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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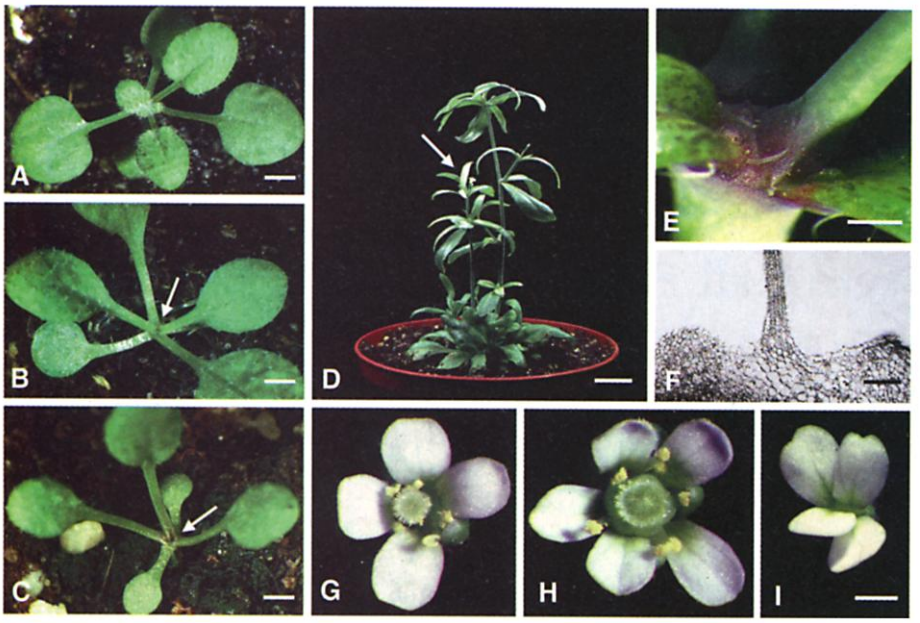

Fig. 1. Constitutive expression of *CLV3* in transgenic plants. **(A)** Three-week-old wild-type plant. **(B)** *CaMV35S::CLV3* transgenic plant. The shoot meristem arrests after initiation of the first leaves (arrow). **(C)** A *wus* mutant plant of the same age. **(D)** Two-month-old *CaMV35S::CLV3* plant. The inflorescence meristem is flattened and initiates leaves and abnormal flowers (arrow). **(E)** A terminated inflorescence meristem. **(F)** Longitudinal section through a terminated meristem as shown in (E). Cells at the apex are large and vacuolated. A single leaf originates from the center. **(G)** Wild-type flower. **(H)** A *clv3-2* mutant flower. Organ number is increased, particularly carpel number. **(I)** Flower of a *CaMV35S::CLV3* plant. Stamens and carpels are missing. Bars (A, B, and C): 2 mm; (D): 2 cm; (E, G, H, and I): 0.5 mm; (F): 50 μm.

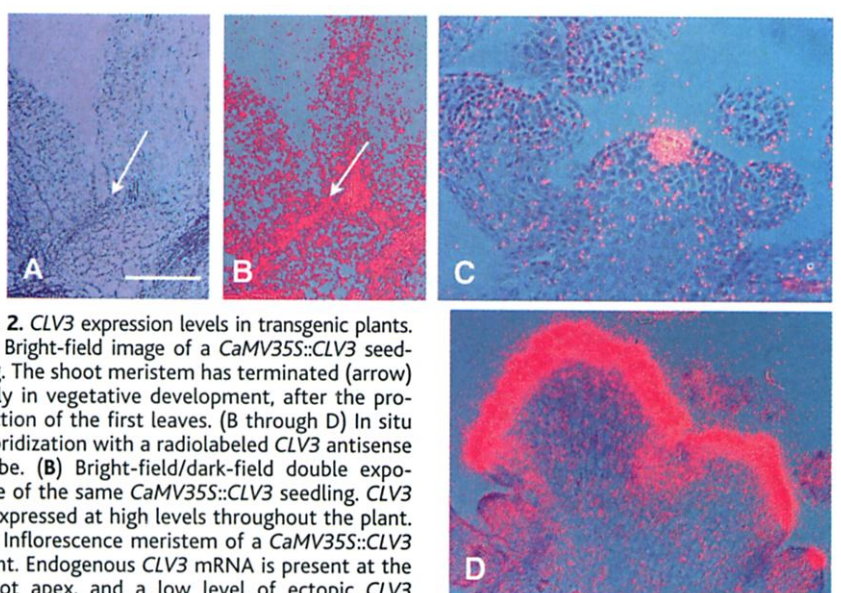

Fig. 2. *CLV3* expression levels in transgenic plants. **(A)** Bright-field image of a *CaMV35S::CLV3* seedling. The shoot meristem has terminated (arrow) early in vegetative development, after the production of the first leaves. (B through D) In situ hybridization with a radiolabeled *CLV3* antisense probe. **(B)** Bright-field/dark-field double exposure of the same *CaMV35S::CLV3* seedling. *CLV3* is expressed at high levels throughout the plant. **(C)** Inflorescence meristem of a *CaMV35S::CLV3* plant. Endogenous *CLV3* mRNA is present at the shoot apex, and a low level of ectopic *CLV3* expression is detectable throughout the rest of the plant. **(D)** Inflorescence meristem of a *CaMV35S::CLV3 clv1-4* plant. *CLV3* is expressed at high levels throughout the plant, particularly in the outermost cell layers of the enlarged meristem. Bar, 50 μm.

introduced into wild-type *Arabidopsis* plants and *clv3-8* mutants that still express *CLV3* mRNA but do not make a functional CLV3 protein product (*14*). The *UFO::CLV3* transgenic plants obtained resembled the *CaMV35S::CLV3* transgenic plants, indicating that CLV3 can act outside of its normal expression domain. Patterns of *CLV3* expression during embryogenesis were analyzed by RNA in situ hybridization (Fig. 3). In the *clv3-8* mutants, *CLV3* RNA is expressed in the central zone of the meristem (Fig. 3A). In torpedo stage *UFO::CLV3* transgenic embryos, *CLV3* RNA was detected in cells underlying and surrounding the central region of the meristem in a pattern typical for expression from the *UFO* promoter (Fig. 3B). However, endogenous *CLV3* expression in the meristem center was barely detectable (Fig. 3B), indicating that *CLV3* signaling from the *UFO* expression domain is sufficient to cause a down-regulation of *CLV3* transcription in the central zone, perhaps by repressing cell division or initiating differentiation in the cells at the apex of the meristem.

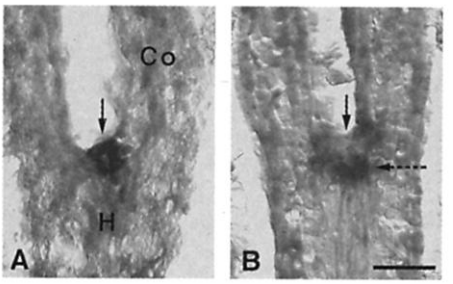

Fig. 3. Feedback regulation of *CLV3*. In situ hybridization with digoxigenin-labeled *CLV3* antisense probe. **(A)** Torpedo stage *clv3* embryo. *CLV3* is expressed in the center of the meristem (arrow). H, hypocotyl; Co, cotyledon. **(B)** Torpedo stage embryo of a *UFO::CLV3* plant. *CLV3* is expressed in the *UFO* pattern (dashed arrow), and expression in the meristem center is reduced (arrow). Bars, 10 μm.

We conclude that targets of the *CLV* signal transduction pathway include factors that promote transcription of the *CLV3* gene in the central zone of the meristem or that control central zone cell identity or division. *CLV3* signaling would act to repress the activity of these regulatory factors, which would themselves promote *CLV3* expression or stem cell maintenance. The *WUSCHEL* (*WUS*) gene product, a homeodomain transcription factor (*15*), promotes stem cell formation and maintenance in the meristem, thereby acting antagonistically to the *CLV* genes. Phenotypically, *wus* mutants strongly resemble our transgenic plants that constitutively express *CLV3* from the *CaMV35S* promoter (compare Figs. 1B and 1C). In both cases, stem cells are not correctly specified during embryogenesis and shoot and floral meristems are not maintained. Genetic analysis revealed that *wus* mutations are almost fully epistatic to *clv* mutations (*15, 16*), indicating that *WUS* could be a target gene that is repressed by the *CLV* signaling pathway. Consistent with this, the *WUS* expression domain expands laterally and into the overlying cell layers in meristems of *clv* mutants (Fig. 4, A and B) (*16*). In addition, we did not detect any *WUS* RNA in the arrested apices of *CaMV35S::CLV3* transgenic plants by in situ hybridization, indicating that one consequence of *CLV* signaling is a severe reduction in the levels of *WUS* transcripts (Fig. 4C). However, because *CLV3* is still expressed at low levels in *wus* mutants

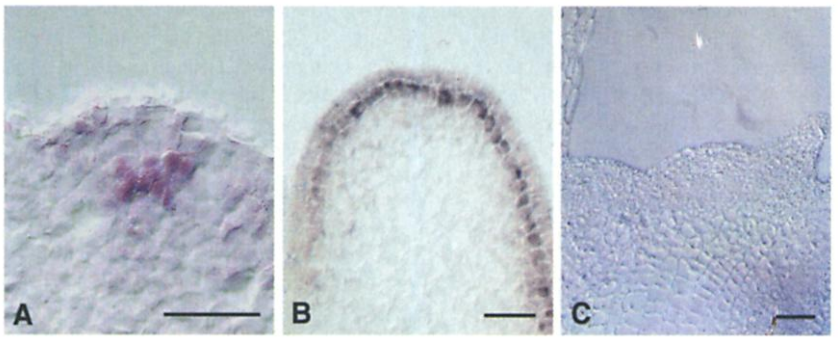

Fig. 4. *CLV3*-dependent regulation of *WUS* mRNA expression. In situ hybridization with *WUS* digoxigenin-labeled antisense probe. (**A**) Longitudinal section through a wild-type shoot meristem. *WUS* is expressed in deep regions of the meristem. (**B**) In the enlarged meristem of a *clv3* mutant, the *WUS* expression domain is expanded. (**C**) Arrested meristem of a *CaMV35S::CLV3* plant. *WUS* RNA is not detectable. Bars, 20 μm.

(*17*), the stem cell–promoting pathway may involve additional factors that may overlap in function with *WUS* and be potential targets for regulation by the *CLV* pathway, such as *POLTERGEIST* (*18*).

In wild-type *Arabidopsis* plants, the CLV3 signal is likely to be released from the stem cells at the apex of the meristem and to activate a CLV1/CLV2 receptor complex in underlying cells (*2*). Signaling through the *CLV* pathway limits *WUS* activity by restricting its expression to a narrow domain of cells in deeper layers of the meristem. Constitutive signaling through *CLV3* enhances this negative pathway, causing down-regulation of *WUS* and complete loss of stem cells. When the negative pathway is disrupted in *clv* mutants, the *WUS* expression domain expands laterally and upward, resulting in, or resulting from, an accumulation of stem cells. Activity of the positive pathway promotes the expression of *CLV3* or maintenance of the *CLV3* expression domain. This mutual regulation, involving positive and negative interactions, provides a feedback system for maintaining the delicate balance required for proliferation of stem cells to proceed at the right time and in the right place.

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9. A *CLV3* full-length cDNA was inserted between the *CaMV35S* promoter and the 3′*NOS* terminator of pRTΩNot I to give pBU4. Clone orientation and fidelity was verified by sequencing. A Pst I restriction fragment of pBU4 was introduced into pGREEN (P. Mullineaux and R. Hellens, John Innes Centre, Norwich, UK) to give pBU6 (*CaMV35S::CLV3*). pBU6 was transformed into *Agrobacterium tumefaciens* strain GV3101 and used to transform *Arabidopsis* plants by vacuum infiltration [N. Bechtold, J. Ellis, G. Pelletier, *C. R. Acad. Sci. Paris Sci. Vie* **316**, 1194 (1993)]. Transformation efficiency ranged from 0.5 to 5.0%.
10. Some 2% of all transgenic plants presumably failed to express the transgene at sufficient levels to give a phenotype.
11. In situ hybridizations were performed as described in (*2*).
12. A 5.5-kb Eco RI genomic DNA fragment containing the *CLV3* gene was cloned into pGREEN, giving gE55*CLV3*, and transformed into *Agrobacterium* strain GV3101. Introduction of gE55*CLV3* into *clv3-2* mutants rescued the mutant phenotype. Plants that carried four copies of the *CLV3* gene were obtained by introducing two copies of gE55*CLV3* into the genome of wild-type plants.
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14. A DNA fragment containing 3.5-kb DNA upstream of the putative transcription start site of the *UFO* gene was used to replace the *CaMV35S* promoter in pRTΩNot I. A *CLV3* cDNA clone was then inserted between the Ω-leader and the *NOS*-terminator giving pBU11. The *UFO::CLV3* gene construct was transferred as a Pst I fragment into a pGREEN T-DNA vector, giving pBU12. Transformation of *Arabidopsis* wild type and *clv3-8* mutants via *Agrobacterium* GV3101 was performed as described above. We analyzed 203 transformants.
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A Structural Model of Transcription Elongation

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The path of the nucleic acids through a transcription elongation complex was tracked by mapping cross-links between bacterial RNA polymerase (RNAP) and transcript RNA or template DNA onto the x-ray crystal structure. In the resulting model, the downstream duplex DNA is nestled in a trough formed by the β′ subunit and enclosed on top by the β subunit. In the RNAP channel, the RNA/DNA hybrid extends from the enzyme active site, along a region of the β subunit harboring rifampicin resistance mutations, to the β′ subunit "rudder." The single-stranded RNA is then extruded through another channel formed by the β-subunit flap domain. The model provides insight into the functional properties of the transcription complex.

Transcription is the major control point of gene expression, and RNAP is the central enzyme of transcription. In bacteria, the essential core RNAP (subunit composition $\alpha_2\beta\beta'$) has a molecular mass of around 400 kD and is evolutionarily conserved among all cellular organisms (*1*). The processive ternary elongation complex (TEC) of core RNAP, DNA template, and RNA transcript is extremely stable, yet RNAP can translocate in both directions on the DNA and disassembles rapidly upon encountering specific termination signals. Empirical models of TEC structure and function have attempted to reconcile these properties (*2–5*), but were not based on

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