Running Head
Expression of CLE polypeptide signaling gene family

Author for Correspondence

Plant Gene Expression Center
USDA-ARS/UC Berkeley
800 Buchanan Street
Albany, CA 94710, USA
tel: +1 510 559 5917
fax: +1 510 559 5678
email: jfletcher@berkeley.edu

Journal Research Area
Genome Analysis
Comprehensive analysis of \textit{CLE} polypeptide signaling gene expression and over-expression activity in Arabidopsis

JiHyung Jun*1, Elisa Fiume*1, Adrienne H. K. Roeder2, Ling Meng3, Vijay K. Sharma1, Karen S. Osmont1, Catherine Baker2, Chan Man Ha1, Elliot M. Meyerowitz2, Lewis J. Feldman3 and Jennifer C. Fletcher1

*co-primary author

1Plant Gene Expression Center
USDA-ARS/UC Berkeley
800 Buchanan Street
Albany, CA 94710, USA
and
Department of Plant and Microbial Biology
University of California, Berkeley
111 Koshland Hall
Berkeley, CA 94720, USA

2Division of Biology, MC 156-29
California Institute of Technology
1200 East California Boulevard
Pasadena, CA 91125, USA

3Department of Plant and Microbial Biology
University of California, Berkeley
111 Koshland Hall
Financial Source
National Science Foundation Arabidopsis 2010 grant MCB 0313546

Present Addresses
Vijay K. Sharma
Monsanto Company
700 Chesterfield Parkway West
Chesterfield, MO 63017

Catherine Baker
Stanford University School of Medicine
300 Pasteur Drive
Stanford, CA 94305

Karen S. Osmont
Biology Department
University of Massachusetts
611 North Pleasant St.
Amherst, MA 01003

Corresponding Author
Jennifer C. Fletcher
jfletcher@berkeley.edu
Abstract
Intercellular signaling is essential for the coordination of growth and development in higher plants. Although hundreds of putative receptors have been identified in Arabidopsis thaliana, only a few families of extracellular signaling molecules have been discovered and their biological roles are largely unknown. To expand our insight into the developmental processes potentially regulated by ligand-mediated signal transduction pathways, we undertook a systematic expression analysis of the members of the Arabidopsis CLAVATA3/ESR-RELATED (CLE) small signaling polypeptide family. Using reporter constructs we show that the CLE genes have distinct and specific patterns of promoter activity. We find that each Arabidopsis tissue expresses at least one CLE gene, indicating that CLE-mediated signaling pathways are likely to play roles in many biological processes during the plant life cycle. Some CLE genes that are closely related in sequence have dissimilar expression profiles, yet in many tissues multiple CLE genes have overlapping patterns of promoter-driven reporter activity. This observation, plus the general absence of detectable morphological phenotypes in cle null mutants, suggests a high degree of functional redundancy exists among CLE gene family members. Our work establishes a community resource of CLE-related biological materials and provides a platform for understanding and ultimately manipulating many different plant signaling systems.
Introduction

Plant growth and survival is critically dependent on the communication of information between cells. Intercellular signaling pathways convey cell fate information, regulate cell division and differentiation processes, propagate and amplify specific signaling states, and coordinate tissue responses and functions. The importance of cell-to-cell communication is underscored by the classification of 10% of the Arabidopsis proteome as playing roles in signal transduction (Initiative, 2000). Yet whereas more than 400 Arabidopsis genes encode receptor-like kinases (Shiu and Bleecker, 2001) that presumably function as transmembrane sensors for extracellular signals, only a few families of putative signaling molecules have been identified.

The CLE genes encode small polypeptides (<15 kD) that share several structural features. Each possesses either an amino terminal signal peptide or membrane anchor sequence, a large variable domain, and a highly conserved 14-amino-acid motif called the CLE domain near the carboxyl terminus (Cock and McCormick, 2001). Biochemical evidence indicates that the full-length CLAVATA3 (CLV3) polypeptide is proteolytically processed (Ni and Clark, 2006) to a mature active 12 or 13-amino-acid arabinosylated glycopeptide consisting of the CLE domain (Kondo et al., 2006; Ohyama et al., 2009). Synthetic peptides corresponding to the CLE motif of other CLE family members also show biological activity in various bioassays (Fiers et al., 2005; Ito et al., 2006), suggesting that such peptides are likely to be the functional CLE gene products.

The biological functions of only a few CLE genes are known. CLV3 is a founding member of the family and plays a key role in the intercellular communication of stem cell fate during Arabidopsis development. CLV3 is specifically expressed in the stem cell population of shoot and floral meristems (Fletcher et al., 1999). Secreted into the extracellular space (Rojo et al., 2002), the CLV3 polypeptide is perceived by CLAVATA1 (Clark et al., 1997; Ogawa et al., 2008) and other transmembrane receptors (Jeong et al., 1999; Muller et al., 2008) in the underlying cells. This signal transduction pathway is a core component of a negative feedback loop linking the stem cell reservoir and the underlying organizing center (Brand et al., 2000). Signaling through the CLV pathway restricts stem cell accumulation by limiting the expression domain of the WOX family transcription factor gene WUSCHEL (Laux et al., 1996), which promotes stem cell activity and CLV3 expression (Schoof et al., 2000).

CLE gene activity also controls stem cell homeostasis in the root meristem. CLE40 transcripts are present at low levels in all Arabidopsis tissues (Hobe et al., 2003), but in roots
CLE40 is specifically expressed in the stele and in differentiating columella cells (CCs) of the protective root cap (Stahl et al., 2009). CLE40 activity emanating from the CCs promotes distal root meristem differentiation by acting through the receptor kinase ARABIDOPSIS CRINKLY4 (ACR4) in a negative feedback loop that limits the expression domain of the WUS-related gene WOX5 (Stahl et al., 2009). WOX5 is present in the quiescent center (QC) and promotes columella stem cell fate in the distal domain of the root meristem. Thus CLE/WOX-mediated signaling modules regulate Arabidopsis stem cell fate in both the shoot and the root apical meristems.

Over-expression studies have uncovered other developmental processes that respond to CLE peptide activity. A number of CLE genes when mis-expressed in the root apical meristem gradually inhibit root meristem maintenance (Casamitjana-Martinez et al., 2003; Fiers et al., 2004; Fiers et al., 2005; Ito et al., 2006; Meng et al., 2010) via a signaling pathway that appears to be distinct from the CLE40 pathway. Plants over-expressing CLE19, CLE21 or CLE25 form miniature rosettes and inflorescences, and display anthocyanin overproduction and developmental delays (Strabala et al., 2006). Over-expression of CLE42 or CLE44 results in bushy, dwarfed plants with delayed development and reduced apical dominance, whereas CLE18 or CLE26 over-expression leads to enhanced root elongation (Strabala et al., 2006). Simultaneous over-expression of CLE6 and CLE41 produces stunted, bushy plants with increased hypocotyl vascular cell proliferation, suggesting that CLE peptides can function synergistically (Whitford et al., 2009). These phenotypes indicate roles for CLE family members in regulating many different aspects of development. Yet although many Arabidopsis CLE genes cause morphological phenotypes when over-expressed, others still remain to be evaluated by this type of study.

Results from over-expression studies as well as in vitro bioassays revealed that multiple CLE peptides have the capacity to generate the same morphological phenotypes. More than a dozen CLE peptides can activate the CLV3 signaling pathway when ectopically expressed in the shoot meristem (Ni and Clark, 2006; Strabala et al., 2006; Jun et al., 2008; Meng et al., 2010). Application of 19 different synthetic CLE peptides in root growth assays can arrest root meristem growth (Ito et al., 2006; Strabala et al., 2006; Whitford et al., 2009), whereas the application of CLE41, CLE42, CLE43 or CLE44 peptides leads to suppressed xylem differentiation (Ito et al., 2006; Whitford et al., 2009). The CLE family has been divided into two functional classes on this basis, with A-type CLE peptides (CLV3, CLE1 through CLE27, and CLE40) being capable of inducing root and/or shoot meristematic cell differentiation whereas B-
type CLE peptides (CLE41 through CLE44) are not (Whitford et al., 2009). These observations also raise the possibility that another key determinant of CLE peptide specificity, in addition to their primary amino acid sequence (Meng et al., 2010), may be their tissue distribution. Yet although RT-PCR studies show that CLE genes are transcribed in many different tissues (Sharma et al., 2003), their specific expression patterns remain to be characterized.

To obtain a more precise understanding of the extent of overlap between CLE genes and gauge their possible functional redundancy, we used reporter assays to obtain high resolution expression data for the entire Arabidopsis A-type CLE gene family. We observed highly specific CLE gene promoter activity patterns in roots, shoots, leaves, stems and flowers, with most tissues expressing multiple CLE promoters. We thus uncover a number of new biological processes that may be regulated by small peptide signal transduction pathways. In addition, we characterized the over-expression phenotypes of previously unstudied CLE genes, and identified hypomorphic or null insertion alleles for eight A-type CLE genes. Morphological examination of homozygous cle single mutant plants revealed no detectable growth or development phenotypes. Taken together, our expression and functional data indicate that CLE family members have diverse activities yet significant functional redundancy exists among them.

Results

Analysis of A-type CLE promoter activity in vegetative tissues

Among the Arabidopsis A-type CLE genes, the expression patterns of CLV3, CLE19 and CLE40 have been already reported (Fletcher et al., 1999; Hobe et al., 2003; Fiers et al., 2004). To determine the expression patterns of the other A-type CLE genes, we generated CLE promoter:GUS or GFP fusion constructs using from 974 - 3398 base pairs of 5’ genomic region upstream of each CLE coding sequence. At least 10 independent transgenic lines were analyzed for each gene promoter to monitor consistent reporter activity, with the exception of the CLE11, CLE12 and CLE13 promoters for which 4-8 independent transgenic lines were analyzed. Each CLE promoter drove GUS expression in one consistent pattern except for CLE10 (see Supplemental Methods). We observed that all but one A-type CLE gene reporter is expressed in vegetative tissues (Table 1, Fig. 1-4).

Seedling expression patterns
We first analyzed CLE promoter activity in 11-day-old Arabidopsis seedlings (Fig. 1). From this analysis the CLE expression patterns could be divided into three groups: (1) those expressed in both shoot and root tissue (15 genes), (2) those expressed only in shoot tissue (2 genes), and (3) those expressed only in root tissue (5 genes) as listed in Table 1. CLE8 was the only CLE gene for which expression was not detected at this stage of development.

Among the CLE genes with promoter activity in aerial tissues, CLE12, CLE18, CLE22, CLE25 and CLE26 all display GUS activity in the vascular tissues (Fig. 1A-E). Among these, CLE12 staining is relatively weak in secondary and tertiary veins compared to primary veins, and is stronger in the root than in the shoot (Fig. 1A; Fig. 2C). CLE18 and CLE22 are expressed uniformly in vascular tissue throughout the rosette leaves, although CLE18 promoter activity begins later during leaf growth than CLE22 (Fig. 1B, C). The patterns of CLE25 and CLE26 promoter activity are complementary during leaf development. CLE25 is strongly expressed in the vascular tissue of young leaf primordia, but is weaker in mature leaves (Fig. 1D; Fig. 2A). CLE26 is initially detected in the leaf tip region where vein patterning initiates, and only later expands into the vasculature throughout the entire leaf (Fig. 1E; Fig. 2B).

The CLE9, CLE16, CLE17 and CLE27 promoters drive GUS activity in rosette leaf blade cells (Fig. 1F-I). CLE9 is expressed specifically in the stomatal developmental lineage, including meristemoid cells, guard mother cells and young guard cells, throughout the aerial portions of the plant (Fig. 1F; Fig. 2I, J). CLE16 promoter activity is detected throughout the blade pavement cells, whereas CLE17 and CLE27 promoter activity is broad in young leaves but becomes predominantly marginal as the leaves mature. CLE16 and CLE17 promoter activities are also detected in the trichomes (Fig. 1G, H; Fig. 2D, E), as is CLE14 promoter activity (Fig. S1D). CLE10 activity is restricted to the rosette leaf margins (Fig. 1J), particularly in the hydathode region (Fig. 2H). The CLE5 and CLE6 promoters also drive specific GUS staining in the hydathode region (Fig. 1K, L; Fig. 2F, G).

Other seedling tissues also express multiple CLE gene reporters. Hypocotyls display GUS activity driven by eight CLE promoters (Fig. 1A, C, E, G, H, I, M; Fig. 2L, M; Table 1). The shoot apex region exhibits GUS activity driven by the CLE3-6, CLE10, CLE11, CLE16, CLE17, CLE21 and CLE27 promoters (Fig. 1G-P). Among these, CLE3 and CLE11 show stipule-specific expression (Fig. 1N, O; Fig. 2K, L). CLE4 is limited to the pith region (Fig. 1Q), whereas CLE5, CLE6, CLE10, and CLE21 expression is restricted to the base of the rosette leaves and is
excluded from the shoot apical meristem (Fig. 1J-M; Fig. 2M-P). CLE16, CLE17 and CLE27 are expressed in initiating leaf primordia (see below). GUS activity driven by the CLE1, CLE2, CLE7 and CLE13 promoters is specific to root tissue (Fig. 1Q-T).

**Root expression patterns**

The promoters of many CLE genes are active in the root system of 11-day-old seedlings. GUS activity from four CLE promoters is detected throughout the primary root cap (Fig. 3A-D). CLE16 activity localizes to the root cap and the elongation zone but is absent from the meristematic division zone (Fig. 3E), whereas CLE17 activity is absent from the root cap but localizes to the meristematic zone and the distal end of the elongation zone (Fig. 3F). CLE22 activity is limited to a single file of newly differentiating vascular cells (Fig. 3G), whereas CLE25 and CLE26 activity is restricted to the vascular parenchyma (Fig. 3H, I). Vascular tissue in the elongation zone exhibits GUS activity from both the CLE1 and CLE18 promoters (Fig. 3A, D).

More mature root tissues express multiple CLE gene reporters in overlapping patterns. We examined two different areas of 11-day-old primary and lateral roots: a younger region with immature hair cells, as well as an older region with fully differentiated hair cells. We found that the stele in both areas of the root exhibit GUS activity driven by five different CLE promoters (Fig. 4A-H; Table 1). pCLE20:CLE20-GFP fusion protein activity is observed in the protoxylem and metaxylem (Fig. S1B), whereas CLE25 and CLE26 promoter activity is restricted to the metaxylem (Fig. S2A and data not shown). CLE22 is strongly detected in the vascular parenchyma (Fig. S2B). Pericycle cells specifically display GUS activity from the CLE4 and CLE18 promoters (Fig. 4I-L; Fig. S2C), whereas both the pericycle and endodermis express CLE7 (Fig. 4M, N; Fig. S2D). CLE1 promoter activity is detected throughout the endodermis and the stele (Fig. 4O, P; Fig. S2E). In contrast, pCLE14:GFP activity is restricted to the root epidermis (Fig. S1F).

Several CLE promoters are active in a spatially and temporally restricted fashion in the primary root. In the less mature region of the root pCLE16:GUS and pCLE17:GUS activity are found exclusively in the epidermis (Fig. 4Q, S), whereas in the older region CLE16 promoter activity localizes to the stele (Fig. 4R) and CLE17 promoter activity expands throughout the root (Fig. 4T). CLE3 promoter activity is patchy in the stele in the less mature root region (Fig. 4U),
but becomes limited to the pericycle and endodermis in older tissue (Fig. 4V). Finally, CLE5 promoter activity is not detected until the primary root is fully differentiated, when very weak activity is observed in the stele (Fig. 4W, X).

CLE promoter activity is highly dynamic during lateral root formation. CLE27 promoter-driven GUS activity is detected in the cortex of the primary root at lateral root inception (Fig. S3A). As the lateral root cells begin to grow out, the CLE27 reporter is expressed throughout the dome but most strongly in a ring around the base (Fig. S3B-C). As outgrowth continues, CLE27 promoter activity is lost from the lateral root tip but remains detectable in the more basal region (Fig. S3D-E). In mature lateral roots, CLE27 promoter activity is found at the base of the lateral root where it joins the primary root (Fig. S3F), as well as in the meristematic zone (Fig. S3G). CLE2 and CLE20 reporter expression is likewise detected at the base of initiating lateral roots (Fig. S3H, I; Fig. S1C, D), although at a slightly later stage than CLE27. As root outgrowth progresses, CLE2 promoter activity becomes confined to the interior cells at the junction between the primary and lateral roots (Fig. S3J). Similarly CLE11 promoter-driven GUS activity is observed in a small group of cells surrounding the initiating lateral roots (Fig. S3K) as well as at the root tip, and is sustained at the junction between the primary and lateral roots (Fig. S3L). CLE5 and CLE6 promoter activity is also seen specifically in the interior cells at the junction between the primary root and the mature lateral roots (Fig. S3M, N).

CLE promoter activity is also dynamic in the lateral root vasculature. CLE22 is expressed earliest during lateral root formation (Fig. S3O-P), followed by CLE25 (Fig. S3Q). pCLE25:GUS and pCLE26:GUS activity both localize to the vasculature of outgrowing lateral roots (Fig. S3Q-R), whereas CLE4 (Fig. S3S) and CLE12 (Fig. S3T) activity is restricted to the mature lateral root vasculature. CLE7 promoter-driven activity is likewise limited to the mature lateral root vasculature, but unlike the others its expression is absent from the junction between the primary and lateral root (Fig. S3U). Thus the expression of a half dozen CLE reporters is induced at various stages during the development of the lateral root vasculature.

Lateral root tips initiate the expression of different CLE reporters at various stages during their formation. GUS activity driven by the promoters of both CLE16 and CLE17 is detected throughout the initiating lateral roots, before becoming confined to the tips and meristematic zones as the roots grow out (Fig. S4A-H). CLE11 (Fig. S4I) and CLE13 (Fig. S4K) activity initiates at the lateral root tip at a slightly later point during outgrowth, followed by CLE18
promoter activity (Fig. S4M) and CLE1 activity (Fig. S4O). The promoters of all six of these CLE genes are active in the root caps of mature lateral roots (Fig. S4D, H, J, L, N, P). Yet unlike the other five, which are localized in all root cap cells, CLE1 promoter activity in lateral roots is restricted to the interior layers of the root cap. CLE18 reporter signal is strong in the root cap and weaker in cells at the very distal end of the elongation zone. In addition, initiating vascular tissues display CLE22 promoter activity (Fig. S4Q) and more mature vascular tissues display CLE25 and CLE26 promoter activity (Fig. S4R, S). Lateral roots therefore express different combinations of CLE gene reporters in overlapping patterns throughout their development.

Eight CLE genes are represented on the ATH1 microarray used to generate a spatiotemporal map of gene expression in the roots of 5-7 day old seedlings (Brady et al., 2007). Comparison of our results with this dataset using the eFP Browser (Cartwright et al., 2009) showed congruent profiles for CLE3, CLE12 and CLE17. However, we did not detect activity of CLE2 in the phloem, CLE6 in phloem companion cells, CLE21 in epidermal cells of the elongation region, or CLE27 in primary root cortex and lateral root cap cells (Brady et al., 2007). Further, CLE26 is reported in the metaphloem whereas we detect it in the metaxylem. These discrepancies likely reflect the increased sensitivity of transcriptional profiling compared to GUS reporter analysis, the use of insufficient CLE regulatory sequence for some reporter constructs, and/or the difference in developmental age between the samples evaluated.

**Analysis of A-type CLE promoter activity in reproductive tissues**

With a few exceptions, such as those for CLE2 and CLE8, most A-type CLE gene reporters are expressed during reproductive growth (Table 2, Fig. 5-7). As in seedlings, each reproductive tissue expresses more than one CLE gene reporter. Conversely, most CLE gene reporters are expressed in more than one reproductive tissue, yet their individual expression patterns are highly specific and restricted.

**Inflorescence expression patterns**

The promoters of many CLE genes are active in inflorescence tissues. GUS activity from the CLE16 promoter is found specifically in the epidermis (Fig. 5A). The CLE12, CLE13, CLE20, CLE22, CLE25 and CLE26 reporters are expressed in the stem vasculature (Fig. 5B-F and data not shown), in each case more strongly in the primary inflorescence branching points than
elsewhere in the stem. Activity from 7 CLE promoters is largely restricted to the primary branching points of the stem (Fig. 5G-M; Table 2), although CLE17 promoter activity is also present in the stem trichomes. Consistent with its expression in vegetative tissues, signal from the CLE9 reporter is detected in the stomata of stem epidermal cells (Fig. 5N). Pedicels display promoter-driven GUS activity from CLE16 in the epidermis (Fig. 5P), from CLE22 and CLE26 in the vasculature (Fig. 5Q, R), and from CLE27 throughout (Fig. 5S).

Eight CLE promoters are active in the cauline leaves. CLE12 and CLE22 promoter activity is strong in the proximal vasculature and at the very tip of the cauline leaf (Fig. 5B, D). The CLE26 promoter drives patchy vascular expression in the blade (Fig. 5F). CLE18-driven expression is detected in the vasculature and at the leaf margins (Fig. 5O), whereas CLE10 and CLE17 promoter-driven GUS activity is limited to the leaf margins (Fig. 5J, K). CLE9 expression is only detected in stomatal cells (Fig. 5N), and CLE3 expression is restricted to stipules (data not shown). Promoter activity from all eight genes is also detected in rosette leaves.

Flower expression patterns
Floral tissues display highly complex CLE promoter activity patterns (Fig. 6). Sepals and petals express nine different CLE gene reporters. CLE9 promoter activity is detected in the sepal stomatal cells, consistent with what is observed in vegetative tissues (Fig. 2J). The CLE14 reporter is expressed in sepal trichomes (Fig. S1G). CLE16 promoter activity is detected in sepal and petal vasculature throughout flower development, although stronger at the distal ends in young flowers (Fig. 6A), and becomes confined to these regions in fully mature flowers (Fig. 6H). CLE18 and CLE26 reporters are expressed in sepal veins, more strongly in their distal portion (Fig. 6B, C). CLE22 promoter activity is observed uniformly in the vasculature of both sepals and petals, although it becomes more pronounced in the sepal veins in fully developed flowers (Fig. 6D). CLE17 promoter activity is detected along the margins of sepals and petals in young flower buds (Fig. 6E). CLE5 and CLE6 reporters are expressed at the base of each flower organ type, above the abscission zone (Fig. 6F, G).

The male reproductive structures express a variety of CLE gene reporters (Fig. 6). Each stamen-expressed reporter is present in either the anther or the filament but not in both. Five CLE reporters are expressed in the filament (Table 2). CLE16 and CLE18 are uniformly detected
throughout the entire filament (Fig. 6H, I). In contrast, CLE6 promoter activity is detected very weakly in the distal region of the filament (Fig. 6G), whereas CLE4 and CLE26 promoter activity is limited to the distal tip of the filament, where it connects to the anther (Fig. 6J, K). CLE4 is the only one of these that is expressed exclusively in the filament and not in other floral tissues.

Anthers express six different CLE gene reporters (Table 2). CLE1 promoter activity is found in both pollen grains and tapetum cells of anthers from their emergence in stage 6 floral buds until maturation (Fig. 6L). The CLE25 reporter is expressed throughout the anthers in young developing flowers (Fig. 6M). In mature flowers CLE25 expression become more restricted, and overlaps with that of CLE7 in the central region of the anther sacs, along the boundaries with the connective tissue (Fig. 6N). CLE11, CLE12 and CLE13 promoter activity is evident solely in pollen grains. The CLE12 reporter is expressed throughout all stages of pollen development (Fig. 6O). However, CLE11 and CLE13 reporters show a dynamic and complementary expression pattern: the CLE11 reporter is expressed only in fully mature pollen grains (Fig. 6P, Q), whereas the CLE13 reporter is expressed only in young developing anthers (Fig. 6R, S).

The female reproductive structure exhibits CLE promoter activity in a variety of complex patterns (Fig. 7). The CLE10 reporter is the most broadly expressed, its activity being detected in the stigma, style, transmitting tract, septum, and ovules (Fig. 7A). The style also displays promoter activity from CLE1, CLE5, and CLE11. Among these, CLE5 and CLE1 reporters are expressed in the transmitting tract of the style (Fig. 7B, C), whereas CLE11 reporter expression is observed in a central region that appears to correspond to the transmitting tract or vascular fans (Fig. 7D). Uniquely, CLE17 promoter activity is detected in a ring at the margin between the stigmatic tissue and the style, in the transmitting tract, and in the septum (Fig 7E). CLE21 promoter activity is observed in the valve margins (Fig. 7F), whereas CLE16 promoter activity occurs in the valves (Fig. 7G). The vasculature of the gynoecium exhibits CLE22, CLE25 and CLE26 promoter activity (Fig. 7H, I, J). CLE25 reporter expression is additionally detected in the septum, funiculi and at the proximal end of the ovules (Fig. 7K), whereas the CLE27 reporter is expressed throughout the funiculi and ovules of fully mature flowers (Fig. 7L). Finally, the abscission zone exhibits promoter activity from CLE10 (Fig. 5J), CLE12 (Fig. 6O), CLE13 (Fig. 6R, S), CLE16 (Fig. 6H), CLE21 (Fig. 7G), CLE22 (Fig. 7H), CLE26 (Fig. 7J), and CLE27 (Fig.
7M). These promoter activity patterns are consistent throughout gynoecium and silique development, with two exceptions. First, \textit{CLE17} reporter expression shifts from the stigma/style region in carpels to the valve margins in siliques (Fig. 7N). Second, \textit{CLE4} reporter expression becomes detectable specifically in the silique receptacle (Fig. 7O). In sum, our observations indicate that different \textit{CLE} promoters drive GUS activity in complex, overlapping spatial and temporal patterns, particularly in the reproductive tissues.

\textit{Detailed analysis of CLE promoter activity in shoot apices}

Promoter activity from ten different \textit{CLE} genes is detected around the shoot apex region of seedlings (Fig. 1, 2). We examined their expression patterns in greater detail by sectioning 10-day-old GUS-stained seedlings. We found that \textit{CLE21} promoter activity is restricted to the stipules (Fig. S5A), whereas \textit{CLE10} promoter activity is detected in stipules and in a proximal, adaxial domain of leaf primordia (Fig. S5B). \textit{CLE4} and \textit{CLE26} promoter activity is located in the pith and the ground cells of the hypocotyl, respectively (Fig. S5C, D).

In contrast, the \textit{CLE16}, \textit{CLE17}, and \textit{CLE27} reporter constructs show consistent activity in or adjacent to the shoot apical meristem (SAM) in multiple independent transgenic lines. Compared to untransformed wild-type plants (Fig. 8A1) and p\textit{CLV3:GUS} plants (Fig. 8B1), \textit{CLE16} promoter activity is detected throughout initiating leaf primordia on the shoot apical meristem flanks (Fig. 8C1). As the leaf primordia develop, \textit{CLE16} GUS activity is stronger in the proximal than the distal region, and in the L1 cells (Fig. 8C1; Fig. S6A, B). A similar pattern is observed in p\textit{CLE17:GUS} leaf primordia (Fig. 8D1). However, \textit{CLE17} promoter activity is also detected in the SAM (Fig. 8D1). Transgenic plants with weak \textit{CLE17} promoter activity show GUS staining in the outer layers of the CZ and the PZ (Fig. S6C). Transgenic plants with strong \textit{CLE17} promoter activity exhibit GUS staining throughout the SAM and initiating leaf primordia, most strongly in the PZ and the outer cell layers (Fig. S6D). \textit{CLE27} promoter activity is exclusively detected in the epidermal layer of developing leaf primordia and young rosette leaves, and in the pith region beneath the rib meristem (Fig. 8E1; Fig. S6E, F). However, \textit{CLE27} reporter expression is excluded from the SAM and the initiating leaf primordia on the meristem flanks. These results are consistent with Arabidopsis SAM transcription profiling data showing that \textit{CLE27} mRNA is absent from the SAM, but that \textit{CLE17} is expressed at low levels in the CZ.
and OC and at moderate levels in the PZ (Yadav et al., 2009). CLE16 is not represented in the profiling dataset.

Because CLE17 reporter expression in the SAM overlapped with the CLV3 expression domain, we tested whether CLE17 could activate the CLV signaling pathway by analyzing the phenotypes of p35S::CLE17 transgenic plants. Ectopic expression of CLV3 causes SAM arrest early during vegetative development (Fig. 8B2, B3) as well as premature floral meristem termination (Brand et al., 2000). In contrast, ectopic expression of CLE17 does not confer a SAM termination phenotype (Fig. 8D2, D3), nor is floral organ formation affected. These data indicate that CLE17 cannot activate the CLV3 signaling pathway in either shoot or floral meristems. Instead, the rosette leaves of CLE17 over-expressing plants show a delayed growth rate, and smaller and epinastic morphology compared to wild-type rosette leaves (Fig. 8D2, D3). Developmental timing is also delayed and apical dominance appears to be reduced. In addition, CLE17 over-expression (Fig. 8H) causes root apical meristem termination (Fig. 8D4, D5), as has been observed in plants that have been treated with exogenous CLE peptides or over-express other CLE genes, such as CLV3 (Fig. 8B4, B5). Transgenic plants over-expressing either CLE16 or CLE27 (Fig. 8G, I) display phenotypes that closely resemble those of p35S::CLE17 plants (Fig. 8C2-5, E2-5).

Alignment of the CLE16, CLE17 and CLE27 peptides with that of CLV3 reveals altered amino acids at key residues in the CLE domain. Compared to CLV3, all three peptides contain an asparagine instead of a histidine at position 12, CLE16 and CLE17 contain a histidine instead of a proline at position 4 as well as an asparagine instead of an aspartate at position 8, and CLE27 contains a cysteine instead of the highly conserved glycine at position 6 (Fig. 8F). These observations suggest that the failure of these three proteins to activate the CLV3 signaling pathway when over-expressed in the SAM may be due to differences in the composition of their CLE motifs.

CLE over-expression phenotypes in shoots and roots

Despite extensive over-expression studies that uncovered other developmental processes responding to CLE peptide activity, the over-expression phenotypes a few CLE genes remain undetermined. To fill in this gap, we generated transgenic plants expressing the coding region of CLE8, CLE12 or CLE22 under the control of the CaMV 35S promoter, and scored them for
shoot and root meristem arrest phenotypes (Table S1). We found that p35S:CLE8 plants showed neither shoot nor root meristem defects (Fig. S7B), whereas both p35S:CLE12 and p35S:CLE22 plants displayed SAM termination (Fig. S7C, D). Reduced root growth and RAM arrest was also observed in CLE12 and CLE22 over-expressing plants (Fig. S7E, F). Our data are consistent with previous work except in the case of CLE8, which has been reported to trigger RAM consumption when over-expressed (Ito et al., 2006). Combined with earlier studies, our results indicate that 16 of 26 A-type CLE genes, including CLV3, can induce SAM termination when over-expressed, and that 18 (or 19) A-type CLE genes can induce RAM termination.

**Mutational analysis of A-type CLE loci**

To begin to determine the functions of additional A-type CLE genes, we obtained cle T-DNA insertion alleles from the publically available collections. We identified one allele each with an insertion within the CLE1, CLE10, CLE16 and CLE18 coding regions, as well as one allele each with an insertion in the CLE3, CLE7, CLE13 and CLE19 5’ UTR, and one allele with an insertion in the CLE17 3’ UTR (Fig. 9). We performed RT-PCR to examine CLE mRNA transcript levels in plants homozygous for each of these cle alleles. No transcripts were detected in cle1-1, cle7-1, cle16-1, and cle18-1 plants (Fig. 9A-D), indicating that they represent null alleles. Reduced transcript levels were detected in cle3-1, cle10-1, cle13-1, cle17-1 and cle19-1 plants (Fig. 9E-I), showing that these are hypomorphic alleles. For cle3-1, we observed a minor but reproducible decrease in CLE3 mRNA levels (Fig. 9E). In addition, we identified T-DNA insertion alleles within 700 base pairs upstream or downstream of the CLE2, CLE4, CLE6, CLE9, CLE17 and CLE21 coding regions, but RT-PCR experiments detected no significant reduction in transcript levels (Table S2). Detailed inspection of seedling, inflorescence, flower and root development revealed no detectable morphological defects in plants homozygous for any of these cle alleles, suggesting that substantial functional redundancy occurs among A-type CLE family members.

**Discussion**

Dozens of potential intercellular signaling molecules as well as hundreds of putative receptors have been cataloged in the Arabidopsis genome, yet relatively little is known about their individual expression patterns or functions. Several members of the A-type CLE family of small
secreted polypeptides act to maintain cell fate in shoot and root apical meristems; however, the
dearth of mutations in the other small *CLE* coding sequences has limited our insight into their
biological activities. In addition, only a single study of A-type *CLE* mRNA transcription profiles
exists (Sharma et al., 2003), and few *CLE* genes are represented on microarrays, another source
of detailed expression data. To address these deficiencies we performed a comprehensive
characterization of A-type *CLE* promoter expression during Arabidopsis vegetative and
reproductive development, and identified null or hypomorphic alleles of seven *CLE* genes.

One important finding from our work is that most Arabidopsis tissues express one or
more *CLE* gene reporters (Fig. 10). This includes highly specialized cell types such as stomata,
trichomes and stipules. Primary roots display *CLE* promoter-driven expression in the root cap,
the root apical meristem, and in each radial cell layer (Fig. 3, 4), and lateral root formation is
associated with the dynamic activity of multiple *CLE* promoters (Fig. S3). The vasculature is
characterized by the expression of 14 *CLE* reporters, half of which are specific to either the root
or the shoot vasculature. Multiple *CLE* gene reporters also are expressed in different tissues of
the inflorescence stem (Fig. 5), as well as in each floral organ. In particular, the reproductive
organs express a variety of *CLE* gene reporters in specific spatial and temporal patterns (Fig. 6,
7). Seven different *CLE* reporters are expressed at the base of the flower, where the cognate
genes may be involved in the signaling process(es) that controls floral organ abscission. These
observations indicate that, beyond their functions in shoot and root apical meristems, *CLE-*
mediated signal transduction pathways are likely to play roles in a wide variety of different
biological processes.

Another intriguing finding is that the A-type *CLE* gene promoters drive highly distinct
and specific patterns of expression. For example, the promoters of *CLE3, CLE5, CLE16* and
*CLE17* are active in a spatially and temporally restricted fashion in the primary root (Fig. 4),
whereas the *CLE1, CLE5, CLE11, CLE16, CLE17* and *CLE21* promoters are active in unique
sub-domains of the developing gynoecium (Fig. 7). No two *CLE* promoters drive expression in
identical patterns throughout the plant, and indeed we observe that even *CLE* genes with very
similar sequences have divergent reporter expression patterns. This is exemplified by *CLE3* and
*CLE4*, which have an identical CLE motif and pair together in the published phylogenies (Ito et
al., 2006; Strabala et al., 2006; Jun et al., 2008; Mitchum et al., 2008). We detect *CLE3* promoter
activity in leaf stipules (Fig. 2K) and the pericycle and endodermis of mature roots (Fig. 4U, V),
whereas *CLE4* promoter activity coincides with *CLE3* in the root pericycle (Fig. 4I, J) but is also found in the hypocotyl pith (Fig. S5C), inflorescence branch points (Fig. 5G), stamen filaments (Fig. 6J) and receptacle (Fig. 7O). Thus while these two genes may function interchangeably in the pericycle, they would appear to have unique activities in the other tissues. The importance of the location of gene expression in conferring functional specificity has also been shown with closely related members of the MADS domain transcription factor family (Pinyopich et al., 2003).

Although many CLE proteins act interchangeably when ectopically expressed in shoots or roots, indicating that tissue distribution important for their functional specificity, the location of gene expression is not the only determinant of CLE function. Studies have shown that the CLE motif itself determines much of the functional specificity of the proteins in different plant tissues (Fiers et al., 2005; Fiers et al., 2006; Ito et al., 2006; Kondo et al., 2006; Ni and Clark, 2006; Meng et al., 2010). We found that the *CLE17* promoter is active in the SAM in a domain that overlaps with *CLV3*, yet its over-expression fails to induce a *CLV3* over-expression SAM termination phenotype (Fig. 8). The CLE peptide of the SAM-expressed *CLE17* gene differs from that of CLV3 at several key residues, including the C-terminal residue (\(12\)His) that plays an essential role in CLV3 peptide function and binding to the CLV1 receptor kinase (Kondo et al., 2008). These data provide an additional piece of evidence that the CLE motif plays a critical role in determining CLE activity and receptor binding specificity *in planta*. Other factors contributing to CLE signaling specificity are likely to include the tissue distribution of their cognate receptors as well as of the enzymes that process the CLE proteins to the active arabinosylated peptides (Ohyama et al., 2009).

To date, assessing the biological roles of small signaling peptide gene families has proven to be a significant challenge, primarily due to a lack of hypomorphic or null alleles. These are not available for most CLE family members because the small size of the genes reduces the target size for T-DNA insertion, and because the mature molecule consists of only a short stretch of amino acids. We have identified null mutations in the *CLE1, CLE7, CLE16* and *CLE18* genes, but in each case the homozygous plants lack detectable morphological phenotypes, indicating that their function may be conditioned by environmental factors and/or masked by redundancy with other CLE peptides. In the future, alternative strategies will be required to specifically target...
CLE genes for down-regulation. Possibilities include artificial microRNAs (Schwab et al., 2006) and increased efficiency homologous recombination (Osakabe et al., 2010; Zhang et al., 2010).

Although each CLE promoter is active in a unique spatial and temporal pattern during Arabidopsis development, we observe that multiple CLE promoters are active in overlapping patterns within a given tissue. These overlapping CLE genes are most likely to have redundant functions and thus our complete gene family promoter expression analysis serves as a guide to identify potential redundant CLE peptides within specific tissues. However, it should be noted that overlapping expression patterns do not necessarily guarantee redundant activities and that CLE genes expressed in the same cell types could potentially send opposite signals, as has been observed for members of the EPF family of small peptide ligands during stomatal development (Abrash and Bergmann, 2010).

Finally, our elucidation of CLE promoter activity throughout Arabidopsis development provides a resource for predicting candidate receptors based on their overlapping or neighboring expression patterns. For instance, the PXY gene encodes an LRR receptor kinase that belongs to the same clade of LRR-RLK subclass XI proteins as CLV1 (Hirakawa et al., 2008). PXY is expressed in dividing vascular procambium cells (Fisher and Turner, 2007) and interacts with the B-type CLE peptide CLE41 (Etchells and Turner, 2010). We find that 14 different CLE promoters drive GUS activity in the vasculature (Table 1). Thus the products of one or more of these CLE genes could act as ligands for PXY, and/or for VASCULAR HIGHWAY1 (VH1), another LRR-RLK expressed in procambial cells throughout the plant (Clay and Nelson, 2002). An LRR-RLK encoded by the EMS1/EXS gene is expressed in the sporogenous and parietal cells of the developing anther, where it controls microsporocyte differentiation and tapetal identity (Canales et al., 2002; Zhao et al., 2002). The CLE1, CLE7, CLE12, CLE13 and CLE25 promoters are all active during early anther formation, making these genes candidates to encode EMS1/EXS ligands. EMS1/EXS is also expressed in developing leaf primordia, inflorescence meristems and young flower buds (Canales et al., 2002). Yet among the CLE promoters active in young anthers, only those of CLE12, CLE13 and CLE25 are also active in leaf primordia and none is active in or adjacent to the inflorescence meristem. Thus broadly expressed receptors such as EMS1/EXS may potentially bind different CLE ligands deriving from different cell types. In conclusion, our systematic analysis of the CLE gene family illustrates the complex expression dynamics of these signaling molecules throughout the Arabidopsis life cycle and
provides a foundation for identifying and characterizing many ligand-receptor mediated signaling pathways during plant development.

**Materials and Methods**

*Plant materials*

*Arabidopsis thaliana* ecotype Columbia (Col) and Landsberg *erecta* (Ler) plants were used in this study. Seeds were imbibed at 4°C for 3 days before sowing and were grown in a greenhouse under long days (16 h light and 8 h dark) with a day/night temperature cycle of 22°C/18°C. Seeds were surface sterilized for 10 min in 5% NaOCl and 0.1% Triton X-100, rinsed in distilled water, and plated on plates containing Murashige and Skoog medium with 0.8% type M agar (Life Technologies, Rockville, MD), 0.5 mM MES pH 5.7, 0.5% Sucrose, 13 B5 vitamins.

*Construction of transgenic plants*

To generate *CLE* promoter::GUS fusion constructs, the 5’ upstream region (from 974 bp to 3398 bp) of each *CLE* gene was PCR-amplified from Col genomic DNA and cloned into a binary vector for transformation. For each construct, the binary vector in the *Agrobacterium tumefaciens* strain GV3101 or ASE was introduced into plants by the floral dip method (Clough and Bent, 1998). Primer sequences are listed in Table S3. Upon request, all novel materials described in this publication will be made available in a timely manner for non-commercial research purposes, subject to the requisite permission from any third-party owners of all or parts of the material. Obtaining any permissions will be the responsibility of the requestor.

*Histochemical assays*

GUS staining of transgenic plants was performed as described (Jefferson et al., 1987), with the modification that 0.5 mM potassium ferrocyanide and 2.5 mM potassium ferricyanide were used. Incubation times ranged from 2 to 16 hours following vacuum infiltration. Subsequent tissue embedding and sectioning were performed as described (Sieburth and Meyerowitz, 1997). For roots and some flower samples, whole-mount clearing was performed in Hoyer’s medium (Liu and Meinke, 1998) and the samples were visualized using a Zeiss Axiophot microscope equipped with Nomarski optics. Whole seedlings, inflorescences and flower specimens were imaged using a Zeiss Stemi 2000-c and a Zeiss Stemi SV11 microscope. GFP fluorescence was visualized.
using a Leica DM LB fluorescence microscope or a Zeiss LSM 510 confocal microscope with 488 nm/530 nm excitation/emission light.

mRNA transcript analysis

cDNA was synthesized from 1~5 µg of total RNA using an oligo(dT<sub>18</sub>) primer and SuperScript III reverse transcriptase (Invitrogen). For RT-PCR, one microliter of the first-strand cDNA reaction was used as a template. The annealing temperature for RT-PCR was 55-60°C for all primer pairs and the number of PCR cycles was EF1α: 23 cycles; TUB4: 26 cycles; CLE2, CLE16, CLE17, CLE27: 30 cycles; CLE1, CLE3, CLE4, CLE6, CLE7, CLE19: 35 cycles; CLE18: 40 cycles. Primer sequences are listed in Table S3.

Supplemental Material

Supplemental Materials and Methods

Table S1. CLE over-expression meristem phenotypes.

Table S2. CLE insertion alleles.

Table S3. Oligonucleotides used in this study.

Figure S1. CLE14 and CLE20 promoter activity in vegetative and reproductive tissues.

Figure S2. Examples of CLE promoter activity in primary root vasculature.

Figure S3. CLE promoter activity during lateral root development.

Figure S4. CLE promoter activity in lateral root tips of 11-day-old-seedlings.

Figure S5. CLE promoter activity in the shoot apex region.

Figure S6. CLE16, CLE17 and CLE27 promoter activity in the shoot apex.

Figure S7. CLE8, CLE12 and CLE22 over-expression phenotypes.

Acknowledgements

We thank Niki Kubat, Vicky Chen and Liz Fong for assistance handling plants and generating constructs; the RIKEN, SAIL, GABI-KAT and SALK collections for supplying indexed insertion mutant lines; and Sheila McCormick and Barbara Baker for helpful discussions.

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Sieburth LE, Meyerowitz EM (1997) Molecular dissection of the AGAMOUS control region shows that cis elements for spatial regulation are located intragenically. Plant Cell 9: 355-365


Figure Legends

**Figure 1.** *CLE* promoter activity in 11-day-old-seedlings. (A) *CLE12*. (B) *CLE18*. (C) *CLE22*. (D) *CLE25*. (E) *CLE26*. (F) *CLE9*. (G) *CLE16*. (H) *CLE17*. (I) *CLE27*. (J) *CLE10*. (K) *CLE5*. (L) *CLE6*. (M) *CLE21*. (N) *CLE3*. (O) *CLE11*. (P) *CLE4*. (Q) *CLE1*. (R) *CLE2*. (S) *CLE7*. (T) *CLE13*. Arrows indicate GUS activity in the vasculature of young leaves in (A-D), leaf margins in (H, J) and hydathodes in (K, L). Arrowheads in (J-P) indicate GUS activity in the shoot apex.

**Figure 2.** *CLE* promoter activity in 11-day-old-seedlings. (A) *CLE25*, (B) *CLE26* and (C) *CLE12* in leaf vascular tissue. (D) *CLE16* in trichomes and leaf blade. (E) *CLE17* in trichomes and leaf margin. (F) *CLE5*, (G) *CLE6*, and (H) *CLE10* in hydathodes. (I) *CLE9* in stomata. (J) Magnified view of the region boxed in (I). (K) *CLE3*, (L) *CLE11* and (M) *CLE21* in stipules. (N) *CLE10*, (O) *CLE5* and (P) *CLE6* in the leaf base. (Q) *CLE4* in the pith. In (A, B), first to fourth rosette leaves are arranged in order from the left. Arrows indicate GUS activity in the vasculature of the basal and apical regions of young leaves in (A) and (B), respectively, and in the midvein in (C), trichomes in (D, E), hydathodes in (F-H) and stipules in (K-M). Black and white arrowheads in (J) indicate a meristemoid cell and a young guard cell, respectively.

**Figure 3.** *CLE* promoter activity in the primary root tips of 11-day-old-seedlings. (A) *CLE1* in the root cap and vascular parenchyma. (B) *CLE11*, (C) *CLE13*, and (D) *CLE18* in the root cap and apical meristem. (E) *CLE16* in the root cap and throughout the elongation zone. (F) *CLE17* in the root apical meristem. (G) *CLE22* in the newly differentiating vascular tissue. (H) *CLE25* and (I) *CLE26* in the vascular parenchyma.

**Figure 4.** *CLE* promoter activity in primary root cell files. (A, B) *CLE12*, (C, D) *CLE22*, (E, F) *CLE25* and (G, H) *CLE26* in the stele. (I, J) *CLE4* and (K, L) *CLE18* in the pericycle. (M, N) *CLE7* and (O) *CLE1* in the pericycle and endodermis. (P) *CLE1* in the stele and endodermis. (Q) *CLE16* in the epidermis. (R) *CLE16* in the stele. (S) *CLE17* in the epidermis. (T) *CLE17* throughout the root. (U, V) *CLE3* and (W, X) *CLE5* in patches around the vascular bundle. Images in (A, C, E, G, I, K, M, O, Q, S, U, W) were taken from regions of 11-day-old primary or
lateral roots where the root hair cells are not fully mature. Images in (B, D, F, H, J, L, N, P, R, T, V, X) were taken from the primary root maturation zone with fully differentiated root hair cells.

**Figure 5.** *CLE* promoter activity in inflorescences and cauline leaves.

(A) *CLE16* in the epidermis. (B) *CLE12*, (C) *CLE13*, (D) *CLE22*, (E) *CLE25* and (F) *CLE26* in the vasculature. The *CLE22* stem is over-stained to visualize GUS activity in the cauline leaf. (G) *CLE4*, (H) *CLE5*, (I) *CLE6*, (J) *CLE10*, (K) *CLE17*, (L) *CLE21* and (M) *CLE27* in the inflorescence branching points. (N) *CLE9* in the stomata. (O) *CLE18* in the cauline leaf vasculature and marginal cells. (P) *CLE16* in the epidermis. (Q) *CLE22* and (R) *CLE26* in the vasculature. (S) *CLE27* throughout the pedicels.

**Figure 6.** *CLE* promoter activity in flowers.

(A) *CLE16*, (B) *CLE18*, (C) *CLE26* and (D) *CLE22* in sepal vasculature. (E) *CLE17* in sepal and petal margins. (F) *CLE5* and (G) *CLE6* at the base of the flower. (H) *CLE16*, (I) *CLE18*, (J) *CLE4*, and (K) *CLE26* in the stamen filaments. (L) *CLE1*, (M) *CLE25* and (N) *CLE7* in the anthers. (O) *CLE12*, (P, Q) *CLE11* and (R, S) *CLE13* in the pollen grains.

**Figure 7.** *CLE* promoter activity in the gynoecium.

(A) *CLE10* in the stigma, style and central tissues. (B) *CLE5*, (C) *CLE1* and (D) *CLE11* in the style. (E) *CLE17* at the stigma/style boundary. (F) *CLE21* in the valve margins. (G) *CLE16* in the valves. (H) *CLE22*, (I) *CLE25* and (J) *CLE26* in the vasculature. Inset in (H) shows *CLE22* in the basal vasculature and abscission zone. (K) *CLE25* in the funiculi and ovules. (L, M) *CLE27* in the ovules and abscission zone, respectively. (N) *CLE17* in the siliqua valve margins. (O) *CLE4* in the siliqua receptacle.

**Figure 8.** *CLE16*, *CLE17* and *CLE27* over-expression phenotypes. (A1-E1) Longitudinal section of 10-day-old wild-type, *pCLV3:GUS*, *pCLE16:GUS*, *pCLE17:GUS* and *pCLE27:GUS* plant shoot apices, respectively. (A2-A5) 13-day-old *Ler* seedlings. (B2-B5) 13-day-old *p35S:CLV3* seedlings. (C2-C5) 13-day-old *p35S:CLE16* seedlings. (D2-D5) 13-day-old *p35S:CLE17* seedlings. (E2-E5) 13-day-old *p35S:CLE27* seedlings. (F) Comparison of CLE peptide sequences. Alignment was performed using MUSCLE (Edgar, 2004). Each color represents a different
amino acid residue. (G-I) RT-PCR analysis of CLE expression in transgenic plants. EFlα is used as a control. (A3-E3) are the magnified views of shoot apex in (A2-E2). Nomarski images of the roots in (A5-E5) show root hair differentiation (arrowheads) close to the root tip. Bars: A1-E1 and A5-E5 100 µm; A2-E2 2.5 mm; A3-E3 1 mm; A4-E4 5 mm.

**Figure 9.** Characterization of CLE insertion alleles. Location of the insertion allele relative to each CLE coding region (grey box), and CLE mRNA transcript levels in two individual wild-type (WT) and homozygous cle mutant plants. TUBULIN4 (TUB4) is used as a control.

**Figure 10.** Summary of A-type CLE promoter activity in Arabidopsis. Shown is a list of the CLE genes expressed in the various tissues of a mature Arabidopsis plant.
Table 1. Summary of pCLE:GUS activity during vegetative development.

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<th>Leaf blade</th>
<th>Other(^b)</th>
<th>Tip(^c)</th>
<th>Vasculature</th>
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\(^a\) Shoot apex includes shoot apical meristem and rosette leaf primordia
\(^b\) Other includes pith, stipules, stomata, hydathodes, leaf margins, trichomes and the leaf base
\(^c\) Root tip includes root cap, root apical meristem and cell division zone
\(^d\) Other includes root hair cells and lateral root branch points
\(^e\) Eight of 15 pCLE10:GUS lines showed root tip expression
Table 2. Summary of pCLE:GUS activity during reproductive development.

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<th>Branching points</th>
<th>Cauline leaves</th>
<th>Sepals/ petals</th>
<th>Stamens</th>
<th>Gynoecium</th>
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¹ Includes receptacle and abscission zone
² Includes valves, replum and septum
³ Siliques only
⁴ Sepals only
⁵ Vasculature
Footnote:

The author responsible for distribution of materials integral to the findings presented in this article in accordance with the Journal policy described in the Instructions for Authors (http://www.plantphysiol.org) is: Jennifer C. Fletcher (jfletcher@berkeley.edu).
Figure 1. CLE promoter activity in 11-day-old-seedlings. (A) CLE12. (B) CLE18. (C) CLE22. (D) CLE25. (E) CLE26. (F) CLE9. (G) CLE16. (H) CLE17. (I) CLE27. (J) CLE10. (K) CLE5. (L) CLE6. (M) CLE21. (N) CLE3. (O) CLE11. (P) CLE4. (Q) CLE1. (R) CLE2. (S) CLE7. (T) CLE13. Arrows indicate GUS activity in the vasculature of young leaves in (A-D), leaf margins in (H, J) and hydathodes in (R).
Figure 2. CLE promoter activity in 11-day-old-seedlings. (A) CLE25, (B) CLE26 and (C) CLE12 in leaf vascular tissue. (D) CLE16 in trichomes and leaf blade. (E) CLE17 in trichomes and leaf margin. (F) CLE5, (G) CLE6, and (H) CLE10 in hydathodes. (I) CLE9 in stomata. (J) Magnified view of the region boxed in (I). (K) CLE3, (L) CLE11 and (M) CLE21 in stipules. (N) CLE10, (O) CLE5 and (P) CLE6 in the leaf base. (Q) CLE4 in the pith. In (A, B), first to fourth rosette leaves are arranged in order from the left. Arrows indicate GUS activity in the vasculature of the basal and apical regions of young leaves in (A) and (B), respectively, and in the midvein in (C), trichomes in (D, E), hydathodes in (F-H) and stipules in (K-M). Black and white arrowheads in (J) indicate a meristematic cell and a young guard cell, respectively.
Figure 3. CLE promoter activity in the primary root tips of 11-day-old-seedlings. (A) CLE1 in the root cap and vascular parenchyma. (B) CLE11, (C) CLE13, and (D) CLE18 in the root cap and apical meristem. (E) CLE16 in the root cap and throughout the elongation zone. (F) CLE17 in the root apical meristem. (G) CLE22 in the newly differentiating vascular tissue. (H) CLE25 and (I) CLE26 in the vascular parenchyma.
Figure 4. CLE promoter activity in primary root cell files. (A, B) CLE12, (C, D) CLE22, (E, F) CLE25 and (G, H) CLE26 in the stele. (I, J) CLE4 and (K, L) CLE18 in the pericycle. (M, N) CLE7 and (O) CLE17 in the pericycle and endodermis. (P) CLE1 in the stele and endodermis. (Q) CLE16 in the epidermis. (R) CLE16 in the stele. (S) CLE17 in the epidermis. (T) CLE17 throughout the root. (U) CLE16. Images in (A, C, E, G, I, K, M, O, Q, S, U, X) were taken from the primary root maturation zone with fully differentiated root hair cells where the root hair cells are not fully mature. Images in (B, D, F, H, J, L, N, P, R, T, V, X) were taken from the primary root maturation zone with fully differentiated root hair cells.
Figure 5. CLE promoter activity in inflorescences and cauline leaves. (A) CLE16 in the epidermis. (B) CLE12, (C) CLE13, (D) CLE22, (E) CLE25 and (F) CLE26 in the vasculature. The CLE22 stem is over-stained to visualize GUS activity in the cauline leaf. (G) CLE4, (H) CLE5, (I) CLE6, (J) CLE10, (K) CLE11 and (L) CLE17 in the inflorescence branching points. (N) CLE9 in the stoma. (O) CLE20 in the cauline leaf vasculature and marginal cells. (P) CLE16 in the epidermis. (Q) CLE22 and (R) CLE26 in the vasculature. (S) CLE27 throughout the pedicels.
Figure 6. *CLE* promoter activity in flowers.
(A) CLE16, (B) CLE18, (C) CLE26 and (D) CLE22 in sepal vasculature. (E) CLE17 in sepal and petal margins. (F) CLE5 and (G) CLE6 in the anthers. (J) CLE4, and (K) CLE26 in the stamen filaments. (L) CLE1, (M) CLE25 and (N) CLE7 in the anthers. (O) CLE12, (P, Q) CLE11 and (R, S) CLE13 in the pollen grains.
Figure 7. CLE promoter activity in the gynoecium.
(A) CLE10 in the stigma, style and central tissues. (B) CLE5, (C) CLE1 and (D) CLE11 in the style. (E) CLE17 at the stigma/style boundary. (F) CLE21 in the valve margins. (G) CLE16 in the valves. (H) CLE22, (I) CLE25 and (J) CLE26 in the vasculature. Inset in (H) shows CLE22 in the basal vasculature and abscission zone. (K) CLE25 in the funiculi and ovules. (L, M) CLE27 in the ovules and abscission zone, respectively. (N) CLE17 in the silique valve margins. (O) CLE4 in the siliqua receptacle.
Figure 8. **CLE16, CLE17 and CLE27 over-expression phenotypes.** (A₁-E₁) Longitudinal section of 10-day-old wild-type, pCLV3:GUS, pCLE16:GUS, pCLE17:GUS and pCLE27:GUS plant shoot apices, respectively. (A₂-A₅) 13-day-old Ler seedlings. (B₂-B₅) 13-day-old p35S:CLV3 seedlings. (C₂-C₅) 13-day-old p35S:CLE16 seedlings. (D₂-D₅) 13-day-old p35S:CLE17 seedlings. (E₂-E₅) 13-day-old p35S:CLE27 seedlings. (F) Comparison of CLE peptide sequences. Alignment was performed using MUSCLE (Edgar, 2004). Each color represents a different amino acid residue. (G-I) RT-PCR analysis of CLE expression in transgenic plants. EF1α is used as a control. (A₅-E₅) Nomarski images of the roots in (A₅-E₅) show root hair differentiation (arrowheads) close to the root tip. Bars: A₁-E₁ and A₅-E₅ 100 μm; A₂-E₂ 2.5 mm; A₃-E₃ 1 mm; A₄-E₄ 5 mm.
Figure 9. Characterization of CLE insertion alleles. Location of insertion allele relative to each CLE coding region (grey box), and CLE mRNA transcript levels in two individual wild-type (WT) and homozygous cle mutant plants. TUBULIN4 (TUB4) is used as a control.
Figure 10. Summary of A-type CLE promoter activity in Arabidopsis. Shown is a list of the CLE genes expressed in the various tissues of a mature Arabidopsis plant.