

# Dual CLAVATA3 Peptides in Arabidopsis Shoot Stem Cell Signaling

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**Abstract** Plant shoot stem cell pool is constantly maintained by a negative feedback loop through peptide-receptor mediated signaling pathway. *CLAVATA3* (*CLV3*) encode a 96 amino-acid protein which is processed to 12-amino-acid or arabinosylated 13-amino-acid peptides, acting as a ligand signal to regulate stem cell homeostasis in the shoot apical meristem (SAM). Although arabinosylated 13-amino-acid CLV3 peptide (CLV3p) shows more significant binding affinity to its receptors and biological activities in the SAM, the physiological function of two mature forms of CLV3p remained an unresolved puzzle in the past decade due to the technical difficulties of arabinosylation modification in the peptide synthesis. Here, we analyzed the role of two mature CLV3 peptides with newly synthesized arabinosylated peptide. Beside shoot meristem phenotypes, arabinosylated CLV3p showed the conventional trait of CLV2-dependent root growth inhibition. Moreover, both 12-amino-acid and arabinosylated 13-amino-acid CLV3 peptides have analogous activities in shoot stem cell signaling. Notably, we demonstrated that non-arabinosylated 12-amino acid CLV3p can affect shoot stem cell signaling at the physiological level unlike previously suggested (Ohyama et al. 2009; Shinohara and Matsubayashi 2013; Shinohara and Matsubayashi 2015). Therefore, these results support the physiological role of the 12-amino-acid CLV3p in shoot stem cell signaling in the deficient condition of arabinosylated 13-amino-acid CLV3p in *Arabidopsis thaliana*.

**Keywords:** *Arabidopsis thaliana*, CLAVATA3, Peptide modification, Shoot apical meristem, Stem cell signaling

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## Introduction

A stable stem cell pool in the shoot apical meristem (SAM) is the self-renewable reservoir to generate new cells for leaf, stem and flower organogenesis, and is dynamically maintained by complex regulatory networks in plants. The CLAVATA-WUSHCEL (CLV-WUS) feedback signaling pathway, first discovered in *Arabidopsis thaliana*, appears to be conserved for stem cell control in the SAM of higher plants (Somssich et al. 2016; Yamaguchi et al. 2016). The CLAVATA3 peptide (CLV3p) produced in the stem cells of the shoot apex functions as a signaling ligand of the CLAVATA1 (CLV1) and related receptor kinases to restrict the level of WUS transcription factor in the organizing center (OC), which promotes *CLV3* expression via cell-to-cell communication through plasmodesmata and the stem cell fate but represses differentiation (Fletcher et al. 1999; Yadav et al. 2011; Yadav et al. 2013; Somssich et al. 2016; Yamaguchi et al. 2016). The signaling components between CLV3-CLV1 and WUS remains enigmatic despite great efforts in the past two decades. Although loss-of-function and various genetic manipulations of *CLV3* expression have facilitated the analysis of long-term phenotypes, exogenous application of physiological CLV3 peptides complementing *clv3* mutants will aid in the discovery of primary and dynamic processes in peptide-receptor signaling (Somssich et al. 2016; Yamaguchi et al. 2016).

Mass spectrometry (MS) analyses have identified two forms of mature CLV3 peptides. The first mature form is the 12-amino-acid CLV3 peptide (MCLV3p) by in situ MALDI-TOF MS analyses from *CLV3* overexpression calli with hydroxyproline residues in the 4<sup>th</sup> and 7<sup>th</sup> positions. MCLV3p binds to the ectodomain of CLV1 in vitro with the dissociation constant (K<sub>d</sub>) values of 17.5–24 nM (Kondo et al. 2006; Ogawa et al. 2008; Ohyama et al. 2009). A secreted arabinosylated 13-amino-acid peptide [Ara<sub>3</sub>]CLV3p is also

identified in liquid medium using nano-LC-MS/MS from *CLV3* overexpression seedlings (Ohyama et al. 2009). Because the high affinity ( $K_d=1$  nM) of the purified [Ara<sub>3</sub>]CLV3p binding to the ectodomain of CLV1 in vitro, it has been assumed that the glycopeptide is the physiological ligand in CLV3-CLV1 signaling (Ohyama et al. 2009). When comparing the biological effects of chemically synthesized peptides in *clv3-1* with enlarged SAM, synthetic [Ara<sub>3</sub>]CLV3p at 30–100 nM leads to SAM size reduction but not the non-arabinosylated 13-amino-acid CLV3p with much lower binding affinity to CLV1 ( $K_d=280$  nM) (Shinohara and Matsubayashi, 2013). However, the performance of chemically synthesized 12-amino-acid MCLV3p and 13-amino-acid [Ara<sub>3</sub>]CLV3p have not been carefully compared due to the difficulties of determining the stereoselective nature of glycosidic linkages in chemical synthesis of the pure [Ara<sub>3</sub>]CLV3 peptides (Shinohara and Matsubayashi 2013). Inevitably, high concentrations of synthetic CLV3/EMBRYO SURROUNDING REGION-related (CLE) peptides in the range of 1–100 mM have been commonly applied in the measurement of SAM size in bioassays in different plant species, leading to concerns about biological relevance of the findings (Somssich et al. 2016; Yamaguchi et al. 2016).

Three studies spanning the past decade have led to unresolved puzzles regarding the physiological function of arabinosylation in the Arabidopsis CLV3 peptides (Kondo et al. 2008; Song et al. 2012; Song et al. 2013; MacAlister et al. 2016). Alanine-scan analyses were conducted using synthetic MCLV3 peptide variants in bioassays and in vitro competitive binding with the CLV1 ectodomain (Kondo et al. 2008), or using in vivo complementation of the SAM size and *WUS* expression in *clv3-2* transgenic plants expressing MCLV3 peptide variants from *CLV3* transgenes (Song et al. 2012). These systematic analyses of peptide mutants in vitro and in vivo failed to support a critical biological role of the 7<sup>th</sup> hydroxyproline residue, where three arabinoses are covalently attached (Kondo et al. 2008; Ohyama et al. 2009; Song et al. 2012; Shinohara and Matsubayashi 2013). Although recent findings on hydroxyproline O-arabinosyltransferases (HPATs), responsible for the arabinosylation of proteins or peptides, have confirmed the importance of peptide arabinosylation in tomato plants (Xu et al. 2015), the thorough genetic and phenotypic analyses of the single, double and triple Arabidopsis *hpat1,2,3* mutants do not support a role of HPATs in stem cell signaling in the SAM (MacAlister et al. 2016).

In this study, we functionally analyzed the role of newly synthesized the arabinosylated 13-amino-acid CLV3p in shoot stem cell signaling. Our results revealed similar biological activities between MCLV3p and [Ara<sub>3</sub>]CLV3p in physiological conditions. These results support the idea of the redundant role of different in vivo CLV3 peptides in the Arabidopsis shoot stem cell signaling and development.

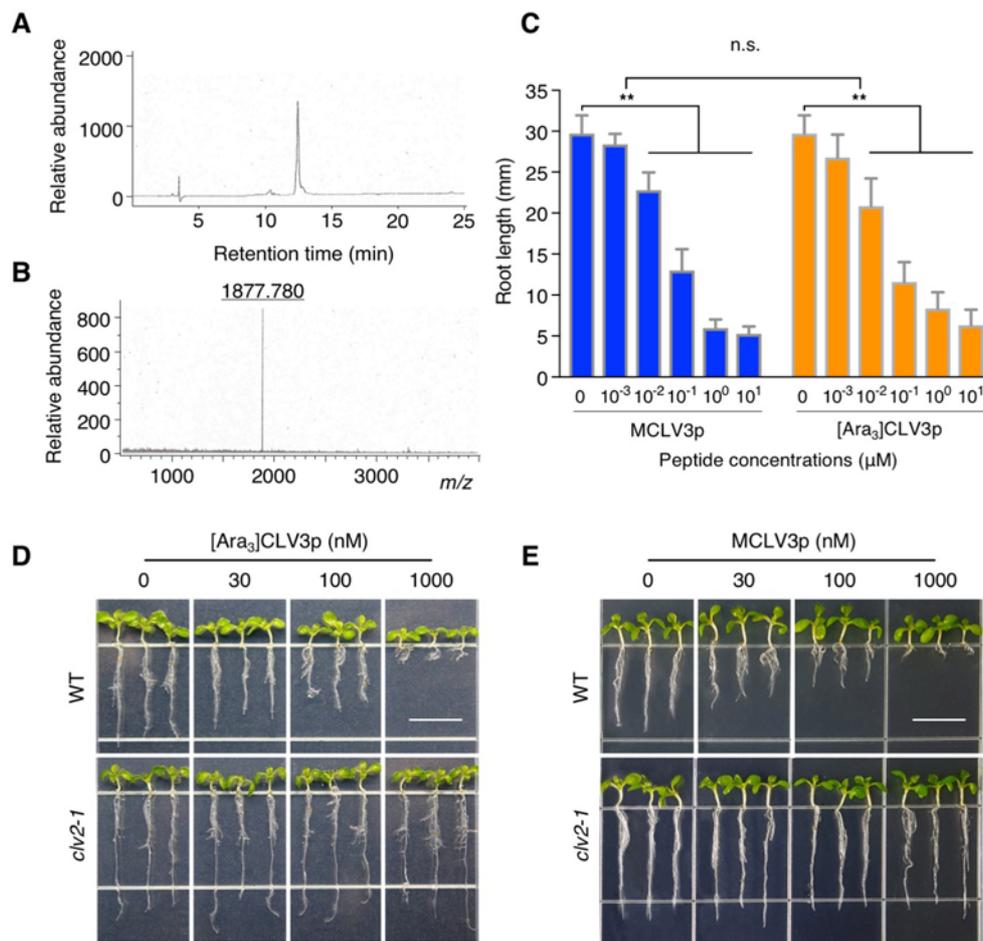
## Results

### Effect of Chemically Synthesized Arabinosylated CLV3 Peptide in Root Growth Inhibition

As the activity of MCLV3p and [Ara<sub>3</sub>]CLV3p at physiological concentrations in bioassays is a prerequisite for integrated analyses of dynamic CLV3 peptide signaling, we chemically synthesized [Ara<sub>3</sub>]CLV3p (Fig. 1A, B; see Materials and Methods) and MCLV3p. Because of the difficulty in the chemical synthesis of glycosylated CLV3 peptides, we tested whether newly synthesized [Ara<sub>3</sub>]CLV3p has a conventional property as a CLV3 signal. The biological activities of [Ara<sub>3</sub>]CLV3p and MCLV3p were first examined in parallel using the well-established and sensitive inhibitory assays in the root apical meristem (RAM) (Kondo et al. 2006; Kondo et al. 2008; Kondo et al. 2011). Both [Ara<sub>3</sub>]CLV3p and MCLV3p but not unarabinosylated 13-amino-acid CLV3p displayed similar and prominent root growth inhibition at 10–100 nM and this inhibition was almost saturated 1 mM or higher concentrations (Fig. 1C) (Kondo et al. 2006; Kondo et al. 2008; Lee et al. 2012). This result indicates that [Ara<sub>3</sub>]CLV3p has similar biological activity in addition to MCLV3p in the consumption of root meristems via the misspecification of cell type (Fiers et al. 2005). Because the primary root growth inhibition by [Ara<sub>3</sub>]CLV3p as well as previously reported MCLV3p was not observed in the *clv2-1* mutant seedlings (Fig. 1D, E) (Lee et al. 2012), the result demonstrates that newly synthesized [Ara<sub>3</sub>]CLV3p also possesses a typical biological trait known as CLV2-dependent root growth inhibition (Fiers et al. 2005).

### Effect of Chemically Synthesized CLV3 Peptides in Shoot Growth Arrest

We next determined the effect of both CLV3 peptides on stem cell signaling in the SAM by applying a physiological range of exogenous MCLV3p and [Ara<sub>3</sub>]CLV3p. The CLV-WUS negative feedback loop controls the homeostasis of stem cell population in the SAM, and it therefore affects the SAM size depending on the balance between cell division and differentiation. To investigate more sensitive effect of CLV3p-mediated SAM growth arrest, we used and measured the size of shoot meristems of *clv3-2*, which is a null mutant lacking the endogenous CLV3 peptide signal (Fletcher et al. 1999; Ohyama et al. 2009; Song et al. 2012; Shinohara and Matsubayashi 2013; Xu et al. 2015). *clv3-2* mutant seedlings were treated with each type of CLV3p with various concentrations for 7 days and then we measured the width and the height between two flanking primordia in the SAM after sectioning paraffin-embedded tissues (Fig. S1). Surprisingly, unlike previous reports (Shinohara and Matsubayashi 2013;



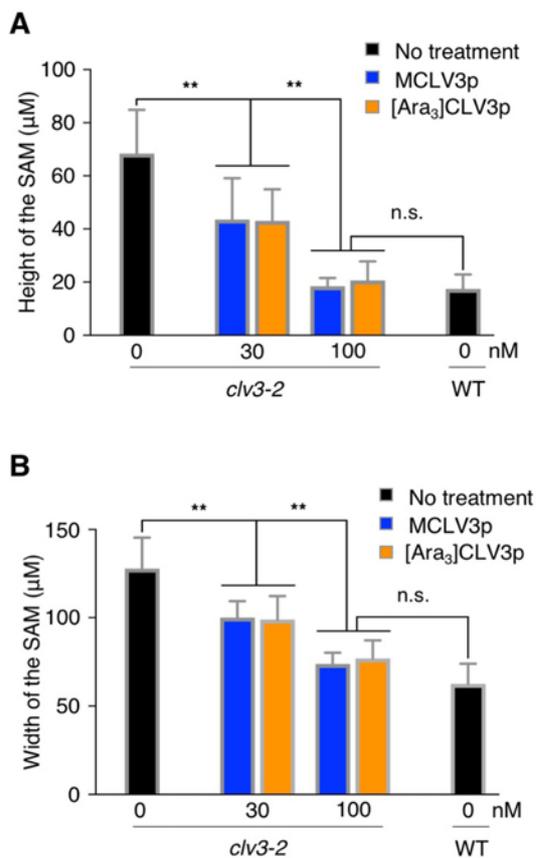
**Fig. 1.** *In vivo* activity of chemically synthesized arabinosylated CLV3 peptides. (A) HPLC purification of the chemically synthesized [Ara<sub>3</sub>]CLV3 peptide. (B) Selected ion at *m/z* 1877.780 that corresponds to the 13-amino-acid [Ara<sub>3</sub>]CLV3 peptide in MS analysis. (C) Similar root growth inhibition mediated by MCLV3p and [Ara<sub>3</sub>]CLV3p. *Ler* seedlings were treated with synthetic peptides (0, 1, 10, 100, 1000, 10,000 nM) for 14 days. Error bars represent SD from repeats (*n* = 10). \*\* indicates significant difference (two-way ANOVA test, *p* < 0.001). n.s., not significant. (D, E) CLV2-dependent root growth inhibition mediated by [Ara<sub>3</sub>]CLV3p and MCLV3p. WT *Ler* and *clv2-1* mutant seedlings were treated without or with 30 nM, 100 nM and 1000 nM [Ara<sub>3</sub>]CLV3p or MCLV3p for 7 days. Scale bar = 10 mm.

Shinohara and Matsubayashi 2015), both MCLV3p and [Ara<sub>3</sub>]CLV3p at 30–100 nM similarly reduced the enlarged SAM in *clv3-2* to the size in WT (Fig. 2A, B). It is possible that different *clv3* alleles and experimental conditions make different effects. Notably, these results demonstrate that MCLV3p can also reduce meristem size at physiologically low concentration in addition to [Ara<sub>3</sub>]CLV3p (Shinohara and Matsubayashi 2013). Consistent with a key role of CLV3p signaling in controlling stem cell numbers in the SAM (Je et al. 2016; Mandel et al. 2016; Somssich et al. 2016; Yamaguchi et al. 2016), CLV3p-mediated reduction of the shoot meristem size was most significantly reflected in the height of the *clv3-2* SAM rather than the width (Fig. 2A, B). In particular, we could observe the different penetrance between [Ara<sub>3</sub>]CLV3p and MCLV3p in causing the depletion of shoot stem cell population. About 18.2 % of *clv3-2* seedlings treated with 100 nM [Ara<sub>3</sub>]CLV3p but not MCLV3p were

manifested as a flat structure in the region of the SAM (Fig. S1, Table 1). Consistent with this, the slightly enlarged shoot meristems in well-known CLV3 receptor mutants, such as *clv1* or *barely any meristem 1 (bam1)*, were also similarly reduced by the treatment of two kinds of CLV3 peptides (Fig. S2). Taken together, these results indicate that both MCLV3p and [Ara<sub>3</sub>]CLV3p have similar biological activities on SAM growth and development despite of more sensitive effect of [Ara<sub>3</sub>]CLV3p.

#### Effect of Chemically Synthesized CLV3 Peptides in the Regulation of Downstream Genes

To investigate molecular responses stimulated by MCLV3p and [Ara<sub>3</sub>]CLV3p, we also performed reverse transcription-quantitative polymerase chain reaction (RT-qPCR) using the SAM tissues from *clv3-2* seedlings, which possess higher



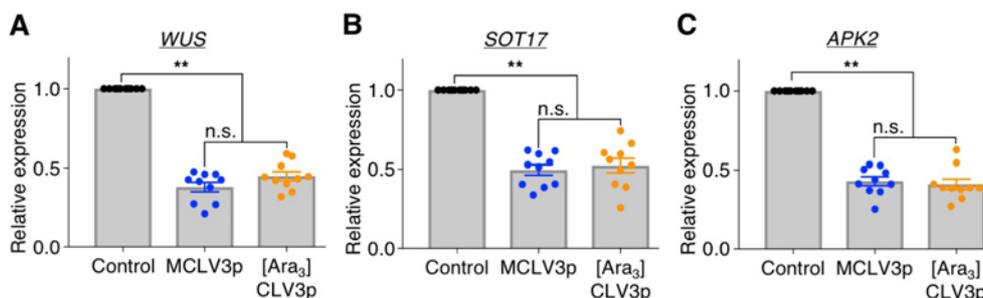
**Fig. 2.** MCLV3p and [Ara<sub>3</sub>]CLV3p complement the SAM size in *clv3-2*. (A, B) The size of shoot meristems was quantified by measuring the height (A) or the width (B). Error bars represent SD from biological repeats (*n* = 11). \*\* indicates significant difference (two-way ANOVA test, *p* < 0.001). n.s., not significant.

basal level of *WUS* expression by the deficient endogenous CLV3 peptide signal for repressing *WUS* expression in the CLV-*WUS* pathway (Somssich et al. 2016). When we applied 100 nM of two different batches of MCLV3p and [Ara<sub>3</sub>]CLV3p for 6 h, both chemically synthesized peptides equivalently decreased about 60% of *WUS* expression in

*clv3-2* (Fig. 3A), which correlated well with the reduced height in the SAM size (Fig. 2A, Table 1). Interestingly, the reduced *WUS* level in the *clv3-2* SAM caused by the treatment of two kinds of CLV3 peptides seemed to be quite similar to the endogenous level of *WUS* gene in the WT *Ler* SAM (Fig. 3A, Fig. S3A). Consistently, the treatment of 100 nM MCLV3p or [Ara<sub>3</sub>]CLV3p almost complemented the enlarged size of the *clv3-2* SAM like that of the WT *Ler* SAM (Fig. 2, Fig. 3, Fig. S3A). In addition, we detected similar reduction of other marker genes co-expressed with *WUS* in the SAM, such as *SULFOTRANSFERASE 17 (SOT17)* and *ADENOSINE-5'-PHOSPHOSULFATE KINASE 2 (APK2)* genes (Fig. 3B, C) (Yadave et al. 2009), which are known to be involved in the promotion of stem cell fate and proliferation in root meristems by glucose-TOR signaling (Xiong et al. 2013). It suggests the reciprocal promotion of stem cell proliferation in Arabidopsis shoot meristems. However, since the endogenous levels of *SOT17* and *APK2* genes in the WT *Ler* SAM were not correlated with the reduced expression caused by two kinds of CLV3 peptides in the *clv3-2* SAM (Fig. 3B, C, Fig. S3B, C), it indicates that *SOT17* and *APK2* genes involved in stem cell proliferation seem to be indirectly affected by the CLV-*WUS* pathway.

**Discussion**

Our findings with the chemically synthesized [Ara<sub>3</sub>]CLV3p and MCLV3p in this study provide compelling phenotypic and molecular evidence to support their analogous biological activities in various *in vivo* analyses (Fig. 1, Fig. 2, Fig. 3, Table 1). These observations are consistent with prior *in vitro* and *in vivo* analyses supporting physiological functions of dual CLV3 peptides identified in calli and liquid medium of seedlings overexpression the Arabidopsis *CLV3* transgene (Kondo et al. 2006; Kondo et al. 2008; Ohyama et al. 2009; Song et al. 2012; Song et al. 2013). Moreover, a recent study of the Arabidopsis loss-of-function mutants of three HPATs



**Fig. 3.** MCLV3p and [Ara<sub>3</sub>]CLV3p suppress marker genes in the SAM of *clv3-2* mutant plants. (A-C) Seedlings (7 days) were treated with 100 nM CLV3 peptides for 6 h, and RNAs from the SAM tissues were analyzed by RT-qPCR. *ACT2* (At3g18780) was used as an internal control to normalize the expression levels of *WUS* (At2g17950) (A), *SOT17* (At1g18590) (B) and *APK2* (At4g39940) (C). Error bars represent SEM (*n* = 10). \*\* indicates significant difference (one-way ANOVA test, *p* < 0.001). n.s., not significant.

**Table 1.** Shoot meristem growth arrested by different CLV3 peptides

Genotype	Peptide	With ( $\mu\text{m}$ )	Height ( $\mu\text{m}$ )	n	
<i>Ler</i>	no treatment	62.5 $\pm$ 13.0	17.4 $\pm$ 6.8	11	
<i>clv3-2</i>	no treatment	127.8 $\pm$ 19.5	68.3 $\pm$ 17.5	11	
<i>clv3-2</i>	MCLV3p	30 nM	100.7 $\pm$ 11.9	43.6 $\pm$ 16.1	11
		100 nM	72.3 $\pm$ 9.9	18.6 $\pm$ 4.3	11
	[Ara <sub>3</sub> ]CLV3p	30 nM	98.9 $\pm$ 14.3	43.3 $\pm$ 12.4	11
		100 nM	78.0 $\pm$ 10.6	20.2 $\pm$ 6.6	11*

\*Some (18.2%) of observed SAMs showed the flat structure.

without overt SAM defect supports the likely physiological role of the 12-amino-acid MCLV3p in shoot stem cell signaling when the 13-amino-acid [Ara<sub>3</sub>]CVL3p cannot be generated (MacAlister et al. 2016). Indeed, since the arbinosylated modification of the 12-amino acid CLE2 peptide was almost disappeared even in the *hpat3* single mutant (Ogawa-Ohnishi et al. 2013), the arbinosylation of CLV3p is not functionally indispensable in the Arabidopsis shoot stem cell signaling as previously suggested (Ohyama et al. 2009; Shinohara and Matsubayashi 2013; Shinohara and Matsubayashi 2015). Therefore, application of the synthetic 12-amino-acid MCLV3p, but not the arbinosylated 13-amino-acid CLV3p, in the *clv3* null mutants will greatly simplify future research in uncovering the primary and dynamic signaling mechanisms in the CLV-WUS pathway in the SAM using the versatile Arabidopsis system (Kondo et al. 2006; Kondo et al. 2008; Song et al. 2012; Song et al. 2013).

Arabidopsis has been known to contain 32 *CLE* genes in the genome (Yamaguchi et al. 2016). Currently, three *CLE* peptides, including CLV3p, CLE2p and CLE9p, are purified with a glycosylated modification of three arabinose residues (Ohyama et al. 2009; Shinohara et al. 2012). Interestingly, only CLV3p has been revealed to have the 13-amino-acid length among Arabidopsis *CLE* peptides (Yamaguchi et al. 2016), suggesting that Arabidopsis has different proteolytic processing to produce different length of *CLE* peptides (Tabata and Sawa 2014). In fact, it has been demonstrated that N-terminal flanking sequence of CLV3p affects its cleavage to produce two mature forms of 12-amino-acid and 13-amino-acid CLV3 peptides (Xu et al. 2013). Similar with our results, both arbinosylated/non-arbinosylated 12-amino-acid CLE9 peptides displayed similar activity in the binding affinity to the BARELY ANY MERISTEM 1 (BAM1) receptor (Shinohara et al. 2012), supporting an idea of the redundant role of two mature CLV3 peptides in Arabidopsis shoot stem cell signaling. Nevertheless, peptide arbinosylation could affect peptide conformation and receptor binding, and modulate peptide stability and trafficking in a context-dependent manner, as has been functionally proven for *CLE*

and related peptides in other plant species (Ohyama et al. 2009; Shinohara and Matsubayashi 2013; Xu et al. 2015; Somssich et al. 2016; Yamaguchi et al. 2016). Different environmental conditions may influence HPAT or other AT enzyme activity or expression at different developmental stages. It remained to be clarified whether peptides with or without arbinosylation will lead to differential signaling via distinct receptors in the Arabidopsis SAM signaling and development.

## Materials and Methods

### Plant Materials and Growth Conditions

Landsberg *erecta* (*Ler*) ecotype was used as wild-type (WT) Arabidopsis plants in this study. The *clv2-1* and *clv3-2* are in *Ler* background. The mutation of *clv2-1* causes the early stop codon at the 33<sup>rd</sup> residue and is a null allele (Jeong et al. 1995). The  $\gamma$ -ray-induced *clv3-2* is a presumed null allele (Fletcher et al. 1999). For liquid culture of Arabidopsis seedlings, 10 seeds were germinated and grew in 6-well plates containing 1 mL of liquid medium (0.5  $\times$  MS and 0.5% sucrose, pH 5.8 adjusted with KOH). To quantify the primary root length of WT in various concentrations of peptides, seedlings were grown in liquid medium for two weeks (12-h light/12-h dark, 100  $\mu\text{mol m}^{-2} \text{ s}^{-1}$ , 22°C $\pm$ 1) (Fig. 1C). To determine the root growth inhibition by [Ara<sub>3</sub>]CLV3p in *clv2-1*, seedlings were grown in liquid medium with various concentrations of peptides for seven days (16-h light/8-h dark, 100  $\mu\text{mol m}^{-2} \text{ s}^{-1}$ , 22°C $\pm$ 1) (Fig. 1D). The SAM tissues from *clv3-2* seedlings grown for seven days in liquid medium without or with 100 nM peptides under long days were used for RT-qPCR analysis of marker genes (Fig. 3).

### CLV3p-mediated SAM Arrest Assay

Ten seeds of *clv3-2* mutants were sowed in 6-well plates containing 1 mL of liquid medium without or with various concentrations of CLV3 peptides. Seedlings were grown at 22°C under the long day condition for seven days. The SAM tissues were harvested and fixed in 1X PBS/4% (w/v) paraformaldehyde/4% (v/v) DMSO at 4°C for overnight. Collected samples were dehydrated through ethanol series (30%, 40%, 50%, 60%, 70%, 85%, 95% for 1 h in each step) at 4°C, and were stained with 0.1% Eosin Y (Sigma) in 100% ethanol at 4°C for overnight. Ethanol was changed through histoclear series (75% ethanol:25% histoclear, 50% ethanol:50% histoclear, 25% ethanol:75% histoclear for 30 min in each step and 100% histoclear twice for 1 h in each step) (National Diagnostics). Histoclear was then gradually replaced with melted paraffin (Leica) at 60°C. Replacement of freshly melted paraffin was performed once a day for four days. Paraffin embedded tissues were poured into the mold and adjusted in appropriate position. Section was carried out with a rotary microtome (Reichert-Jung) at 8  $\mu\text{m}$  thickness. Sectioned ribbons were placed on poly-prep slides (Sigma) with pre-warm water and incubated on slide warmer (Chang Shin Science, Korea) at 42°C for overnight. For meristem staining, paraffins were removed by 100% histoclear twice for 10 min. Sectioned tissues were hydrated through reverse ethanol series (100%, 95%, 80%, 60%, 30% ethanol and water for 2 min in each step). Sections were stained with 0.1% Giemsa (Sigma) for 2 min and rinsed briefly with water. Stained sections were dehydrated through ethanol series (2 min in each step). For microscopic analysis, samples were dried and mounted with Cytoseal 60 (Richard-Allan Scientific) before observing and measuring the height and width of

the SAM using a microscope (Olympus X22LED).

#### RT-qPCR Analysis

Total RNA was isolated from SAM tissues with TRIzol reagent (Invitrogen). To harvest SAM tissues, other tissues including cotyledons, hypocotyls and roots were removed using fine forceps and single-edged blades. Harvested SAM tissues were immediately frozen by liquid nitrogen and ground in a microtube using a blue pestle powered by a motor drill. First strand cDNA was synthesized from 1 µg of total RNA with M-MLV reverse transcriptase (Promega). All RT-qPCR analyses were performed by Step One Plus™ real time PCR detection system with Power SYBR® green master mix (Applied Biosystem). *ACT2* (At3g18780) was used as a control gene. Following primers were used: ACT2-F: 5'-TCCCT CAGCA CATC CAGCA GAT-3' and ACT2-R: 5'-AACGA TTCCT GGACC TGCCT CATC-3' for *ACT2* gene expression; WUS-F: 5'-TGCA GCTCA GGTAC TGAAT G-3' and WUS-R: 5'-ATGAT CCATG TTTGC CCATC-3' for *WUS* (At2g17950) gene expression; SOT17-F: 5'-GGAAG AGGAG GGAAA TGTTG-3' and SOT17-R: 5'-CAGCA GGACG ATCTT CTCTA TC-3' for *SOT17* (At1g18590) gene expression, APK2-F: 5'-TCCGT ACCGG AGAGA CAGAG-3' and APK2-R: 5'-GGATC TCTCG ACTCG CACAC-3' for *APK2* (At4g39940) gene expression.

#### Synthesis of [Ara<sub>3</sub>]CLV3 Peptides

The [Ara<sub>3</sub>]CLV3 peptide was synthesized by solid-phase peptide synthesis using a PS3 peptide synthesizer (Protein Technology). Starting with 0.1 mmole (0.166 g) of HMP (p-hydroxymethyl phenoxymethyl polystyrene) resin (1.66 mmole g<sup>-1</sup>) (CYW, unpublished), the synthesis was performed using a stepwise Fast Moc protocol. The amino acids were introduced using the prepacked cartridges (0.4 mmole each). The Fmoc-Hyp(Ara<sub>3</sub>) was introduced using the prepacked cartridges (0.2 mmole each) and stirring overnight. After synthesis, 0.412 g peptide resin was placed in a round-bottom flask containing a micro stirring bar. The cool mixture containing 0.375 g crystalline phenol, 0.125 mL EDT, 0.25 mL thioanisole, 0.25 mL water, and 5 mL TFA was put into the flask and stirred for 1 h at 0°C. The peptide was further lyophilized. Then the peptide was rinsed with 100 mL cold ether and filtered. The peptide was purified by HPLC using a C18 column (5 µm particle size, 250 10 mm, Supelco) with a gradient (9% to 11% buffer B in 24 min) using buffer A (0.1% TFA in water) and buffer B (0.1% TFA in acetonitrile) at a flow rate of 2.5 mL min<sup>-1</sup> and monitored by absorbance at 214 nm. Mass spectra were determined using a Ultraflex II TOF/TOF (Bruker). All reagents and solvents were obtained commercially as reagent grade and used without further purification. The amino acid sequence of [Ara<sub>3</sub>]CLV3p is RTVHypSGHyp(3Ara)DPLHHH. Hyp indicates a hydroxyproline residue.

#### Statistical Analysis

Statistical analysis was performed with one-way or two-way analysis of variation (ANOVA) with Prism software (version 7.0b) and significant differences were determined by Turkey's multiple comparison test.

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#### Author's Contributions

HL and JS designed the research and wrote the manuscript. HJK and HL performed the experiments. HMY and CYW performed the chemical synthesis of CLV3 peptides.

#### Supporting Information

**Fig. S1.** CLV3 peptides complement *clv3-2* in the SAM homeostasis. **Fig. S2.** MCLV3p and [Ara<sub>3</sub>]CLV3p similarly reduce the SAM size in *clv1* and *bam1* mutants.

**Fig. S3.** Endogenous expression levels of *WUS*, *SOT17* and *APK2* genes in the SAM of *clv3-2* and WT *Ler* seedlings.

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