genomic region indicating the sequences used to attempt rescue of the *clv1–1* mutant phenotype. E, EcoRI; K, KpnI; X, XbaI; S, SacI; H, HindIII. The HindIII site on the right end of the map in (B) corresponds to the HindIII site at the far left end of the map shown in Figure 2. The internal HindIII sites are not shown.

transmembrane domain. The putative intracellular domain contains all of the conserved residues found among serine/threonine protein kinases (Figure 4), and recombinant CLV1 protein has protein kinase activity (RWW, SEC, and EMM; unpublished data).

The *CLV1* gene was amplified and sequenced from DNA isolated from plants homozygous for seven different *clv1* mutant alleles (Table 1). In each sequence, a single substitution relative to wild-type was uncovered. Two of the strong mutant alleles, *clv1–4* and *clv1–8*, contained missense mutations in the LRR domain. For the five other alleles, all weak to intermediate in phenotype, we identified lesions in the kinase domain. Four of the alleles, *clv1–1*, *clv1–2*, *clv1–5*, and *clv1–9*, all contain missense mutations. Interestingly, one of the weakest known alleles, *clv1–6*, contains an insertion of an A near the beginning of the kinase domain that would theoretically replace most of the kinase domain with 16 novel amino acids.

CLV1 is similar in sequence to a number of other genes coding for LRR kinases. The most closely related complete gene in the database is *RLK5* (Walker, 1993), an LRR kinase of unknown function in Arabidopsis. CLV1 and RLK5 are 37% identical and 58% similar at the amino acid level over their entire length. The kinase domains are 47% identical, and the LRR domains are 36% identical at the amino acid level. The majority of the identical residues in the putative receptor domain are at conserved LRR positions; at nonconserved positions, the CLV1 and RLK5 LRR domains are only 22% identical. Other similar Arabidopsis genes include *TMK1* (Chang et al., 1992), *TMKL1* (Valon et al., 1993), and the *ERECTA* gene, which is required for proper internode elongation (Torii et al., 1996). A non-Arabidopsis plant gene of identical structure to *CLV1* and *RLK5* is *Xa21* (Song et al., 1995). *Xa21* was isolated from rice based on its ability to provide resistance to the bacterium Xanthomonas oryzae pv. oryzae race 6.

In addition to the known proteins, there are several Arabidopsis expressed sequence tags (ESTs) that are similar to regions of the CLV1 protein. These include LRR-containing sequences and kinase domain sequences. Some of these sequences are more similar to CLV1 than RLK5 is to CLV1, indicating that the Arabidopsis genome contains many *CLV1*-like genes.

Phylogenetic Analysis

A number of plant kinases, many of which are involved in development and disease-resistance pathways, have been isolated over the past several years. To determine the relationship of these kinases, as well as their relationships to kinase domains found in animals, we performed a phylogenetic analysis using a heuristic tree-building program. After calculating a single shortest tree using no weighting for similar amino acids, this tree was used as the starting point for a heuristic analysis using a matrix that weighted amino acid changes based on the similarity of the altered residue. A single shortest tree was produced, and six other trees, shorter than the starting tree, were also identified. The strict consensus of the seven trees is shown in Figure 5C.

Several relationships are supported in the consensus tree. First, all the plant kinases analyzed, except for CTR1, form a separate lineage distinct from the animal kinases. Most of the S-receptor-like kinases cluster together, but the same is not true for the LRR kinases. The kinases involved in disease resistance, Pto and Xa21, do not form a distinct group. Pto, which has a kinase but no extracellular domain, belongs to an LRR kinase group including CLV1, RLK5, and ERECTA.

CLV1 Expression Patterns

Since we know so little about how and where receptors of any type function in plants, we sought to establish the pattern of *CLV1* mRNA expression. To avoid cross-hybridization to closely related members of the LRR kinase gene family, we defined a *CLV1*-specific probe. The region coding for the C-terminus of the kinase domain (the last 300 nt of the ORF) showed little sequence homology to known kinases, and the corresponding probe hybridized only to *CLV1* in a low stringency genomic DNA blot hybridization experiment (data not shown). ^{35}S-labeled sense and antisense riboprobes were generated from this fragment and were hybridized to sections of wild-type Arabidopsis inflorescence tissue (see Experimental Procedures).

In the shoot meristem, *CLV1* was expressed in a patch of cells across the center of the meristem. It was not expressed in the L1 layer, as can be seen from both longitudinal serial sections (Figures 6B–6F) and transverse sections (data not shown). *CLV1* signal was not detected on the flanks of the shoot meristem in cells that are presumably flower anlagen. In the most central region, *CLV1* may also be expressed in the L2 layer.

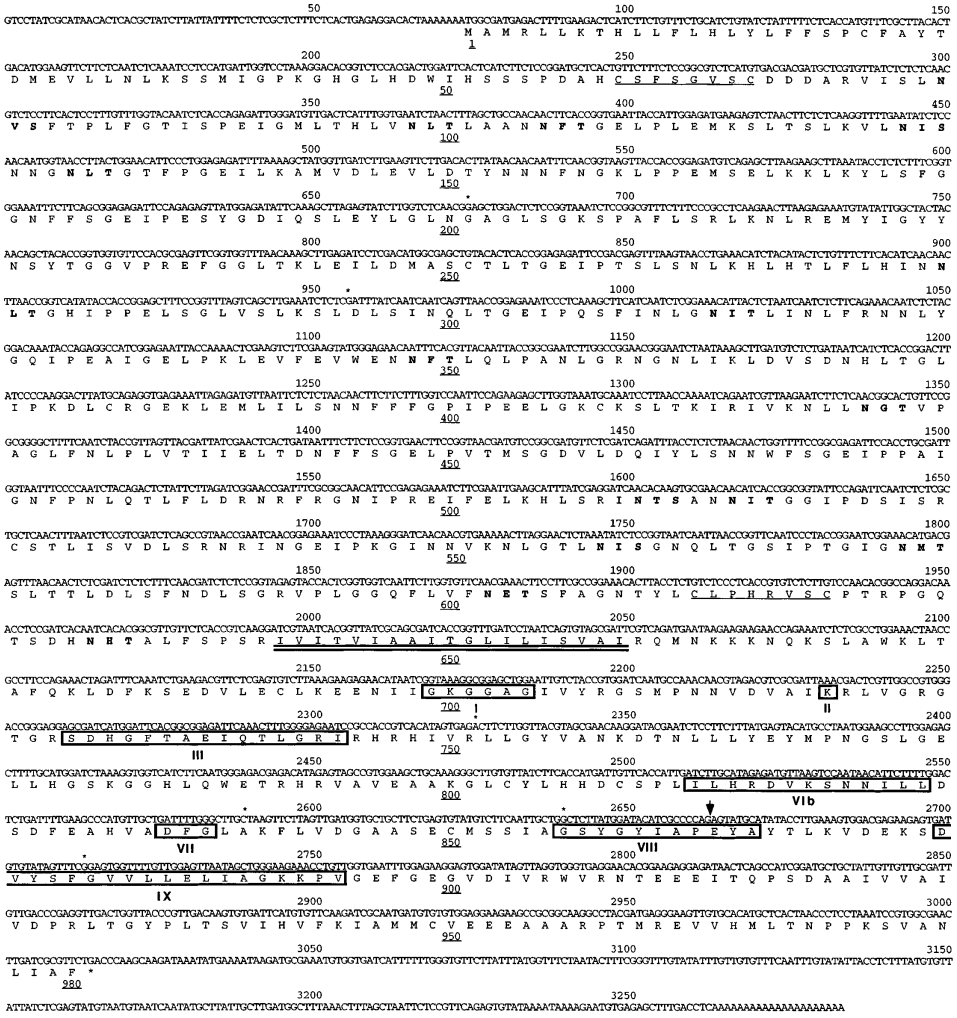

Figure 4. The *CLV1* Gene and Protein Sequence

The DNA sequence corresponding to the *CLV1* transcript is shown, with the predicted amino acid sequence shown below. The nucleotide numbering is listed above the nucleotide sequence, and the amino acid numbering (underlined) is given below the protein sequence. The paired cysteines flanking the LRR region are underlined, and potential N-linked glycosylation sites in the extracellular domain are in bold lettering. The predicted transmembrane sequence is double underlined and flanked by charged residues. In the kinase domain, the conserved regions are boxed and indicated with Roman numerals, following the nomenclature of Hanks and Quinn, 1991. The arrow indicates the position of the intron, and the asterisks indicate the positions of the mutations listed in Table 1.

Sense controls reveal that this signal was specific for *CLV1* and was not due to background (Figures 6G–6I).

In the floral meristem, *CLV1* expression seemingly changes preceding organ initiation. *CLV1* expression is down-regulated in the floral anlagen, and no expression is detected in stage 1 flowers (Figures 6B–6D; all stages according to Smyth et al., 1990). In stage 2 floral meristems, signal was detected. This was observed in a central region but was absent from the presumed sepal anlagen (Figure 7D). Further down-regulation preceded organ initiation. By stage 4, *CLV1* expression was absent from whorl 2 and at least the outer portion of whorl 3 in the petal and stamen anlagen (Figures 7A–7C and 7E). By stage 5, *CLV1* expression was only detected in the very center of the floral meristem (Figures 7A–7C), and no signal was detected after this stage (Figure 7F). As in the shoot meristem, *CLV1* mRNA was not detected in the L1 layer of the flower meristem, and we were unable to determine if it is expressed in the L2 layer cells.

Discussion

CLV1 as a Receptor Kinase

We have cloned the Arabidopsis *CLV1* locus based on its map position, and sequence analysis indicates that it may function as a receptor kinase. The presumed extracellular domain is composed almost exclusively of LRRs, which are known to be involved in protein–protein interactions. The crystal structure of the LRR-containing ribonuclease inhibitor (RI) complexed with RNase A has been solved (Kobe and Deisenhofer, 1995). Each repeat forms a section of β sheet and a section of α helix, and when combined with other repeats, the protein forms a donut-shaped structure, with an inner face containing the parallel β sheets that contact the RNase A molecule

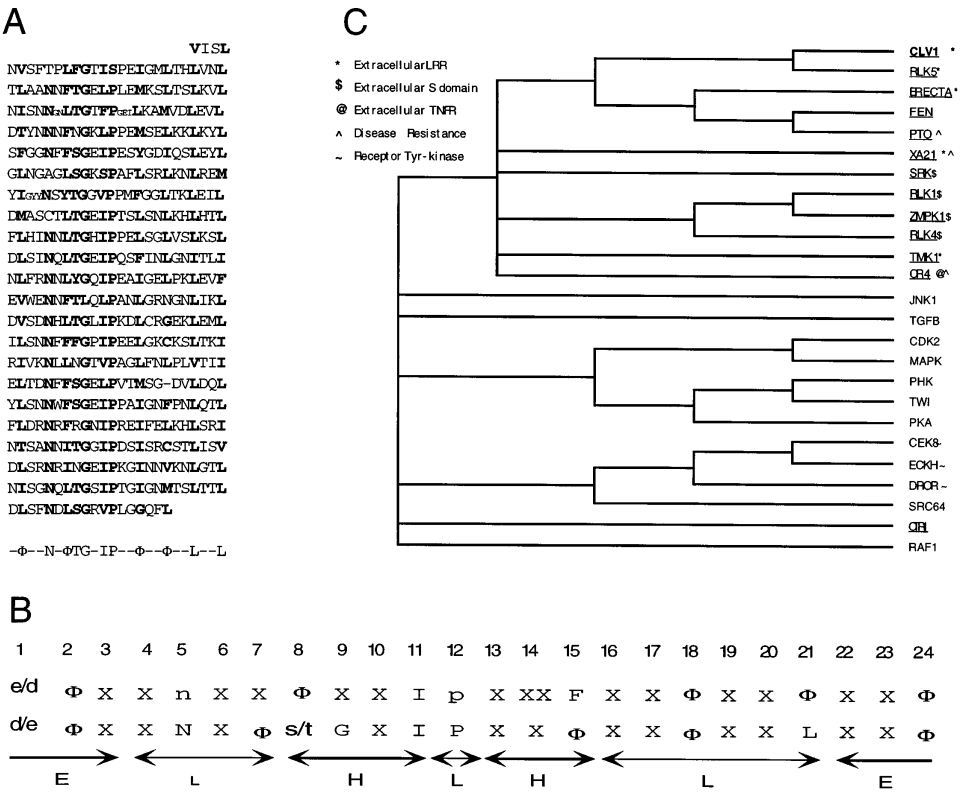

Figure 5. Analysis and Comparison of the CLV1 Protein Sequence

(A) The LRR domain of CLV1 is presented, with the 21 complete and 2 partial repeats aligned.

(B) The CLV1 LRR consensus (bottom) is compared to the animal hormone-receptor repeat structures (top). "X" indicates any amino acid; other capital letters indicate consensus amino acids (Φ = hydrophobic), and lower case letters indicate residues frequent at a position. Aspartic acid or glutamic acid is present at position 1 in 8 of 21 CLV1 repeats and 7 of 21 animal repeats (only those repeats analyzed in Jiang et al., 1995, were included in this calculation). Asparagine is present in 16 of 21 animal repeats at position 5 or 6. Proline is present in 11 of 21 animal repeats at position 12 or 13. The region of each repeat predicted to be in a β sheet (E), loop (L), or α helical (H) conformation is indicated below the consensus alignments.

(C) Phylogenetic relationships between plant and animal kinase domains showing the consensus of the seven shortest trees. The kinase sequences used in the analysis were as follows: CLV1 (this article), RLK1, RLK4, RLK5 (Walker, 1993), TMK1 (Chang et al., 1992), ER (Torii et al., 1996), and CTR1 (Kieber et al., 1993) from Arabidopsis; SRK (Stein et al., 1991) from Brassica napus; PTO (Martin et al., 1993) and FEN (Loh and Martin, 1995) from tomato; Xa21 (Song et al., 1995) from rice; ZMPK1 (Walker, 1993) and CR4 (Becraft et al., 1996) from maize; DROR (Wilson et al., 1993), TWICHIN, and SCR64 (Hoffmann et al., 1983) from Drosophila; JNK1 (Sanchez et al., 1994), TGFβR (Wrana et al., 1992), MAPK (Payne et al., 1991), PKA (Uhler et al., 1986), RAF1 (Bonner et al., 1986), HuCDK2 (Tsai et al., 1991), HuPHK (Hanks, 1989), and HuECKH (Lindberg and Hunter, 1990) from humans; and CEK8 (Sajjadi and Pasquale, 1993) from chickens. Plants proteins are underlined, and CLV1 is in bold lettering.

and an outer face containing the α helix portions. Glycoprotein hormone receptors from animals, including luteinizing hormone receptor (LHR; McFarland et al., 1989), thyroid-stimulating hormone receptor (TSHR; Parmentier et al., 1989), and follicle-stimulating hormone receptor (FSHR; Sprengel et al., 1990), all contain extracellular LRRs that are responsible for high affinity binding of their respective ligands. The repeat structure of

Table 1. *clv1* Alleles

Allele	Lesion	Predicted Effect	Phenotype	Selected Reference
clv1–1	G→A	Gly-856→Asp	intermediate	1, 2, 3
clv1–2	G→A	Gly-881→Glu	intermediate	1
clv1–3	n.s.		strong	1
clv1–4	G→A	Gly-201→Glu	strong	2, 4
clv1–5	G→A	Gly-856→Asp	intermediate	2
clv1–6	frameshift	see text	weak	2
clv1–7	n.s.		weak	2
clv1–8	G→A	Asp-295→Asn	strong	5
clv1–9	C→T	Ala-839→Val	weak	6

References: 1, Leyser and Furner, 1992; 2, Clark et al., 1993; 3, Koornneef et al., 1983; 4, McKelvie, 1962; 5, Medford et al., 1992; 6, this work. n.s., not sequenced.

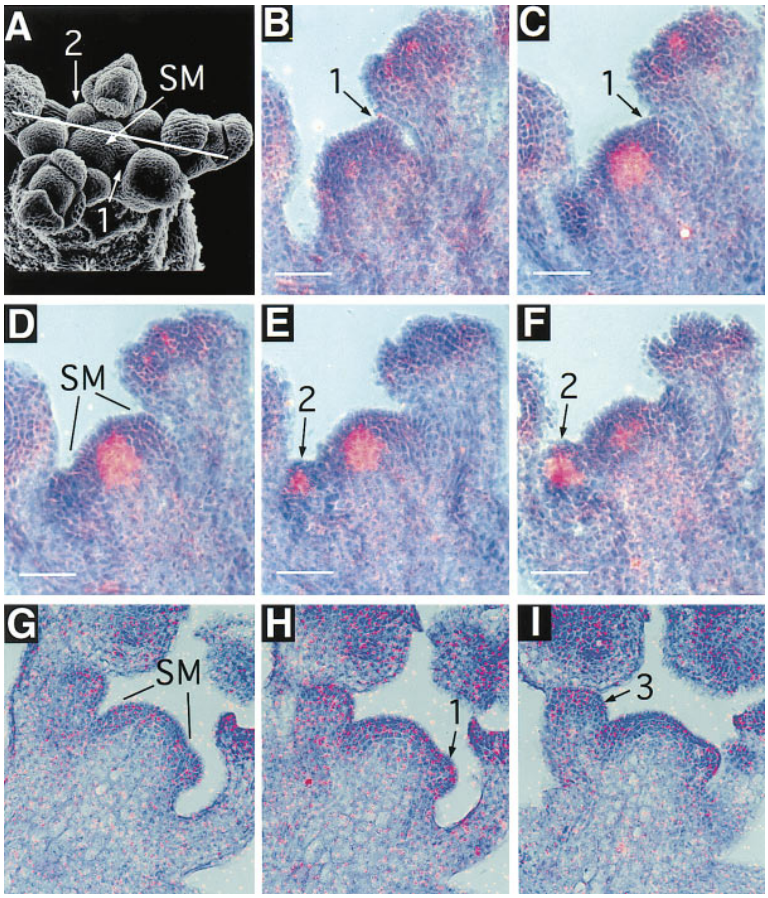

Figure 6. *CLV1* mRNA Expression Patterns in the Shoot Meristem

A labeled *CLV1*-specific RNA probe was hybridized to serial sections of shoot meristem tissue from wild-type plants. In (A), the line indicates the approximate orientation of serial sections in (B–F). Combined bright-field and dark-field exposures (signal is indicated by red grains) from sections hybridized to antisense (B–F) and sense (G–I) probes are shown. Numbers indicate flower stages (all stages according to Smyth et al., 1990). SM, shoot meristem. Scale Bar = 40 μm.

this class of receptors has been compared to that of porcine RI (Jiang et al., 1995), and computer predictions suggest a common structure. CLV1 has an LRR repeat pattern very similar to that of the glycoprotein hormone receptors (Figure 5B). Based on this analysis, the strong *clv1–4* and *clv1–8* alleles result in amino acid substitutions in the β-sheet face (*clv1–4* at position 4 and *clv1–8* at position 1). Thus, the severe phenotype of these alleles might be due to reduced ligand binding.

The presumed intracellular domain coded by *CLV1* contains all of the conserved residues found among serine/threonine protein kinases (Hanks and Quinn, 1991); furthermore, recombinant CLV1 protein has protein kinase activity (RWW, SEC, and EMM; unpublished data). The most closely related sequences in the database are several Arabidopsis ESTs and the intracellular domains of RLK5 and TMK1, which have been shown to autophosphorylate on serine/threonine residues in vitro (Chang et al., 1992; Horn and Walker, 1994). The three-dimensional structures of several protein kinases have been solved and shown to be similar to one another (De Bondt et al., 1993; Hu et al., 1994; Zhang et al., 1994). The location of the lesions in *clv1* mutants was analyzed based on the protein kinase A structure (PKA; Knighton et al., 1991). The amino acids altered in the weak/intermediate *clv1* alleles *clv1–1* (and the identical lesion in *clv1–5*), *clv1–2*, and *clv1–9* are all in residues that are conserved in PKA. For *clv1–1* and *clv1–9*, the altered residues lie in the activation loop, a region shown to be important in regulating kinase activity in a number of protein kinases (Johnson et al., 1996). The lesion in *clv1–2* is located 3′ to the portion of the gene coding for the activation loop.

By analogy to animal receptor tyrosine kinases, CLV1 could act to bind a ligand, leading to homodimerization. Previous genetic evidence has suggested that CLV1 acts as a multimer (Clark et al., 1995). Dimerization would bring the kinase domains in close proximity, which may result in intermolecular phosphorylation. The nature of the *clv1–1* and *clv1–6* alleles also support such a model. The *clv1–1* mutation is semidominant (Clark et al., 1995) and results in an amino acid substitution in a conserved residue in the kinase-activation loop. Thus, the clv1–1 protein may function in a dominant-negative manner by binding to and preventing intermolecular phosphorylation of a wild-type partner protein in heterozygous plants. Biochemical studies with RLK5 have demonstrated that the kinase domain can form dimers and transphosphorylate. Furthermore, an excess of catalytically inactive RLK5 can inhibit the kinase activity of wild-type RLK5 protein (Horn and Walker, 1994). The *clv1–6* mutation, which results in a truncated protein with most of the kinase domain deleted, has a weak phenotype, indicating that the kinase domain may be partially redundant with another protein or that readthrough occurs.

After phosphorylation, the kinase domain would bind specific factors, analogous to SH2-containing proteins in animals, that would transmit the signal to downstream

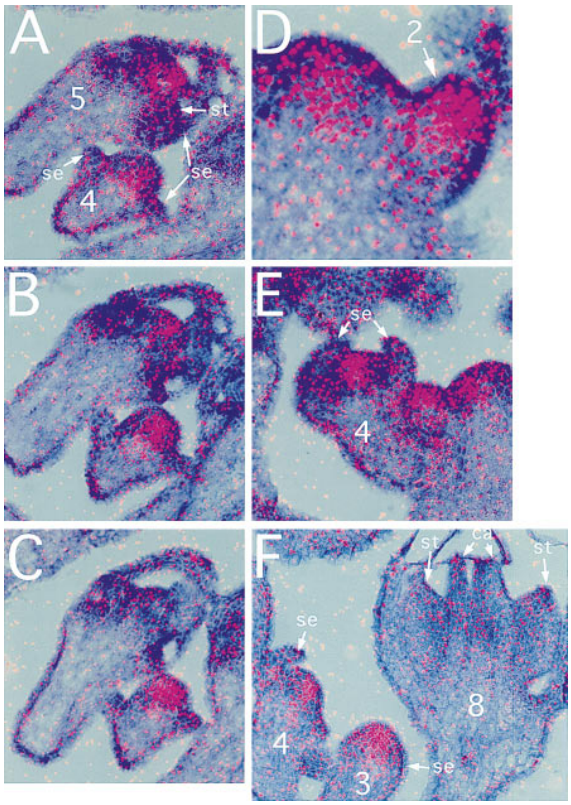

Figure 7. *CLV1* mRNA Expression Patterns in the Flower Meristem

A labeled *CLV1*-specific antisense RNA probe was hybridized to sections of flower meristem tissue from wild-type plants. (A–C) are serial sections through the same flowers. (D) is a close-up of a stage 2 flower. (E) shows *CLV1* expression in a stage 4 flower, and (F) shows a stage 8 flower. All images are shown at the same magnification except for panel D, which is shown at a higher magnification. Numbers indicate flower stages; se, sepal primordium; st, stamen primordium; ca, carpel primordium.

targets. One protein that could be involved in binding the phosphorylated CLV1 kinase domain is KAPP (Stone et al., 1994). KAPP contains a kinase-interaction (KI) domain linked to a type 2C protein phosphatase domain. The KI domain has been shown to associate with the phosphorylated kinase domain of RLK5 but not to a catalytically inactive form of the RLK5 kinase domain (Stone et al., 1994). The specificity of the KAPP–RLK5 interaction and the role of KAPP binding in vivo are unknown.

CLV1 Signaling

If CLV1 acts as a receptor, the question arises as to what in vivo signal it responds. There are two potential models of CLV1 action—regulating cell division or cell differentiation—that could fit with CLV1 acting to perceive some signal. Cell division rates are tightly regulated in the shoot meristem; for example, the cells in the CZ divide much more slowly than those in the PZ (Steeves and Sussex, 1989; Lyndon, 1990). That the shoot meristem remains nearly the same size throughout the life of the plant indicates that the rate of division of central undifferentiated cells is closely balanced with the proportion of cells undergoing differentiation on the flanks. Organ-forming cells on the flanks are derived from the central undifferentiated cells; thus, differences in cell-division rates are presumably controlled based on positional cues. In *clv1* mutants, there is a gradual accumulation of undifferentiated stem cells. Because the pool of stem cells is increased by proliferation of these cells and decreased by incorporation of these cells into organ primordia, CLV1 could function either to repress cell division of the stem cells or to promote their incorporation into organ primordia. Thus, CLV1 could act to perceive positional information regulating stem cell-division rates. Alternatively, if CLV1 acts to promote the transition of cells reaching the flanks of the meristem toward organ formation and an eventual differentiated state, CLV1 could act to receive information regulating this transition. There are extra cells in the shoot meristem of the mature embryo in *clv1* mutants (Running et al., 1995), presumably before any differentiation has yet occured, thus adding support to the cell-division model. However, the *CLV1* expression pattern could fit either of these models (see below).

If CLV1 represents one component of a novel signal-transduction pathway, there must be a number of other components in the signaling pathway. Some could function specifically in the CLV1 pathway, while others may function in several signaling cascades. Candidates for other components of the CLV1 pathway include the gene products of *CLAVATA2* (CLV2; Koornneef et al., 1983), *CLV3*, and *WUSCHEL* (*WUS*; Laux et al., 1996). Mutations at *CLV2* and *CLV3* have similar phenotypes as *clv1* mutants, suggesting that these three genes could act on the same processes. In addition, we have previously concluded that CLV3 likely functions in the same pathway as CLV1, in part because the *clv1* and *clv3* mutants exhibit dominant interactions (Clark et al., 1995). Thus, CLV2 and/or CLV3 could be required for ligand generation or function as downstream components. Mutations at *WUS* exhibit phenotypes largely opposite those of *clv* mutants—failure to establish or maintain functioning stem cells in the shoot meristem and reduced numbers of central organs/whorls in the flower meristem (Laux et al., 1996). Furthermore, *wus* mutations are epistatic to *clv1–4*. These data indicate that either CLV1 functions to regulate negatively WUS activity or that WUS is required to establish the undifferentiated cells upon which CLV1 acts. Under the first hypothesis, WUS could be a target of CLV1 signaling.

CLV1 is similar in structure to Xa21, which confers resistance to Xanthomonas oryzae pv. oryzae race 6 in rice (Song et al., 1995). The tomato genes *Cf-9* and *Pto* are known to be involved in pathogen recognition and defense, and each has sequence similarity to various domains of the CLV1 protein (Cf-9 to the LRRs and Pto to the kinase; Martin et al., 1993; Jones et al., 1994). Thus, both disease resistance and developmental signaling share similar components, suggesting a common origin for these pathways in plants. A similar observation has been made in animals. Components of the mammalian IL-1/NF-κB-mediated inflammatory response and the Drosophila Toll/Dorsal-mediated patterning pathways also share proteins with sequence similarity. Furthermore, proteins in the Drosophila Toll signal-transduction pathway are used to activate antifungal genes

in adult flies (Lemaitre et al., 1996). It is interesting to speculate that in two separate multicellular lineages, components of preexisting regulatory pathways may have been recruited for pathogen defense, or that ancient environmental sensors have been recruited for use in cell–cell communication.

CLV1 Expression

In situ RNA-hybridization experiments revealed that, in the inflorescence, *CLV1* is specifically expressed in the shoot and floral meristems. *CLV1* mRNA is found in the presumptive shoot meristem during embryo development and is not detected in the root meristem (S. E. C. and H. Sakai, unpublished data). *CLV1* is expressed in the shoot meristem in a region comprising a subset of the central stem cells and the inner portion of the organ-forming region (based on expression of floral anlagen-specific genes such as *LEAFY*; Weigel et al., 1992). This expression pattern fails to distinguish between the two main models for CLV1 action: regulating stem cell division or promoting the transition of cells reaching the flanks toward differentiation. *CLV1* is down-regulated on the outer edge of the organ-forming region and in the earliest flower primordia. Expression is reestablished in the flower meristem, so that by stage 2, expression is at a level comparable to that of the shoot meristem. In the flower meristem, the expression pattern is again consistent with both models for *CLV1* action: regulating cell-division rates in the center of the flower meristem or promoting the transition of flanking flower meristem cells toward organ primordia formation and differentiation.

That the expression of *CLV1* would be so tightly restricted was not expected a priori. Many receptors in a variety of systems are expressed ubiquitously, with their spatial regulation being conferred by the ligand. One possible reason for regulating *CLV1* expression might be to provide unidirectional signaling from a secreted ligand. If the information to which CLV1 is responding emanates from the flanks or the superficial cell layers, then limiting *CLV1* expression to a region interior to this signal would ensure that the signal is only perceived in a unidirectional fashion. Another possibility is that the presence of CLV1 in other cell types might interfere with other signaling pathways.

Another observation is that *CLV1* is not expressed in the L1 layer of the shoot and floral meristems. In *clv1* mutants, undifferentiated cells accumulate in all layers of the shoot and flower meristem. This indicates that CLV1 has a cell-nonautonomous function. Several explanations are possible for the mechanism of the nonautonomous function. One could imagine, for example, that CLV1 mediates signaling from the L1 cells to deeper layers, allowing the clonally distinct but adjacent cells to coordinate their division and/or differentiation patterns. Induction between different cell layers requiring communication of this sort has been observed in plant development. Two notable findings include the specification of a nearly normal flower in Antirrhinum plants in which only the L3 layer contains a wild-type *FLORICAULA* gene (Hantke et al., 1995) and in tomato plants whose floral meristem size appeared to be largely determined by the genotype of the L3 (Szymkowiak and Sussex, 1992). Alternatively, the CLV1 protein may be physically transported between cell layers. The maize *KNOTTED-1* gene is transcribed in the corpus, but the protein is found in the corpus and tunica (Smith et al., 1992; Jackson et al., 1994). The KNOTTED-1 protein has been shown to move through plasmodesmata in leaf mesophyll cells (Lucas et al., 1995). Similarly, the MADS box proteins DEFICIENS and GLOBOSA may be transported between cell layers in the Antirrhinum floral meristem (Perbal et al., 1996). A similar process may account for CLV1 affecting L1 cell division and/or differentiation.

These examples of cell–cell communication of division and differentiation information in plants raise a more general possibility. Throughout plant development, the division and differentiation of adjacent cells must be closely coordinated. The absence of cell migration in plant development implies that the form of plant organs and the shape of meristems depend entirely on the number and patterns of cell divisions and cell expansion. In addition, plant organ structure depends on coordination of cell fates and cellular differentiation. To achieve the necessary coordination of the activities of cells such as those in the L1 and L2, which are clonally only distantly related, requires a cell–cell communication mechanism for exchanging and/or relaying cell division or cellular-differentiation information. The nature of the *CLV1* mutant phenotype and of the protein coded by the gene perhaps provides a single example of a general mechanism. The plant LRR kinase family of genes is large, as indicated not only by the other genes already identified (in Arabidopsis, *TMK1*, *RLK5*, and *ER*, as well as *CLV1*), but possibly by additional partial cDNA sequences found in the Arabidopsis EST database. It could be that such receptor kinases play a general role in plants, with each layer or region of cells having its own family members. The general coordination of cellular activities in plant development may depend upon signaling from all cells to their neighbors via members of the LRR kinase family. In this respect, this family of receptors, found so far only in plants, may be functionally similar to the receptor-tyrosine kinases used so extensively in animals, but so far found only in animals.

Experimental Procedures

Plant Material

The *ap1–1*, *clv1–1*, and *ga2* mutants are all in the Landsberg-*erecta* ecotype. The isolation of the *clv1–1* through *clv1–7* alleles has been previously described (Koornneef et al., 1983; Leyser and Furner, 1992; Clark et al., 1993). The *clv1–8* allele was originally called *fully fasciated* (Medford et al., 1992). The *clv1–9* allele was an EMS-generated allele kindly provided by Steven Jacobsen.

Seeds were sown on a 1:1:1 mix of soil:perlite:vermiculite and imbibed for 3–5 days at 4°C. Plants were grown at 25°C under constant light. Plants were fertilized once a week and sprayed with insecticide as needed.

Cloning and Sequencing of *CLV1*

Meiotic recombination breakpoints were generated near *CLV1* by screening for recombinants between *clv1–1* mutants and the flanking markers *ap1–1* and *ga2*, when *clv1–1 ap1–1* and *clv1–1 ga2* double mutants in the L-*er* ecotype were crossed to wild-type Col-O plants. These recombinants were used to map RFLP clones relative to the *CLV1* gene by Southern blot hybridization. m532 was used as a probe to isolate cosQ and cosT by colony hybridization. CosT

was used to isolate cDNA clones from a flower library (Weigel et al., 1992). Each gene was PCR amplified and sequenced directly from several *clv1* alleles. The 5′ end of the *CLV1* transcript was defined by PCR–RACE using a kit (GIBCO–BRL) as outlined in the manufacturer's directions. The MacVector program version 4.1.4 by Kodak IBI was used to identify ORFs and nucleic acid alignments. The protein databases were searched using the BLAST program.

Rescue of *clv1* Mutant Phenotype

Regions of the *CLV1* genomic region were subcloned into the T-DNA-containing vector pCGN1547 (McBride and Summerfelt, 1990) and transformed into E. coli. Recombinant plasmids were identified by restriction-enzyme analysis and then transformed into Agrobacterium tumefaciens strain ASE. Whole plants were then transformed using the vacuum-infiltration method (Bechtold et al., 1993). Transgenic seedlings were selected by resistance to kanamycin and transplanted to soil. Transgenic plants were then grown and allowed to set seed as normal.

Phylogenetic Analysis

Amino acid sequences of all the kinase domains used for tree building were aligned using the Clustal V program and modified by hand. Regions sharing very little similarity among the kinase domains were removed from the sequences for phylogenetic analysis. The regions used for analysis correspond to residues 691–712, 716–723, 733–757, 762–781, 786–808, 813–841, 856–867, and 871–890 of CLV1. The aligned sequences were analyzed using the Paup 3.1.1 program using heuristic searches owing to the large number of genes involved. We first started with a random tree for a heuristic analysis using amino acid identities; i.e., amino acid changes were of equal cost regardless of the residue substituted. After 3 days of computation, the 8000 shortest trees were used for another heuristic search. A single shortest tree was generated from this analysis and was used as the starting tree for a heuristic search using a weighted matrix based on the BLOSUM 62 matrix (M. Frohlich, personal communication). The weighting takes into account amino acid similarities. Seven trees shorter than the starting tree were identified, although it should be noted that these trees are not shorter when using amino acid identities to calculate tree lengths. Bootstrapping was not performed because the large number of sequences involved meant that, starting with a random tree, it took over 3 days of computation to identify the shortest tree.

In Situ Hybridization

A 330 bp PCR product spanning the last 100 codons of the ORF and 30 bp of 3′UTR hybridized to a single band on a genomic Southern blot when washed at 50°C with 2× SSPE. This PCR product was cloned into the TA cloning vector (Invitrogen) to generate the pBW4KD4 plasmid. Antisense RNA probes were generated by linearizing pBW4KD4 with XbaI and synthesized with SP6 RNA polymerase. Sense control probes were synthesized using T7 RNA polymerase on pBW4KD4 linearized with HindIII. The remainder of the in situ procedure was done according to Drews et al. (1991), with modifications by Sakai et al. (1995).

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GenBank Accession Numbers

The GenBank accession numbers for the sequences reported in this article are U96879 for *CLV1* and U96877 and U96878 for *AtVPS-35*.