The Cysteine Pairs in CLV2 are Not Necessary for Sensing the CLV3 Peptide in Shoot and Root Meristems

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Abstract

Receptor-like proteins (RLPs) are involved in both plant defense and developmental processes. Previous genetic and biochemical studies show that the leucine-rich repeat (LRR) receptor-like protein CLAVATA2 (CLV2) functions together with CLAVATA1 (CLV1) and CORYNE (CRN) in *Arabidopsis* to limit the stem cell number in shoot apical meristem, while in root it acts with CRN to trigger a premature differentiation of the stem cells after sensing the exogenously applied peptides of CLV3p, CLE19p or CLE40p. It has been proposed that disulfide bonds might be formed through two cysteine pairs in the extracellular LRR domains of CLV1 and CLV2 to stabilize the receptor complex. Here we tested the hypothesis by replacing these cysteines with alanines and showed that depletions of one or both of the cysteine pairs do not hamper the function of CLV2 in SAM maintenance. *In vitro* peptide assay also showed that removal of the cysteine pairs did not affect the perception of CLV3 peptides in roots. These observations allow us to conclude that the formation of disulfide bonds is not needed for the function of CLV2.

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Introduction

Leucine-rich repeat (LRR) receptor kinases (LRR-RLKs) and LRR receptor-like proteins (LRR-RLPs) are probably the most important cell surface receptors in plants. In the *Arabidopsis* genome, the former are represented by 220 members and the latter have 57 members. Both of them carry an N-terminal secretion signal peptides, variable number of LRR, and a single trans-membrane domain, with (LRR-RLKs) or without (LRR-RLPs) the cytoplasmic kinase domain (Shiu and Bleecker 2001). Available evidences suggest that LRR-RLPs play important roles in many defense and development processes (Jones and Jones 1997; Wang et al. 2008; Fradin et al. 2009). The first identified functional LRR-RLP is Cf-9 from tomato, which confers resistance against the fungal pathogen *Cladosporium*

fulvum (Jones et al. 1994; Dixon et al. 1996, 1998; Thomas et al. 1997). TOO MANY MOUTH (TMM), an LRR-RLP in *Arabidopsis*, regulates the stomata distribution by controlling meristemoid formation and the initiation of stomata precursor cells (Nadeau and Sack 2002; Yang and Sack 1995).

CLV2 is a constitutively expressed RLP with 21 LRRs, which functions in both shoot and root meristems of *Arabidopsis* through perception of CLV3 peptides to regulate the stem cell activities (Jeong et al. 1999; Fiers et al. 2005, 2006, 2007). Mutations of *CLV2* result in enlarged shoot and floral meristems, and increased number of the carpels and stamens (Kayes and Clark 1998). In addition, *clv2* mutants also show significantly reduced sensitivity to the CLE peptides such as CLV3p, CLE19p and CLE40p in root meristems (Fiers et al. 2005). *CLV1* encodes a typical LRR-RLK, with 23 LRR and a

cytoplasmic kinase domain (Clark et al. 1997). The phenotype of *clv1* is similar to that of *clv2,* with enlarged shoot and floral meristems and increased organ number (Clark et al. 1993). *CRN/SOL2*, identified through suppressor screenings, encodes a receptor kinase that lacks an extracellular domain (Casamitjana-Martinez et al. 2003; Müller et al. 2008; Miwa et al. 2008). *CLV3* encodes a stem cell-specific extracellular protein in which a functional peptide ligand CLV3p is derived from the conserved 14-amino acid (AA) CLE motif that is shared by over 30 CLE genes in the *Arabidopsis* genome (Fletcher et al. 1999; Fiers et al. 2005, 2006, 2007; Hirakawa et al. 2010). *In vitro* applications of the CLV3p complement the *clv3* defects in shoot apical meristem (SAM) and trigger the premature differentiation of the stem cells in root meristem (Fiers et al. 2005, 2006). The endogenous CLV3 peptide is first identified as a 12-AA peptide from the CLE motif by *in situ* matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) analyses (Kondo et al. 2006), and a recent work argues that it is a 13-AA arabinosylated glycopeptide (Ohyama et al. 2009).

Biochemical studies show that CLV2 is essential for the stability of CLV1 (Jeong et al. 1999), in which CLV1 and CLV2 may form a disulfide-linked heterodimer of 185 kD, while in the presence of CLV3, a larger complex of 450 kD is formed (Jeong et al. 1999; Trotochaud et al. 1999). With firefly luciferase complementation imaging assays, it has been demonstrated that CLV2 directly interacts with CRN in the absence of CLV3, and CLV1 can interact with CLV2-CRN heterodimer (Zhu et al. 2010). CLV1 forms homodimer independently of CLV2 and CRN at the plasma membrane, as shown by the fluorescence resonance energy transfer (FRET) assay (Bleckmann et al. 2010). The cysteine pairs flanking the LRR domain are rela- tively conserved in LRR-RLKs and LRR-RLPs. The subdomain involved in the dimerization of AtSERK1 is located in a region containing the upstream of LRRs and the first cysteine pair (Shah et al. 2001). To elucidate if the formation of disulfide bonds between CLV2 and its partner proteins such as CLV1 through the cysteine pairs is necessary for the function of CLV2, we introduced point mutations in CLV2 to specifically abolish one or both of the cysteine pairs. We then carried out *in vivo* functional assay through transformation of *clv2-1* and *in vitro* peptide assays in the transgenic plants to see if the mutated CLV2 can still execute CLV2 functions. Our results showed that the cysteine pairs are not necessary for executing the CLV2 function, as shown by complementation of the *clv2* defects in SAM and the perception of CLV3p in root meristem. These results suggest that CLV2 is unlikely to form a disulfide-bound complex with itself or its partners, or the formation of disulfide bonds, if it is really formed, is not necessary for its functions.

Results

Replacement of cysteine pairs by alanines does not affect CLV2 functions

The disulfide linkage within a protein or between two proteins usually relies on cysteine pairs. Cysteine pairs flanking the LRR domain is a common feature of LRR-RLKs such as CLV1 and LRR-RLPs such as CLV2. In order to elucidate whether the cysteine pairs are essential to the CLV2 function in the SAM maintenance, either one or both of the cysteine pairs that existed in CLV2 were replaced by alanines (**Figure 1**) and then constructs obtained were used to transform *clv2-1* to examine whether these replacements affect the function of CLV2. *clv2-1*
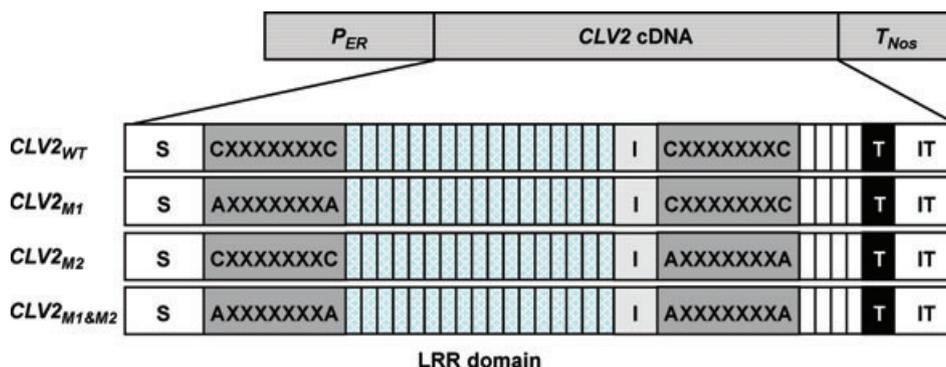
Figure 1. Schematic representation of the *CLV2WT*, *CLV2M1*, *CLV2M2* and *CLV2M1&M2* constructs.

CLV2WT, the original full-length *CLV2* expressed under the control of *ERECTA* (*ER*) promoter; *CLV2M1*, mutated *CLV2,* with the N-terminal cysteine pair replaced by alanines; *CLV2M2*, mutated *CLV2,* with the C-terminal cysteine pair replaced by alanines; *CLV2M1&M2*, mutated *CLV2,* with both the N- and the C-terminal cysteine pairs replaced by alanines. In all constructs, *ER* promoter and *Nos* terminator were used as the regulatory elements. A, alanine; C, cysteine; IT, intracellular tail; I, island domain; S, signal peptide; T, transmembrane domain. Not to scale.

is most likely to be a null mutant as the point mutation at the 97th nucleotide of the CLV2 coding region results in a stop codon (Jeong et al. 1999). Although it has been reported previously that *CLV2* is expressed constitutively at a low-level in all tissues tested (Jeong et al. 1999), the promoter region of *CLV2* has not been defined yet. In order to achieve a suitable expression of the *CLV2* transgenes, we used the rather constitutively expressed *ERECTA* (*ER*) promoter to carry out the *in vivo* complementation experiments (Yokoyama et al. 1998).

The most evident phenotype of *clv2-1* is the increased carpel number in the silique. The pistil of the wildtype *Arabidopsis* plants carries consistently two carpels, while pistil of *clv2-1* has 3.25 ± 0.38 carpels. At least 30 independent *clv2-1* plants carrying $CLV2_{WT}$, $CLV2_{M1}$, $CLV2_{M2}$ or $CLV2_{M1\&M2}$ were obtained, allowing us to perform statistical analysis of the functions of these constructs. Six siliques from the upper,

middle and lower portions of the primary inflorescences in the T1 generation were excised for counting the carpel number. The results showed that *clv2-1* transformed with $CLV2_{WT}$, $CLV2_{M1}$ or $CLV2_{M2}$ exhibits a complete restoration of the *clv2-1* mutant phenotypes (**Figure 2A**). All T1 plants carrying any of these three constructs showed wild type-looking pistils, with only two carpels, as compared with 3.25 ± 0.38 carpels in *clv2-1* (**Figure 2A–D**). Further, 98% of *clv2-1* plants transformed with $CLV2_{M1\&M2}$ were reverted to the two-carpel silique phenotype (**Figure 2A, E**). This observation was further confirmed through progeny analyses, showing that the complementation was tightly linked to the transgene. These results indicated that removal of one or both of the two cysteine pairs does not affect the function of CLV2 in the SAM maintenance. We also tried to insert the hemagglutinin (HA) and EYFPc double tags to the downstream of the signal peptide of the $CLV2_{WT}$,
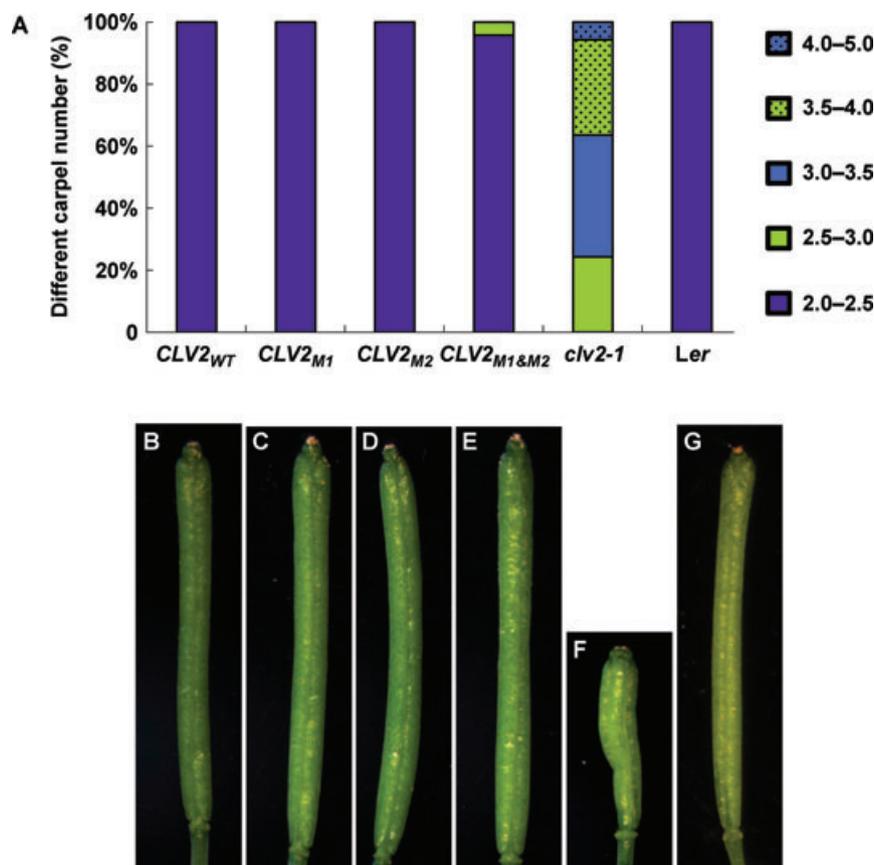
Figure 2. Complementation of *clv2-1* by $CLV2_{WT}$, $CLV2_{M1}$, $CLV2_{M2}$ and $CLV2_{M1\&M2}$ constructs.

(A) Complementation efficiencies of different constructs of $CLV2_{WT}$, $CLV2_{M1}$, $CLV2_{M2}$ and $CLV2_{M1\&M2}$, as indicated by the percentages of transgenic *clv2-1* plants with different carpel numbers. A full complementation leads to a conversion of plants with three to five carpels as in *clv2-1* to two as in the wild type. The carpel numbers were counted in the T1 plants.

(B) The morphologies of transgenic *clv2-1* carrying $CLV2_{WT}$ (B), $CLV2_{M1}$ (C) or $CLV2_{M2}$ *and* $CLV2_{M1\&M2}$, as compared to *clv2-1* (F) and L*er* (G). Note that 98% of *clv2-1* plants transformed with $CLV2_{M1\&M2}$ had two carpels, while *clv2-1* has on average 3.25 ± 0.38 carpels.

$CLV2_{M1}$, $CLV2_{M2}$ and $CLV2_{M1\&M2}$. The results showed that although constructs with single mutations (*HA-EYFPc-CLV2_{M1}* and *HA-EYFPc-CLV2_{M2}*) showed effective restorations of the two-carpel phenotype, less than 60% of the transgenic plants carrying the *HA-EYFPc-CLV2_{M1\&M2}* exhibited two carpels (Figure S1), suggesting that additional tags affected the function of CLV2 in SAM.

Depletion of the cysteine pairs in CLV2 does not affect its function in peptide sensing in roots

Mutation of *CLV2* impairs the CLV3p-, CLE19p- and CLE40p-triggered premature differentiation of the root stem cells, which has allowed us to propose that CLV2 interacts with unknown receptor kinases to perceive the peptide signals (Fiers et al. 2005). The facts that CRN/SOL2 acts as a CLV2-interacting receptor kinase and the mutation of *CRN/SOL2* suppresses the *CLV3*-overexpression phenotypes suggest that *CRN/SOL2* is a good candidate for the interacting partner of CLV2 (Müller et al. 2008; Zhu et al. 2010). Beside the synthetic 14-AA CLV3p, CLV3 peptides with 12 or 13 AA derived from the same CLE motif are also active *in vitro* (Kondo et al. 2008). We examined whether depletions of the cysteine pairs in *clv2-1* carrying *CLV2_{M1}*, *CLV2_{M2}* or *CLV2_{M1\&M2}* interrupted such responses. Synthetic 12-AA CLV3 peptides ($CLV3p_{12}$) were applied in the root assay as described previously (Kondo et al. 2008). After growing the T3 transgenic and L*er* seedlings on media containing different concentration of $CLV3p_{12}$ for 9 d, the main roots were measured and examined. On media containing 10 μM, 1 μM, 100 nM or 10 nM $CLV3p_{12}$, the average lengths of the main roots of L*er* were 0.69 ± 0.09, 0.71 ± 0.08, 0.83 ± 0.16 and 1.35 ± 0.47 cm, respectively (Figure 3A), while the *clv2-1* showed significantly compromised sensitivity to $CLV3p_{12}$, with the average root lengths of 2.64 ± 0.50, 2.69 ± 0.48, $2.74 \pm$ and 2.72 ± 0.54 cm for these concentrations, respectively (Figure 3A). The root length of *clv2-1* carrying *CLV2_{WT}* was similar with that of L*er* when $CLV3p_{12}$ was applied at the concentrations of 10 nM and 100 nM. But the roots of *clv2-1* carrying *CLV2_{WT}* were 1.13 ± 0.22 cm and 1.20 ± 0.27 cm when $CLV3p_{12}$ was applied at the concentrations of 1 μM and 10 μM, respectively, which was slightly longer than that of L*er* but significantly shorter than that of *clv2-1* (Figure 3A), suggesting that the expression of *CLV2* under the control of *ER* promoter has partially compromised its sensitivity to $CLV3p_{12}$. When the same assays were performed in *clv2-1* plants carrying *CLV2_{M1}*, *CLV2_{M2}* or *CLV2_{M1\&M2}* constructs, essentially the same responses as that carrying *CLV2_{WT}* were observed (Figure 3A). These results demonstrate that the eliminations of one or both of the cysteine pairs did not affect the functions of the CLV2 in $CLV3p_{12}$ peptide responses in roots, implying that the cysteine pairs in CLV2 are not essential for its function in roots to perceive the $CLV3p_{12}$ peptide signal.

Our previous work shows that the peptide-induced short root phenotype is resulted from consumptions of the root meristem (Fiers et al. 2005). Roots of transgenic plants carrying *CLV2_{WT}*, *CLV2_{M1}*, *CLV2_{M2}* or *CLV2_{M1\&M2}* constructs treated with 10 μM $CLV3p_{12}$ for 9 d were examined under a differential interference contrast (DIC) microscope. When no peptide was applied, the L*er*, *clv2-1* and *clv2-1* carrying *CLV2_{WT}*, *CLV2_{M1}*, *CLV2_{M2}* or *CLV2_{M1\&M2}* constructs were indistinguishable from each other (data not shown). Under peptide treatments, the root meristems of *clv2-1* (Figure 3H) resembled that without treatment, while L*er* (Figure 3G) and transgenic plants carrying *CLV2_{WT}*, *CLV2_{M1}*, *CLV2_{M2}* or *CLV2_{M1\&M2}* had significantly reduced sizes of root meristem (Figure 3C–F), which is consistent with the results obtained from root length measurements.

The expression of *ER* in roots has not been detected in the previous report (Yokoyama et al. 1998), while our results showed that the expression of *CLV2* under the control of the *ER* promoter was able to restore partially the sensitivity of *clv2-1* to CLV3 peptides. To decipher this scenario, we examined the expression of *ER* in Col-0 with reverse transcriptase-polymerase chain reaction (PCR). The results showed that indeed *CLV2* was lowly expressed in roots, as compared with the expression levels in flowers, seedlings, stems and leaves (Figure 3B).

Discussion

LRR-RLP and LRR-RLK represent the most abundant cell surface receptors in the plant kingdom (Morillo and Tax 2006; Wang et al. 2008). CLV2 belongs to LRR-RLPs that have no cytoplasmic kinase domain, while CLV1 belongs to LRR-RLKs with a cytoplasmic kinase domain. CRN is a cytoplasma membrane-localized receptor kinase that has no extracellular domain. Genetic data suggested that CLV1, CLV2 and CRN may function together to receive the CLV3 peptide to restrict the size of SAM, while in root meristems CLV2 and CRN may act together (Clark et al. 1993; Kayes and Clark 1998; Müller et al. 2008). As being a constitutively expressed gene with functions in both SAM and root meristem (Jeong et al. 1999; Fiers et al. 2005), CLV2 may act as a general partner for multiple receptor complexes. It has been hypothesized previously that CLV2 forms disulfide bonds with its partners such as CLV1 in SAM through the cysteine pairs flanking the LRR domain (Jeong et al. 1999; Trotochaud et al. 1999). Here we showed that depletion of these cysteine pairs does not affect the function of CLV2 in SAM maintenance and peptide sensing in root meristems, suggesting it is very unlikely the case.

In animals, studies have shown that receptor kinase activation usually involves ligand-induced homo- or hetero-dimerization. The epidermal growth factor receptor (EGFR) was the first receptor tyrosine kinase discovered
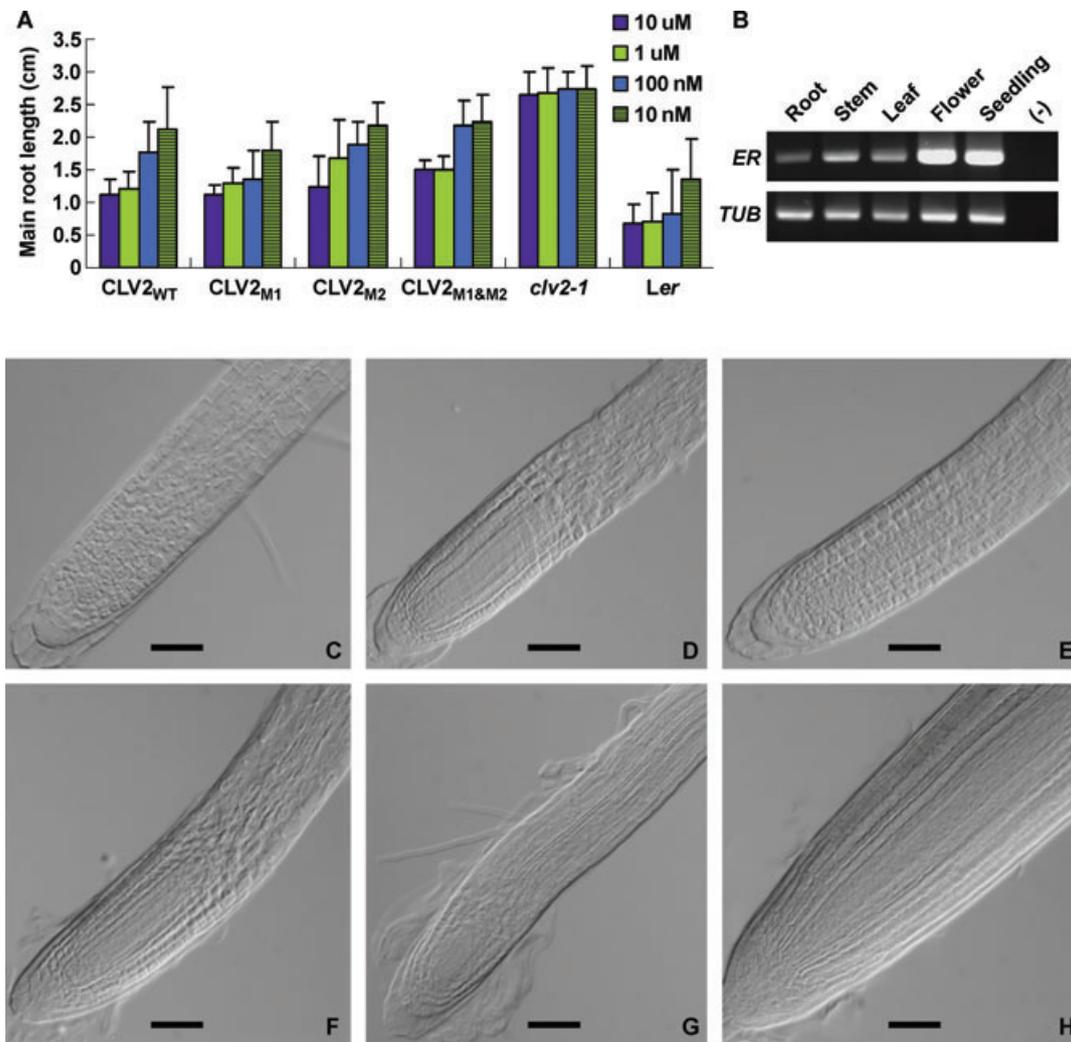
Figure 3. Transformations of *clv2-1* by *CLV2_WT_, CLV2_M1_, CLV_M2_* or *CLV2_M1&M2_* restored its sensitivities to the CLV3 peptide.

(A) Transgenic *clv2-1* plants carrying *CLV2_WT_, CLV2_M1_, CLV_M2_* or *CLV2_M1&M2_* construct were sensitive to CLV3p$_{12}$, as showed by the reduced lengths of the main roots after treatments with 10 μM, 1 μM, 100 nM and 10 nM CLV3p$_{12}$ for 9 d, as compared with *clv2-1*. Note that although no significant difference was observed in plants carrying normal or mutated *CLV2*, showing reduced root lengths, but they were generally longer than that of L*er*, suggesting that expression cassette using *ER* promoter and *Nos* terminator may not give the same expression level as the endogenous *CLV2*.

(B) *ER* promoter is active in roots. Reverse transcription-polymerase chain reaction (RT-PCR) analysis of the *ER* expression in different tissues of Col-0. *TUBULIN* (*TUB*) was used as the control. −, RNA without reverse transcription.

(C–F) The consumptions of root meristems after treatments with the CLV3 peptide.

Note that the root meristems of *clv2-1* transformed with *CLV2W_T_* (C), *CLV2_M1_* (D), *CLV2_M2_* (E) and *CLV2_M1&M2_* (F) were significantly shorter and thinner than that of *clv2-1* **(H)** under peptide treatments, but comparable with L*er* **(G)**. The photos were taken after treatments with CLV3p$_{12}$ for 9 d. Bar, 50 μm.

(Carpenter et al. 1978). It is composed of an extracellular ligand binding domain, a single transmembrane domain and a conserved cytoplasmic tyrosine kinase domain (Lax et al. 1989). The dimerization of EGFR with two molecules of EGF and two EGFR is formed from a stable intermediate complex with one EGF and one EGFR (Lemmon et al. 1997). EGFR is also known to undergo heterodimerization with ErbB2, ErbB3, or ErbB4 in response to EGF stimulations, resulting in tyrosine phosphorylations (Graus-Porta et al. 1997; Schlessinger 2002). The extracellular domain of all members in the EGFR family contains two conserved cysteine-rich domains (Abe et al. 1998).

The crystal structure of the extracellular domain of ErbB3 (Cho and Leahy 2002) shows that these two cysteine-rich domains are responsible for ligand binding (Cho and Leahy 2002). In *Arabidopsis*, homodimerization of BR receptor BRI1 is first detected in the plasmembrane of cowpea protoplasts (Russinova et al. 2004; Kim and Wang 2010), where BR is not needed for the dimerization (Hink et al. 2008). However, BR treatments in *Arabidopsis* leads to an increased co-immunoprecipitation of BRI1-GFP and BRI1-FLAG, indicating that BR promotes or stabilizes BRI1 homodimerization (Wang et al. 2005). The ethylene receptor ETR1, a histidine kinase, is isolated from membranes as a dimmer of 147 kDa. Treatment with the reducing agent dithiothreitol (DTT) converts it to a monomer of 79 kDa, indicative of a disulfide linkage between monomers (Schaller et al. 1995). In column chromatography, CLV1 with the molecular weight of about 105 kDa is separated as a tightly joint protein complex of 185 kDa, and the addition of reducing agents such as DTT to the extraction or column buffer led to the elution of CLV1 exclusively at the monomeric size of 105 kDa. In addition, more than 90% of the CLV1 protein was absent in the *clv2* mutant, suggesting that the *clv2* mutation affected the accumulation of the 185 kDa complexes (Jeong et al. 1999; Trotochaud et al. 1999). These results have allowed Trotochaud et al. (1999) to propose that CLV1 may form a heterodimer with other proteins such as CLV2 through covalent disulfide bonds between cysteine residues. Direct interactions of three proteins have been revealed by recent reports that without the existence of CLV3 peptides, CLV2 and CRN can interact with each other and CLV1 can interact with CLV2-CRN heterodimer *in vitro* (Zhu et al. 2010; Bleckmann et al. 2010). In this paper, our transgenic studies demonstrated that mutated *CLV2,* where one or both of the cysteine pairs were replaced by alanine, can fully complement the *clv2-1* morphological defects, restoring its carpel number from the average of 3.25 ± 0.38 to 2.00, as in the wild type. *clv2-1* plants carrying $CLV2_{M1}$, $CLV2_{M2}$ or $CLV2_{M1\&M2}$ constructs also had restored their sensitivities to the $CLV3p_{12}$ in roots, which further confirmed that the cysteine pairs are not necessary for the functions of CLV2. These observations allow us to conclude that the disulfide bound complex is either not present in CLV2-containing receptor complex, or if it is formed, not necessary for its function. We believe that the tightly linked complexes containing CLV1, CLV2 and CRN may be formed through non-covalent interactions.

Materials and Methods

Plant materials and growth conditions

Arabidopsis thaliana ecotypes L*er* and Col-0 (wild type), and *clv2-1* mutant were grown in soil at $21 \pm 2\,^{\circ}$C in a greenhouse with a 16 h light/8 h dark cycle. Seeds were surface-sterilized

in a desiccator for 2 h with a mixture of 50 mL commercial kitchen bleach (containing 5% sodium hypochlorite) and 5 mL concentrated HCl, and then germinated on 1/2 MS salts medium (Duchefa, Shanghai, China) with or without antibiotic. 20 mg/L hygromycin was supplemented for the selection of transgenic plants. Transformation was done via *Agrobacterium tumefaciens*-mediated vacuum infiltration (Clough and Bent 1998).

Constructions of CLV2 with alanine-replacements in the cysteine pairs

The *CLV2* cDNA fragment was first amplified as $CLV2_{WT}$. Point mutations were introduced into CLV2 to replace two cysteines with alanines (**Figure 1**) through three cycles of PCR amplification with primers *CLV2-FP, CLV2-RP, CLV2-M1-FP, CLV2-M1-RP, CLV2-M2-FP* and *CLV2-M2-RP* (Table S1). As such, three mutated *CLV2* fragments, $CLV2_{M1}$, $CLV2_{M2}$ and $CLV2_{M1\&M2}$, were amplified to eliminate the N-terminal, C-terminal and both the N- and the C-terminal cysteine pairs, respectively (**Figure 1**). A 1, 255 bp region upstream of the *ER* coding sequence (Yokoyama et al. 1998) was amplified from Col-0 and cloned into *pCAMBIA1300* vector, and a 247 bp *Nos* terminator was amplified from the *pBI101* vector and cloned into *pCAMBIA1300* vector. The primers used are listed in Table S1. At last, $CLV2_{WT}$, $CLV2_{M1}$, $CLV2_{M2}$ and $CLV2_{M1\&M2}$ fragments were inserted between the *ER* promoter and the *Nos* terminator individually. Transgenic plants were obtained using the floral dip method as reported (Clough and Bent 1998).

Constructs *HA-EYFPc-CLV2, HA-EYFPc-CLV2_{M1}, HA-EYFPc-CLV2_{M2}* and *HA-EYFPc-CLV2_{M1\&M2}* were made by separating the *CLV2* signal peptide with the rests through three partly overlapped primers. The *HA-EYFPc* was amplified from the *pSPYCE-35S* vector (Walter et al. 2004) and inserted between the signal peptide and the rests of the *CLV2* coding sequences from *CLV2_{WT}, CLV2_{M1}, CLV2_{M2}* and *CLV2_{M1\&M2}* constructs.

Root assay with $CLV3p_{12}$

Seeds were gas-sterilized as mentioned above and vernalized at 4 $^{\circ}$C for one day in sterilized water, and then plated on 1/2 Murashige and Skoog salt mixture (Duchefa), 1% sucrose, 0.5 g/L 2-(N-Morpholino) ethanesulfonic (MES), 1.2% agar (Duchefa), pH 5.8, and different concentrations of chemically synthesized 12-AA $CLV3p_{12}$ peptides (N-RTVPSGPDPLHH-C, AuGCT, Beijing, China), with more than 80% purity.

Whole-mount clearing and meristem measurement

Roots of 9-d-old seedlings grown on plates supplied with 10 μM $CLV3p_{12}$ were excised, cleared and observed under

a Leica DIC microscope as described before (Fiers et al. 2005).

RNA extractions and RT-PCR

RNAs were extracted from different organs of Col-0 with a RNA extraction Kit (Tiangen, Beijing, China), and reverse transcribed to cDNA with a cDNA synthesizing Kit (Toyobo, Beijing, China). A pair of primers, *ER-RT-FP* and *ER-RT-RP* (Table S1) that amplify a 910 bp *ER* cDNA fragment were synthesized and used for the reverse transcription (RT)-PCR analysis.

Acknowledgements

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Constructs with HA-EYFPc tag showed compromised complementation efficiencies.

Note that although all *clv2-1* planttransformed with *HA-EYFPc-CLV2$_{WT}$*, *HA-EYFPc-CLV2$_{M1}$* and *HA-EYFPc-CLV2$_{M2}$* showed 2-carpel phenotype, as in L*er*, less than 60% of *clv2-1* plants transformed with *HA-EYFPc-CLV2$_{M1\&M2}$* had two carpels, suggesting the tag has partially affected the function of *CLV2*.

Table S1. Primer list

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