These uses have accounted for an almost constant 80% of SF_6 sales (*8*); the increasing use by the electronics industry has been largely compensated by the decreasing use in magnesium smelting (*8*). We speculate that SF_5CF_3 originates as a breakdown product of SF_6 in high-voltage equipment. Such systems likely contain fluoropolymers, which provide a source of CF_3 groups that may be attacked by SF_5 radicals formed by high-voltage discharges.

Despite the large GWP of SF_5CF_3, the amount currently in the atmosphere is so small that the contribution of this molecule to overall radiative forcing is very minor ($<10^{-4}$ W m^{-2}). The present-day burden of SF_5CF_3 is $\sim 3.9 \times 10^3$ metric tons, with emissions rising by ~270 metric tons $year^{-1}$ (from the trend shown in Fig. 1 and assuming negligible atmospheric loss). This emission is equivalent in GWP terms (for a 100-year time horizon) to ~1% of the annual UK emission of CO_2 (*16*). SF_5CF_3, however, has a much longer lifetime than that of CO_2 (atmospheric CO_2 equilibration time of 50 to 200 years), and its rate of growth may be accelerating. There is, therefore, potential for an almost irreversible accumulation of this gas in the atmosphere. SF_5CF_3, unlike CO_2, has no natural sources and therefore has the potential for substantial emission control after its sources have been identified. If it is indeed formed in high-voltage equipment, then there may already be substantial amounts "stored" in electrical installations worldwide. We think that it is important to continue monitoring the atmospheric concentration of SF_5CF_3 in order to determine and control its sources and to guard against an undesirable accumulation of this strong greenhouse gas in the atmosphere.

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2. Air samples (200 to 800 ml) were cryofocused at −186°C with liquid argon, desorbed at 90°C, and separated on a temperature-controlled alumina PLOT capillary column. The Micromass "AutoSpec" configuration comprised an electron ionization source, a magnetic field sector, pre– and post–electric field focusing sectors, and a photomultiplier detector.
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5. First, pure SF_5CF_3 (Flura Corporation, Newport, TN) was successively diluted in large stirred aluminum drums to 1 part per billion by volume, followed by injection of microliter amounts into the GC-MS using a small sample loop filled to varying pressures to span the range of concentrations that we measured in ambient air. These measurements were referenced to a synthetic calibrated 10-ppb SF_6-in-nitrogen standard ["Heidelberg" scale (*17*)] that was prepared and analyzed in the same way. Second, the relative abundances of two ions common to both SF_6 and SF_5CF_3 (masses of 89 and 127) were determined from full-scan mass spectra of diluted pure gases and compared with the relative peak areas in the NOAA standard. The second method is independent of errors arising from dilution effects, but it assumes invariant ionization efficiency. The first method yielded a concentration in the NOAA standard (collected at Niwot Ridge, CO, in spring 1994) of 0.072 ± 0.009 ppt, whereas the second method gave a concentration of 0.116 ± 0.018 ppt.
6. Air was extracted by drilling a bore hole to successive depths, each time letting down an inflatable sleeve to seal off the hole near the bottom, and then pumping air out from beneath the seal (*18*) into fused silica-lined stainless steel sample flasks (SilcoCan canisters, Restek Corporation, Bellefonte, PA). Integrity of the pumped air was assured by continuous in situ monitoring of CO_2.
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CLAVATA3, a Multimeric Ligand for the CLAVATA1 Receptor-Kinase

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The CLAVATA1 (CLV1) and CLAVATA3 (CLV3) proteins form a potential receptor and ligand pair that regulates the balance between cell proliferation and differentiation at the shoot meristem of *Arabidopsis*. CLV1 encodes a receptor-kinase, and CLV3 encodes a predicted small, secreted polypeptide. We demonstrate that the CLV3 and CLV1 proteins coimmunoprecipitate in vivo, that yeast cells expressing CLV1 and CLV2 bind to CLV3 from plant extracts, and that binding requires CLV1 kinase activity. CLV3 only associates with the presumed active CLV1 protein complex in vivo. More than 75% of CLV3 in cauliflower extracts is bound with CLV1, consistent with hypotheses of ligand sequestration. Soluble CLV3 was found in an approximately 25-kilodalton multimeric complex.

Although several biologically active putative ligands and putative receptors have been identified in plants (*1*), no proteinaceous ligand and cognate receptor pair have been identified at present. The CLV1 and CLV3 gene products are likely candidates for such a pair. The *CLV1* and *CLV3* genes function in the same pathway, and *clv1* and *clv3* mutations exhibit dominant interactions (*2*). *CLV1* encodes a predicted receptor-like protein kinase with an extracellular domain composed of 21 tandem leucine-rich repeats (LRRs) and an intracellular protein kinase domain that has been shown to act as a serine kinase (*3–5*). *CLV3* encodes a predicted small, secreted protein (*6*).

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Moreover, CLV3 is required for activation of the CLV1 receptor in vivo. CLV1 is present in two distinct protein complexes in vivo (*7*). The smaller 185-kD CLV1 complex is primarily a disulfide-linked multimer that may be a heterodimer between the CLV1 receptor kinase and the receptor-like protein CLV2, which is predicted to contain extracellular LRRs but only a short cytoplasmic domain (*8*). In the absence of CLV2, the CLV1 protein does not accumulate (*8*). The larger 450-kD CLV1 complex contains the 185-kD multimer plus kinase-associated protein phosphatase (KAPP) and a Rho guanosine triphosphatase–related protein (*7*). KAPP contains a forkhead-associated homology domain (*9–11*) that may be required for binding to one or more phosphoserine residues on the CLV1 kinase domain and a type 2C protein phosphatase domain that is capable of dephosphorylating CLV1 in vitro (*4*). Misexpression studies have revealed that KAPP is a negative regulator of CLV1 activity (*4, 5*). The Rho-related protein that is a component of the 450-kD complex may relay signal to downstream targets (*7*). In both *clv1* and *clv3* mutant plants, CLV1 is found primarily or exclusively, depending on allele strength, in the 185-kD complex, indicating that the 185-kD CLV1 complex is the inactive form and that CLV3 is required for CLV1 activation (*7*).

CLV1 and *CLV3* are transcribed in largely nonoverlapping cell layers of the shoot meristem. *CLV3* is expressed primarily in the epidermal L1 layer and the subepidermal L2 layer (tunica), whereas *CLV1* is expressed primarily in the underlying L3 layer (corpus) (*3, 6*). In addition, *CLV3* is capable of acting noncell autonomously, based on clonal studies (*6*). Taken together, however, these data still leave open several possible roles for CLV3 in CLV1 signaling. Roles for CLV3 in producing the ligand, in associating with CLV1 to assist in ligand-binding, or in acting as the ligand are all consistent with current data. These models would predict that CLV3 does not associate with CLV1, that it associates with both the inactive and active forms of CLV1, or that it associates only with the active form of CLV1, respectively.

To test these predictions, we generated polyclonal antibodies to *Escherichia coli*–expressed CLV3 fusion protein (*12*). The resulting immune, but not preimmune, antiserum cross-reacted with a single ~6-kD polypeptide from both wild-type *Arabidopsis* and cauliflower extracts (Fig. 1A) [Web Fig. 1 (*13*)]. To test if this cross-reaction was indeed to CLV3, extract from plants homozygous for the *clv3-2* null allele was also tested. In this case, no cross-reactivity was observed, indicating that the antiserum was specific for CLV3 (Fig. 1A).

To determine if CLV3 associated with CLV1 and, if so, to which complexes CLV3 might be bound, cauliflower extract was separated by column chromatography, and fractions containing the 185-kD and 450-kD CLV1 complexes were pooled separately. Each pool was precipitated with antibody to CLV3 (anti-CLV3) immune and preimmune antisera, and the precipitated proteins were used in Western blots with both anti-CLV1 and anti-KAPP (Fig. 1, B and C). In each case, CLV1 and KAPP were found only among proteins precipitated with the anti-CLV3 immune antiserum from the fractions containing the 450-kD complex precipitated with the anti-CLV3 immune antiserum. This indicates that CLV3 is only associated with the 450-kD CLV1 complex. This was confirmed by reversing the immunoprecipitation. In this case, CLV3 was only detected among proteins immunoprecipitated from the 450-kD complex with anti-CLV1 immune antisera (Fig. 1D).

To examine the range of complexes CLV3 might be associated with, and to examine the CLV1/CLV3 association in *Arabidopsis*, fractionations were performed on extracts from wild-type and mutant *Arabidopsis* plants. Fractions were used in dot blot analyses to test for the presence of CLV3. In wild-type *Arabidopsis*, CLV3 was observed in the same fractions that contained the CLV1 450-kD complex (Fig. 2A; 12 to 13 ml elution volume), as well as a very small molecular mass range (see below). We occasionally observed both CLV1 and CLV3 elution near the void volume (Fig. 2A; 7 to 8 ml elution volume). It is unclear if the inconsistent presence of CLV1 and CLV3 proteins in this larger size range represents a less stable complex or simply protein aggregation. No cross-reactivity was observed in extracts from *clv3-2* plants, again indicating that the cross-reactivity from the anti-CLV3 antiserum is specific for CLV3.

To test if either CLV3 complex is membrane-bound, extracts were generated in the absence of detergent. In this case, only the lower molecular mass CLV3 complex was detected (Fig. 2B). This indicates that CLV3 in the 450-kD complex is membrane-associated, whereas the lower molecular mass CLV3 complex is soluble.

The *clv1-4* and *clv1-8* alleles contain missense mutations in their predicted extracellular domains (*3*). We previously detected clv1-4 and clv1-8 monomers in extracts from homozygous mutant plants (*7*), suggesting that the lesions may primarily interfere with stable multimerization and/or disulfide linkage. Extracts from these plants exhibit a reduction in the proportion of CLV1 in the 450-kD complex, although this complex is still formed at detectable levels (*7*). To test whether the 450-kD complex present in these plants contained CLV3, we examined CLV3 elution in extracts from *clv1-4* and *clv1-8* plants. In both cases, CLV3 associated with the 450-kD CLV1 complex, supporting the idea that these lesions in the extracellular domain do not prevent ligand binding (Fig. 2B).

The *clv1-10* allele contains two missense mutations in the kinase domain (*14*). When the clv1-10 kinase domain was expressed in *E. coli*, it exhibited no autophosphorylation activity (*15*). We previously demonstrated that in extracts from *clv1-10* plants, the clv1-10 protein was found exclusively in the inactive 185-kD complex. When CLV3 elution was examined in extracts from *clv1-10* plants, it was only detected in the very low

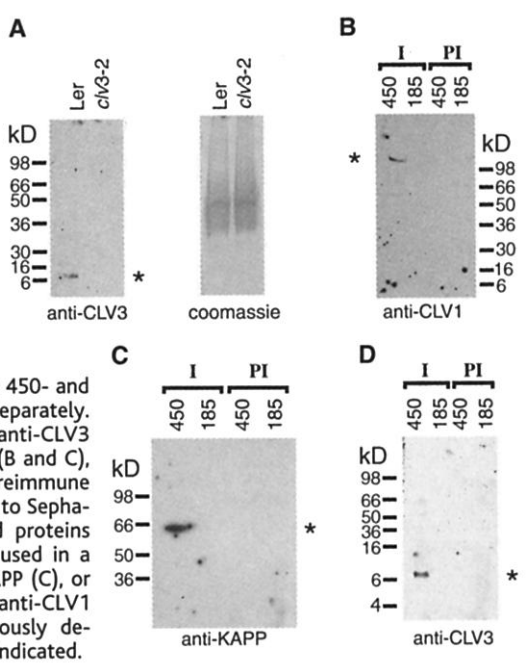

Fig. 1. CLV3 associates with CLV1 in vivo. (**A**) Total protein extracts from wild-type (Ler) and *clv3-2* mutant (*clv3-2*) *Arabidopsis* inflorescences were isolated as previously described (*7*), separated by SDS-PAGE, and subjected to Western blot analysis using anti-CLV3 immune antiserum (left panel) (*12*). Right panel shows the same extracts separated and stained with Coomassie to confirm equal amounts of loaded protein. (**B** through **D**) One hundred fifty milliliters of cauliflower extract was concentrated with an ultrafiltration cell (Amicon) and separated by gel chromatography. Fractions containing the 450- and 185-kD CLV1 complexes were pooled separately. Each pool was immunoprecipitated with anti-CLV3 immune (I) and preimmune (PI) antisera (B and C), or with the anti-CLV1 immune and preimmune anitsera (D), that had been crossed-linked to Sepharose beads as described (*7*). The eluted proteins were then separated by SDS-PAGE and used in a Western blot with anti-CLV1 (B), anti-KAPP (C), or anti-CLV3 (D) antisera. The production of anti-CLV1 and anti-KAPP antisera has been previously described (*7*). Molecular mass markers are indicated.

size range, indicating that CLV3 elution in the 450-kD size range was entirely dependent on CLV1 (Fig. 2B). This also indicated a lack of stable interaction between CLV3 and the clv1-10 protein, despite the fact that clv1-10 protein accumulates to significant levels, contains no lesions in the extracellular domain, and appears to be assembled into the correct disulfide-linked multimer (*7*). This lack of interaction between CLV3 and clv1-10 raises the possibility that intracellular kinase activity is required to stabilize the association between CLV1 and CLV3 (see below).

Arabidopsis extracts were also fractionated over G-50 Sephadex resin to provide better resolution in the lower molecular mass range. When extracts from wild-type *Arabidopsis* were tested, the majority of CLV3 eluted in the void volume (7 to 8 ml elution volume), as would be expected for CLV3 associated with the 450-kD complex (Fig. 3A). CLV3 also eluted in fractions corresponding to 8.5 to 9.5 ml elution volumes, which would give the low molecular mass CLV3 a predicted size of ~25 kD when compared to standards. However, we could not rule out the possibility that CLV3 elution in fractions 8.5 to 9.5 adjacent to the void volume was not the result of incomplete separation of the 450-kD CLV1 complex. To make this distinction, we examined elution of CLV3 over the G-50 column, using extracts from *clv1-10* plants. In these extracts, CLV3 was not associated with clv1-10 protein, so that elution of CLV3 should represent protein that has not associated with the receptor. In this case, we observed CLV3 primarily in fractions 8.5 to 9.5, suggesting that CLV3 was part of a ~25-kD multimeric complex in vivo (Fig. 3B). To determine if the presence of detergent in the extraction buffer alters CLV3 mass, the low molecular mass CLV3 complex was isolated from extracts lacking detergent and tested over the G-50 column. Again, CLV3 eluted in fractions representing masses significantly larger than the CLV3 monomer (Fig. 3C). The CLV3 mature protein is predicted to contain no cysteines (*6*) and, hence, could not be a member of the cysteine-knot class of proteinaceous ligands (*16*).

If the CLV3 multimer is a heteromultimer, it would imply that CLV3 activation of CLV1 would occur where concentrations of CLV3 and its partner protein(s) are the highest, not just where CLV3 is produced. Thus, understanding where CLV1 is activated within the shoot meristem will require understanding the expression of both CLV3 and its partner(s). In addition, the binding affinities of CLV3 for CLV1 or CLV1/CLV2 cannot be properly tested until the partner(s) are identified. However, we could test directly whether the CLV3 multimer recognizes the CLV1/CLV2 extracellular domains on the plasma membrane. We expressed CLV1 and CLV2 in yeast cells (*17*), which made detectable amounts of CLV1 (Fig. 4A). Untransformed and CLV1/CLV2-expressing yeast cells were incubated with detergent-free cauliflower extracts, to ensure that only the soluble CLV3 multimer was added to the cells. After pelleting the cells, extracts were tested in Western blots to detect the presence of CLV1 and CLV3. Although CLV3 protein did not bind to untransformed yeast cells, it did bind to yeast cells expressing CLV1/CLV2 (Fig. 4A). The yeast extracts were analyzed with an enzyme-linked immunosorbent assay (ELISA) to quantitate the amount of CLV3 in extracts from the CLV1/CLV2-expressing yeast cells and the control cells (Table 1). The ability of the CLV3 multimer to bind only to intact CLV1/CLV2-expressing yeast cells indicates that this complex recognizes CLV1/CLV2 at the plasma membrane surface.

Because CLV3 did not bind the kinase-inactive clv1-10 protein in vivo, we tested

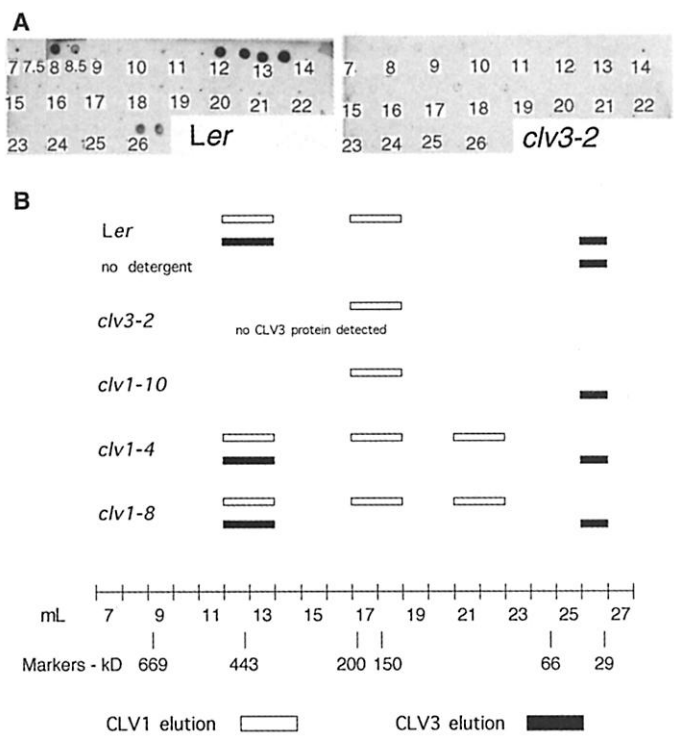

Fig. 2. Fractionation of CLV3 in wild-type and mutant *Arabidopsis* extracts. (**A**) Extracts from wild-type (left) and *clv3-2* mutant (right) *Arabidopsis* plants were separated by gel chromatography as described (*7*) and 0.5-ml fractions were collected. The presence of CLV3 in individual fractions was assayed by dot blot analysis (*7*). Elution of CLV3 near the void volume (7 to 8 ml) in wild-type plants was inconsistently observed. (**B**) The elution of CLV1 and CLV3 in extracts from wild-type and mutant *Arabidopsis* plants was assayed by dot blots. The elution volume is shown at the bottom. The fractions consistently containing CLV1 over a minimum of four repetitions are indicated with open boxes and the fractions consistently containing CLV3 are indicated with filled boxes. The elution of molecular mass size standards is indicated at the bottom. For one extraction ("no detergent"), no Triton was added to the extraction buffer.

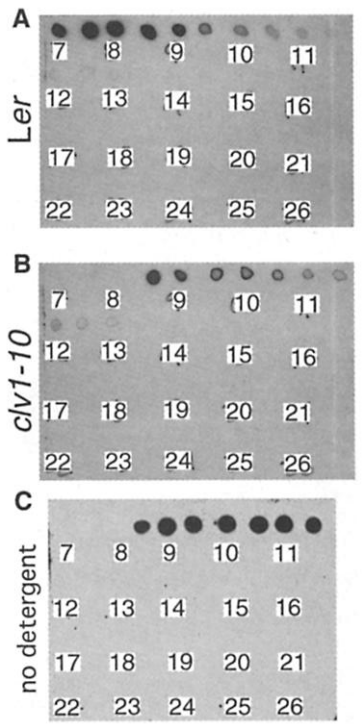

Fig. 3. CLV3 is present as a multimer. Extracts from wild-type (**A**) and *clv1-10* mutant (**B**) *Arabidopsis* plants were separated by gel chromatography over a G-50 Sephadex column. The G-50 column was generated by loading an HR 10/30 column (Pharmacia, Picataway, New Jersey) with Sephadex fine G-50 resin (Sigma, St. Louis, Missouri). Fast protein liquid chromatography over the G-50 column was carried out identically as that over the superose 6 column (*7*). Extracts from wild-type plants in which no Triton was added to the extraction buffer were also assayed (**C**). The presence of CLV3 in the fractions was assayed by dot blots. The elution of molecular mass markers (Sigma) was as follows: 2000 kD dextran (i.e., void volume) at 7.5 ml; 29 kD carbonic anhydrase at 8.8 ml; 12.4 kD cytochrome c at 11.8 ml; and 6.5 kD aprotinin at 14.9 ml.

whether kinase activity was required for CLV3 binding in the yeast system. Yeast were transformed with CLV1 in which the Lys^{720} residue critical for kinase activity was substituted with an Asp (*5*). After incubation of yeast expressing wild-type and kinase-inactive CLV1 with plant extracts, coimmunoprecipitations were performed between CLV1 and CLV3. Only wild-type CLV1 bound to CLV3, indicating that kinase activity was required for ligand binding (Fig. 4B). We know of no other receptor for which ligand binding requires cytoplasmic kinase activity.

The ability to assess CLV3-CLV1 association in vivo afforded an opportunity to measure the proportion of free and bound CLV3. Fractions containing the 450-kD and ~25-kD CLV3 complex were pooled separately, and the amount of CLV3 present in each complex was measured by ELISA analysis (Table 2). Seventy-six percent of CLV3 was bound with CLV1.

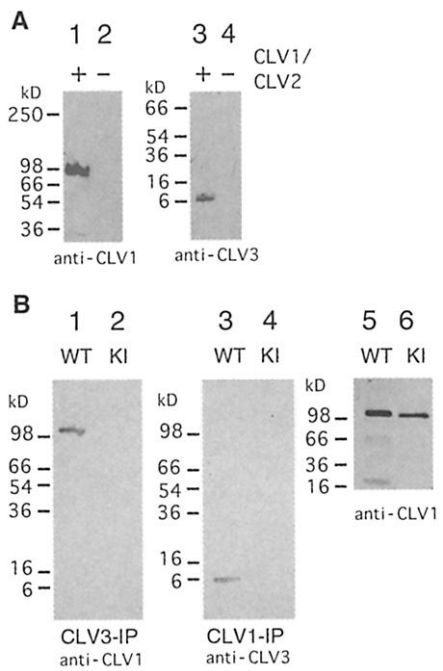

Fig. 4. CLV3 bound to yeast expressing kinase-active CLV1/CLV2. (**A**) Yeast expressing CLV1 and CLV2 (lanes 1 and 3) and untransformed yeast (lanes 2 and 4) were incubated with detergent-free cauliflower extract (*17*). Yeast cells were isolated, and the resulting protein extracts were used in Western blots to detect CLV1 (lanes 1 and 2) and CLV3 protein (lanes 3 and 4). (**B**) Yeast expressing either CLV1 and CLV2 (lanes 1, 3, and 5) or kinase-inactive CLV1 and CLV2 (lanes 2, 4, and 6) were incubated with cauliflower extract. Yeast cells were isolated, and the resulting protein extracts were immunoprecipitated with anti-CLV3 (lanes 1 and 2) or anti-CLV1 (lanes 3 and 4) immune anitsera. The eluted proteins were then separated by SDS-PAGE and used in a Western blot with anti-CLV1 (lanes 1 and 2) or anti-CLV3 (lanes 3 and 4) antisera. Expression of the wild-type and kinase-inactive proteins was assessed by Western blots with anti-CLV1 antisera (lanes 5 and 6). Molecular mass markers are indicated.

The high proportion of receptor-associated CLV3 raises the possibility that this acts as a mechanism to limit the range of diffusion of CLV3 within the meristem. For many ligands, including Hedgehog, Spätzle, Trunk, and Lin-3, sequestration by the receptor is thought to limit the range of ligand signal (*18–20*). Despite considerable genetic evidence supporting this idea, there have been no quantitative measurements in vivo of the proportion of bound ligand in these or many other signaling systems. Thus, CLV3 may represent an opportunity to address this issue, both on the basis of binding-affinity once the CLV3 partner protein is known and also by in vivo measurements of free and bound concentrations of CLV3.

Can we conclude that the CLV3 multimer acts as the ligand for CLV1? Three lines of evidence support the hypothesis that CLV3 is rate-limiting in the activation of CLV1: (i) *clv3* mutations become semidominant in *clv1/CLV1* heterozygote plants (*2*); (ii) *clv3-3*, which contains a transferred DNA insertion outside the coding region that reduces expression, has a reduced proportion of CLV1 in the active complex (*6, 7*); and (iii) the observation that the majority of CLV3 is bound to CLV1 (Table 2) suggests that all CLV3 that diffuses to CLV1-expressing cells becomes bound with CLV1. Previous studies also revealed that CLV1 and CLV3 function in the same pathway (*2*), that CLV1 and CLV3 are expressed in adjacent cell populations (*3, 6*), and that CLV1 activation is entirely dependent on CLV3 (*7*). In light of these previous findings, the data we present that CLV1 and CLV3 coimmunoprecipitate from the active CLV1 complex, that membrane association of CLV3 is entirely dependent on CLV1, and that CLV3 binds to CLV1/CLV2-expressing yeast cells, combine to provide strong evidence that CLV3 acts as the ligand for CLV1 as part of a multimeric complex.

Table 1. Yeast expressing CLV1/CLV2 pull down CLV3. Samples were prepared as described in Fig. 4. ELISA analysis as described (*7*) was used to detect CLV1 and CLV3. Mean OD_{450} reading is presented with standard error for eight measurements.

Signal	Untransformed yeast	CLV1/CLV2-expressing yeast
CLV1	0 ± 0.002	0.061 ± 0.006
CLV3	0.006 ± 0.002	0.057 ± 0.023

Table 2. The majority of CLV3 is receptor-bound. Fractions corresponding to the 450- and 25-kD CLV3 complexes were pooled separately, and the amount of CLV3 was measured by ELISA analysis. The mean ELISA absorbance of 12 measurements for each complex is presented, adjusted to account for the difference in volumes. Standard error is indicated.

CLV3 complex	ELISA absorbance	Percent of total
450 kD	2.95 ± 0.13	76%
~25 kD	0.95 ± 0.02	24%

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17. BY4705 yeast were transformed with CLV2 cloned in p416 GPD and CLV1 cloned in p415 GPD (*21*). In the CLV1 construct, the CLV1 signal peptide had been replaced with the preproalpha signal peptide derived from YCpG-MFalpha1-100 up to the Kex2 cleavage site (*22*) [Web fig. 2 (*13*)]. We concentrated 500 ml of 50 mg/ml cauliflower protein extracted with 50 mM Hepes (pH 7.4), 1 mM EDTA, 1× protease inhibitor cocktail (Sigma, P-9599) to 100 ml with an Amicon ultrafiltration cell 3000D MWCO and centrifuged it at 100,000*g* for 20 min. Untransformed BY4705 yeast and CLV1/CLV2-expressing BY4705 yeast were grown to $OD(A_{600})$ 1.0 and centrifuged at 5000*g* for 10 min. We resuspended 100 ml of cells in 10 ml of 100 mM tris-SO_4 (pH 9.4). The cells were agitated slowly (<200 rpm) for 15 min at 30°C and then centrifuged and gently resuspended in 10 ml of sphereroplasting buffer [100 mM tris-SO_4 (pH 9.4), 1.2 M sorbitol, 1× protease inhibitor cocktail, 50 mg/ml zymolase] and incubated for 30 min at 30°C. The cells were then collected by centrifugation for 5 min at 3000*g*. The cells were gently resuspended in 50 ml of cleared cauliflower extract and rocked gently for 2 hours at room temperature. The cells were centrifuged at

5000*g* for 10 min. The pellet was resuspended in a minimal volume of 50 mM Hepes (pH 7.4), 1 mM EDTA, 0.1% Triton, and 1× protease inhibitor cocktail; ground using a mortar and pestle on ice; and centrifuged at 5000*g* for 10 min. The supernatant was removed. Finally, 30 μl was loaded on a 4 to 20% gradient SDS-PAGE (Bio-Rad Ready Gels) and transferred to 0.2 μm of nitrocellulose (Bio-Rad) for a Western blot.

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Dependence of Stem Cell Fate in *Arabidopsis* on a Feedback Loop Regulated by *CLV3* Activity

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The fate of stem cells in plant meristems is governed by directional signaling systems that are regulated by negative feedback. In *Arabidopsis thaliana*, the *CLAVATA* (*CLV*) genes encode the essential components of a negative, stem cell–restricting pathway. We used transgenic plants overexpressing *CLV3* to show that meristem cell accumulation and fate depends directly on the level of CLV3 activity and that CLV3 signaling occurs exclusively through a CLV1/CLV2 receptor kinase complex. We also demonstrate that the *CLV* pathway acts by repressing the activity of the transcription factor *WUSCHEL*, an element of the positive, stem cell–promoting pathway.

The shoot apical meristem can initiate organs and secondary meristems throughout the life of a plant. A few cells located in the central zone of the meristem act as pluripotent stem cells: They divide slowly, thereby displacing daughter cells outwards to the periphery where they eventually become incorporated into organ primordia and differentiate (*1*). The maintenance of a functional meristem requires coordination between the loss of stem cells from the meristem through differentiation and replacement of cells through division. In *Arabidopsis*, the *CLAVATA* (*CLV1*, *CLV2*, and *CLV3*) genes play a critical role in this process, since loss-of-function mutations in *CLV1*, *CLV2*, or *CLV3* cause an accumulation of stem cells and a progressive enlargement of shoot and floral meristems (*2*–*7*). The *CLV3* gene encodes a small and potentially extracellular protein that is expressed in the stem cells of the shoot and floral meristems (*2*). The other *CLV* genes encode a leucine-rich repeat (LRR)–receptor protein kinase (CLV1) and a LRR-receptor–like protein (CLV2) that are assumed to form a membrane-bound receptor protein complex, together with other intracellular components (*5, 6, 8*). It has been proposed that the CLV3 protein is secreted from the outermost meristem cell layers and interacts with the CLV1/CLV2 receptor complex in deeper cell layers (*2, 6*) to restrict the size of the stem cell population. In *clv1*, *clv2*, or *clv3* mutants, the expression domains of *CLV1* and *CLV3* enlarge coordinately. A simple interpretation is that this coordinated expansion is controlled by a positive, stem cell promoting pathway, which in turn is negatively regulated by the stem cell restricting *CLV* pathway (*2, 3*). The stem cell promoting pathway would then also promote the expression of the *CLV* genes by causing enlargement of the cell populations in which they are expressed.

We separated these two pathways by expressing a *CLV3* cDNA from the constitutively active cauliflower mosaic virus *35S* promoter (*CaMV35S*) in transgenic *Arabidopsis* plants (*9*). In 82% of the 599 *CaMV35S*::*CLV3* transgenic plants studied, the shoot meristem ceased initiating organs after emergence of the first leaves (Fig. 1, A and B). In some plants, the meristem resumed activity and initiated misshapen leaves at random positions, including the central zone that harbors the stem cells in wild-type plants (Fig. 1, D through F). Another 16% of the lines formed inflorescence meristems and produced flowers that failed to initiate the inner whorls with stamens and carpels (Fig. 1, G and I) (*10*). The opposite phenotype is observed in *clv3* loss-of-function mutants, where stem cells accumulate in the center of shoot and floral meristems and additional organs or undifferentiated tissue are formed (Fig. 1H).

We hypothesized that the phenotypic differences between the two classes of *CaMV35S*::*CLV3* transgenic plants might be caused by differences in the expression levels of the *CaMV35S*::*CLV3* transgene. We therefore analyzed the levels of *CLV3* mRNA by in situ hybridization (*11*) and found that transgenic lines showing an early meristem arrest expressed high levels of *CLV3* mRNA, whereas lines that formed shoots with flowers showed weaker ectopic *CLV3* expression (Fig. 2, A through C). This indicates that stem cell fate in the meristem center is controlled by the level of the *CLV3* signal.

We then tested whether *CLV3* signaling requires *CLV1* and *CLV2* by introducing the *CaMV35S*::*CLV3* transgene into *clv1* or *clv2* mutants. The resulting transgenic plants expressed *CLV3* at high levels, but exhibited the typical *clv* mutant phenotype (Fig. 2D). Thus, *CLV3* signaling requires functional CLV1 and CLV2, and the phenotypes observed in the *CaMV35S*::*CLV3* transgenic plants are the result of enhanced *CLV3* signaling through the CLV1/CLV2 receptor complex.

To increase the expression level of *CLV3* under control of its own promoter, we increased the copy number of the *CLV3* gene in the genome. Plants containing four functional copies of the *CLV3* gene (*12*) exhibited no alterations in shoot and floral meristem development, suggesting that increased gene dosage does not lead to enhanced *CLV3* signaling. The observed compensation of the gene dosage could be explained if one indirect consequence of wild-type *CLV3* signaling is the down-regulation of or failure to maintain transcription from the *CLV3* promoter in the stem cells. To test whether *CLV3* expression itself is an indirect target of the *CLV3* signaling pathway, we used the *UNUSUAL FLORAL ORGANS* (*UFO*) promoter to control *CLV3* expression. The *UFO* gene is expressed outside of the normal *CLV3* expression domain in a group of cells underneath the central zone of the shoot apical meristem (*13*). A *UFO*::*CLV3* transgene was

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