The CLAVATA receptor FASCIATED EAR2 responds to different CLE peptides by signaling through different downstream effectors.

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Abstract.

Meristems contain groups of indeterminate stem cells that are critical for organ initiation throughout plant development. The shoot apical meristem (SAM) maintains itself and initiates all shoot organs, such as leaves, floral organs and axillary branch meristems. Development and balanced proliferation of the SAM is regulated by a feedback loop between *CLAVATA* (*CLV*) and *WUSCHEL* (*WUS*) signaling. CLV signaling is initiated by secretion of the CLV3 peptide ligand, which is perceived directly or indirectly by a number of Leucine-Rich-Repeat (LRR) receptor-like kinases, including CLV1 and BARELY ANY MERISTEM (BAM) 1-3, and RECEPTOR-LIKE PROTEIN KINASE 2 (RPK2), as well as the receptor-like protein CLV2 in a complex with the CORYNE (CRN) pseudokinase. However, CLV2, and its maize ortholog FASCIATED EAR2 (FEA2) appear to function in signaling by several related CLV3/EMBRYO-SURROUNDING REGION (CLE) peptide ligands, including CLV3. Nevertheless, it remains unknown how CLV2 or FEA2 transmit specific signals from distinct CLE peptides. Here we show that FEA2 is involved in signaling from at least 2 distinct CLE peptides, ZmCLE7, a maize CLV3 ortholog, and ZmFON2-LIKE CLE PROTEIN1 (ZmFCP1), a newly identified CLE peptide functioning in SAM regulation. Signaling from these 2 different CLE peptides appears to be transmitted through 2 different candidate downstream effectors, COMPACT PLANT2 (CT2), the alpha subunit of the maize heterotrimeric G protein, and maize CRN. Our data provide a novel framework to understand how diverse signaling peptides can activate different downstream pathways through common receptor proteins.

Introduction.

Stem cell proliferation and differentiation throughout plant life is regulated by a feedback loop between the homeodomain transcription factor *WUS* and *CLV* ligand-receptor signaling (Mayer et al. 1998; Brand et al. 2000; Schoof et al. 2000; Yadav et al. 2011; Daum et al. 2014). The secretion of the diffusible glycopeptide CLV3 from the central zone (CZ) stem cells of the SAM is believed to initiate signaling through LRR receptors (Fletcher et al. 1999; Rojo et al. 2002; Kondo et al. 2006; Ohyama et al. 2009; Nimchuk et al. 2011b), which transmit the signal to restrict the expression of WUS in the organizing center (OC) cells. To balance this system, WUS non-cell-autonomously promotes stem cell fate by activation of *CLV3* expression (Yadav et al. 2011; Daum et al. 2014). CLV3 is thought to be perceived by multiple receptor kinase and receptor like proteins, including the CLV1 LRR receptor-like kinase (Clark et al. 1993; Clark et al. 1997; Brand et al. 2000; Ogawa et al. 2008) and the related BAM receptors (DeYoung et al. 2006; Deyoung and Clark 2008; Nimchuk et al. 2015; Shinohara and Matsubayashi 2015), or by a heterodimer of the receptor like protein CLV2 and the transmembrane pseudokinase CRN (Kayes and Clark 1998; Jeong et al. 1999; Miwa et al. 2008; Muller et al. 2008; Bleckmann et al. 2010; Zhu et al. 2010; Nimchuk et al. 2011a), or by the receptor-like kinase RPK2 (Mizuno et al. 2007; Nodine et al. 2007; Kinoshita et al. 2010). The relationship between CLV1 and CLV2 is not clear- CLV1 can form homodimers, or higher order complexes with CLV2/CRN, to signal co-operatively in the SAM (Guo et al. 2010; Somssich et al. 2015), but it seems that CLV2/CRN is not essential for CLV3 perception or for CLV1 signaling (Muller et al. 2008; Nimchuk et al. 2011b; Nimchuk 2017). In contrast to CLV1, CLV2 does not bind CLV3 peptide directly (Shinohara and Matsubayashi 2015), and its expression is not restricted to the SAM, suggesting that it might function as a co-receptor in additional pathways beyond CLV3 signaling. Indeed, CLV2 appears to be involved in signaling by several CLE peptides (Fiers et al. 2005; Meng and Feldman 2010; Hazak et al. 2017) and in biotic interactions (Replogle et al. 2011; Hanemian et al. 2016), suggesting it plays diverse functions in plant development and immunity (Pan et al. 2016). The multiple roles of CLV2 promote the question of how it confers signal specificity. Two candidate downstream effectors of CLV2 have been identified. One is the

transmembrane pseudokinase CRN, discovered in *Arabidopsis,* and the second is COMPACT PLANT2 (CT2), the heterotrimeric G protein alpha subunit, discovered in maize (Bommert et al. 2013a). However, since CRN and CT2 were identified in different species, their molecular and genetic interactions remain unknown.

The CLV-WUS pathway is widely conserved (Somssich et al. 2016; Soyars et al. 2016). In maize, *THICK TASSEL DWARF1* (*TD1*) and *FEA2* are *CLV1* and *CLV2* orthologs, and function similarly to restrict SAM proliferation (Taguchi-Shiobara et al. 2001; Bommert et al. 2005). In rice, *FLORAL ORGAN NUMBER 1* (*FON1*), the *CLV1* ortholog, and *FON2,* the *CLV3* ortholog, similarly function in floral development in a common pathway, as expected (Suzaki et al. 2004; Chu et al. 2006; Suzaki et al. 2006; Suzaki et al. 2008; Suzaki et al. 2009), whereas a second rice CLE peptide gene, *FON2-LIKE CLE PROTEIN1 (FCP1)* controls stem cell proliferation independent of *FON1* (Suzaki et al. 2008). The activity of CLE peptides can be assayed using synthetic peptide treatments, which suppress the growth of the maize SAM and root apical meristem (Bommert et al. 2013a; Je et al. 2016), as in *Arabidopsis* (Ito et al. 2006; Kondo et al. 2006).

How specificity is achieved is a common question in signal transduction pathways. Recently, we identified a distinct CLV receptor, *FASCIATED EAR3 (FEA3)* in maize and *Arabidopsis*, and found that FEA3 controls responses to the maize FCP1 (ZmFCP1) CLE peptide (Je et al. 2016). Here, we show that the maize CLV2 ortholog FEA2 also participates in ZmFCP1 signaling, in addition to controlling responses to the maize CLV3 ortholog, ZmCLE7 (Je et al. 2016). To ask how specificity from these different CLE peptide inputs is achieved, we first isolated mutant alleles of the maize *CRN* gene. Consistent with results in *Arabidopsis* (Miwa et al. 2008; Muller et al. 2008; Bleckmann et al. 2010; Zhu et al. 2010; Nimchuk et al. 2011a), we found that *fea2* was epistatic to *Zmcrn* in control of meristem size, but *Zmcrn;ct2* double mutants showed an additive enhanced phenotype, suggesting they act in parallel pathways, despite the fact that FEA2 binds both ZmCRN and CT2 in co-immunoprecipitation (co-IP) experiments. Strikingly, *ct2* and *Zmcrn* mutants were resistant to different CLE peptides, ZmCLE7 and

ZmFCP1, respectively, but *fea2* was resistant to both, suggesting that FEA2 controls responses to different CLE peptides by acting through different downstream effectors.

Results

Both *fea3* and *fea2* mutants are resistant to the ZmFCP1 peptide

We recently described a new CLE signaling pathway in maize, in which ZmFCP1 peptide signals through FEA3 to restrict ZmWUS1 expression from below its organizing center expression domain (Je et al. 2016). To test this model, we used a 2-component transactivation system (Wu et al. 2013; Je et al. 2016) to drive ZmFCP1 expression in developing primordia, below the ZmWUS1 domain (Je et al. 2016; Nardmann and Werr 2006). As previously described, this expression reduced meristem size of wild type SAMs (Je et al. 2016), however we found that meristem size was only partially rescued when *ZmFCP1* expression was transactivated in a *fea3* mutant background (**Figure 1A and B**), suggesting that ZmFCP1 signals through additional receptors. We therefore conducted peptide response assays using *fea2* mutants, and found that they were also insensitive to ZmFCP1 peptide treatment, as well as to ZmCLE7, the maize CLV3 ortholog (**Figure 1C**) (Je et al. 2016). Interestingly, *fea2;fea3* double mutants restored the size of ZmFCP1 treated meristems to control levels, suggesting that ZmFCP1 signaling is transmitted predominantly through both

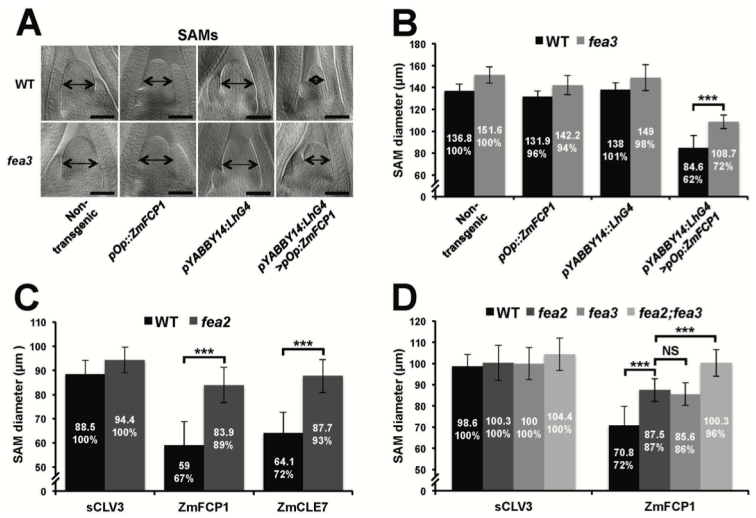

Figure 1. Both *fea3* and *fea2* mutants are resistant to ZmFCP1 peptide. (A) Transactivation of ZmFCP1 in primordia using a pYABBY14:LhG4 driver led to a strong reduction in vegetative SAM size as compared to a non-transgenic control, but this effect was only partially rescued in a *fea3* mutant background; SAM diameter was quantified (B). In CLE peptide treatments, *fea2* mutants were resistant to ZmFCP1, as well as to ZmCLE7 (C), and *fea3;fea2* double mutants showed additive resistance to ZmFCP1, restoring SAM size to normal (D). Scale bars; 100 µm in A. n = 20 (B, C) and 30 (D) plants for each genotype. Data in B, C and D are shown as means ± s.d. and shown as absolute value as well as % with each untreated control set to 100%: ***P value < 0.0001, two-tailed, two-sample t test.

FEA2 and FEA3 (**Figure 1D**). *fea3* mutants are resistant only to ZmFCP1, and not to ZmCLE7 (Je et al. 2016), so we next asked how FEA2 might transmit signals from different CLE peptides.

Zmcrn mutants are fasciated

In maize, FEA2 signals through CT2, the alpha subunit of the heterotrimeric G protein (Bommert et al. 2013a), but in *Arabidopsis* the FEA2 ortholog CLV2 is thought to signal through a membrane bound pseudokinase, CRN (Miwa et al. 2008; Muller et al. 2008; Bleckmann et al. 2010; Zhu et al. 2010; Nimchuk et al. 2011a). To ask if CRN also functions in CLV signaling in maize, we identified maize CRN (ZmCRN) by phylogenic analysis (**Figure supplement 1A**). As is the case for *Arabidopsis CRN*, *ZmCRN* was also predicted to encode an inactive pseudokinase (**Figure supplement 1B**) (Boudeau et al. 2006; Nimchuk et al. 2011a). We identified a predicted null allele as a *Mu* transposon insertion from the Trait Utility System in Corn (TUSC) resource (McCarty and Meeley 2009), 52bp downstream of the predicted translation start site (**Figure 2A**). We backcrossed

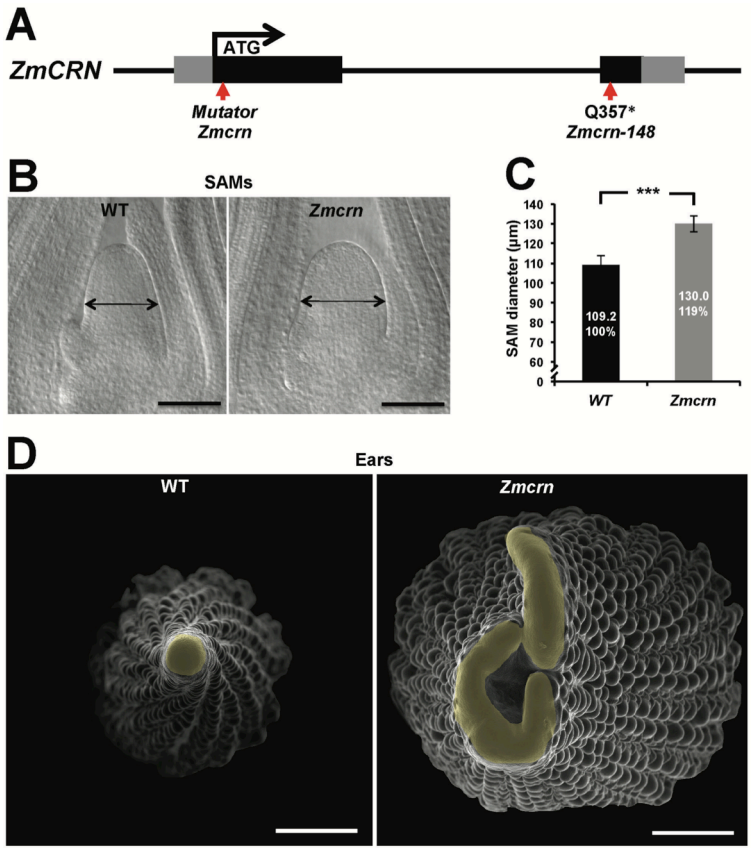

Figure 2. *Zmcrn* mutants develop fasciated ears. (A) Schematic of the *Zmcrn* mutant alleles. The arrows indicate the position of the Mutator transposon insertion and premature stop codon mutation. (B) Cleared SAMs from wild type (WT) and *Zmcrn* plants. The *Zmcrn* SAM has a larger diameter (double-headed arrows), SAM diameter was quantified (C). (D) Scanning electron microscopy images of WT and *Zmcrn* ear primordia (inflorescence meristems in yellow). The *Zmcrn* ear shows an enlarged and fasciated inflorescence meristem. Scale bar: 100 µm in B, 500 µm in D. n = 30 (C) plants for each genotype. Data in C are shown as means ± s.d.: ***P value < 0.0001, two-tailed, two-sample t test.

this *Mu* insertion line 3 times to the standard B73 inbred line, and dissected homozygous mutant or normal sib samples for meristem analysis. The maize *crn* (*Zmcrn*) mutants had larger vegetative shoot meristems (130 ± 4.1 μm, compared to 109 ± 4.6 μm for normal sibs, P value <0.0001, two-tailed t test, **Figure 2B and C**), and developed fasciated ear primordia with enlarged and split inflorescence meristems (**Figure 2D**), reminiscent of other *fasciated ear* mutants (Taguchi-Shiobara et al. 2001; Bommert et al. 2005; Bommert et al. 2013a; Je et al. 2016). Concurrently, we identified a second candidate allele by map-based cloning of a fasciated mutant, *fea*148* (**Figure supplement 2A**), from an ethyl methyl sulfonate (EMS) screen in the B73 background (hereafter *Zmcrn-148*). *Zmcrn-148* introduced a stop codon within the predicted pseudokinase domain (**Figure 2A**), and plants homozygous for this mutation developed a similar fasciated ear phenotype (**Figure supplement 2C**). We next crossed heterozygous *Zmcrn-148* plants with *Zmcrn* mutants. The F1 plants developed fasciated ears, while *Zmcrn/+* or *Zmcrn-148 /+* heterozygotes had normal ear primordia, suggesting that these mutations are allelic (**Figure supplement 3**), and confirming that *CRN* functions in shoot meristem size control in maize, similar to its role in *Arabidopsis*. Next, since *fea2* and other *fea* mutants are is associated with quantitative variation in kernel row number (KRN) (Bommert et al. 2013b), we took advantage of the identification of *ZmCRN* to ask if it is also associated with this yield trait. We conducted a candidate gene association study using a maize association panel of 368 diverse inbred lines (Li et al. 2013; Liu et al. 2015). We found that three SNPs in the 3'UTR region of *CRN* showed significant association with KRN in multiple environments, below the threshold P-value < 0.001 (**Figure supplement 4 and Table supplement 1**). These results suggest that natural variation in *ZmCRN* may underlie subtle variation in inflorescence meristem size sufficient to enhance KRN, with the potential to benefit maize yields.

ZmCRN and *FEA2* function in a common pathway

In *Arabidopsis*, CRN is thought to signal downstream of CLV2 and correspondingly the double mutants show an epistatic interaction (Muller et al. 2008). To ask if this relationship was conserved in

maize, we measured the SAM size in a segregating double mutant population. As expected, both *Zmcrn* and *fea2* vegetative meristems were larger than normal (166.3 ± 8.3 μm, or 176.1 ± 9.8 μm respectively, compared to 139.7 ± 4.8 μm for normal sibs, P value <0.0001, two-tailed t test, **Figure 3A and B**), and the *Zmcrn; fea2* double mutants (177.2 ± 13.3 μm) were similar to the *fea2* single mutants (**Figure 3A and B**). We also characterized ear inflorescence meristems and found that *fea2* had stronger fasciated ears than those of *Zmcrn*, but the double mutants resembled *fea2* single mutants (**Figure 3C**). Together, these results indicate that *fea2* is epistatic to *Zmcrn,* suggesting that *FEA2* and *ZmCRN* function in a common pathway in maize, as in *Arabidopsis*.

ZmCRN and *CT2* function in different pathways

We next asked if *ZmCRN* and *CT2* function in the same or in different pathways, again by double mutant analysis. Both *Zmcrn* and *ct2* mutants had larger SAMs compared with their normal sibs (161.5 ± 10.6 μm, or 157.1 ± 11.8 μm respectively, compared to 139.7 ± 8.5 μm for normal sibs, P value <0.0001, two-tailed t test, **Figure 3D and E**), but the SAMs of double mutants were significantly larger than each single mutant (191.8 ±

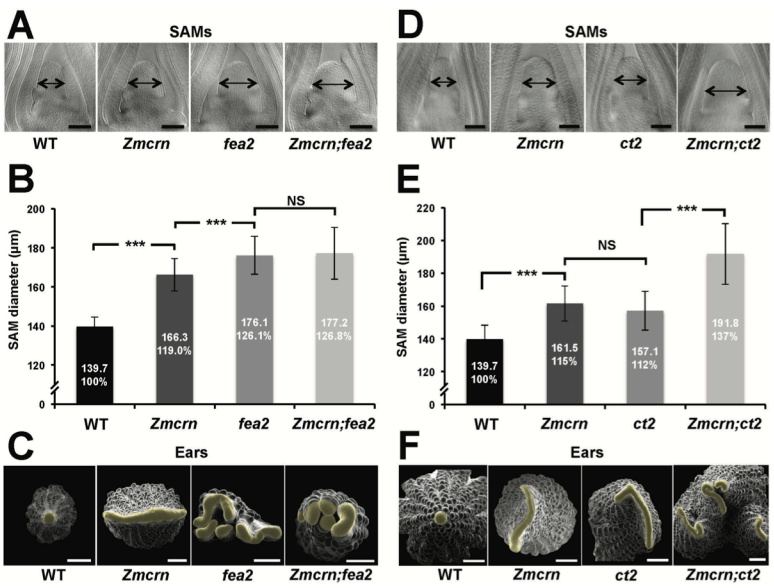

Figure 3. ZmCRN acts in a common pathway with FEA2, but not with CT2. (A) Cleared SAMs from wild type (WT), *Zmcrn*, *fea2*, and *Zmcrn;fea2* double-mutant plants. SAMs from *Zmcrn* and *fea2* plants were significantly wider than in wild type (double-headed arrows), but SAM size was not significantly different between *fea2* and *Zmcrn;fea2* double mutants, SAM diameter was quantified (B). (C) Ear meristems of *fea2;Zmcrn* double mutants resemble *fea2* single mutants. (D) Cleared SAMs from wild type, *Zmcrn*, *ct2*, and *Zmcrn;ct2* double-mutant plants. SAMs from *Zmcrn* and *ct2* plants were significantly wider than in wild type, and were additively increased in *Zmcrn;ct2* double mutants; SAM diameter was quantified (E). (F) *Zmcrn;ct2* double mutants had enhanced fasciation of ear primordia. Scale bars: 100 μm in A and D, 500 μm in C and F. n = 30 (B, E) plants for each genotype. Data in B and E are shown as means ± s.d.: ***P value < 0.0001, two-tailed, two-sample t test, NS, not significant.

18.6 µm, P value <0.0001, two-tailed t test, **Figure 3D and E**), suggesting an additive interaction. *Zmcrn; ct2* double mutant ear inflorescences also showed additive enhancement in fasciation, compared to each single mutant (**Figure 3F**), confirming the additive interaction between *ct2* and *Zmcrn*. In summary, double mutant analyses and quantification of meristem sizes indicated that *ZmCRN* functions in the same pathway as *FEA2* and, as previously reported, *CT2* also functions in the same pathway as *fea2* (Bommert et al. 2013a), but *CT2* and *ZmCRN* themselves function in different pathways. This result is most easily explained by the hypothesis that *FEA2* functions in two different pathways, one with *CT2* and a second with *ZmCRN*.

FEA2 interacts physically with CT2 and with ZmCRN

To test the two-pathway hypothesis, we tested protein-protein interactions using co-IP assays. We used an internal YFP fusion of CT2 that we found to be biologically active (Bommert et al. 2013a), and C terminal mCherry or Myc fusions of ZmCRN or FEA2, respectively, which are predicted to be correctly localized and active, based on similar fusions (Bleckmann et al. 2010; Nimchuk 2017). We first confirmed the expected plasma-membrane localization of ZmCRN-mCherry by transient expression and plasmolysis (**Figure 4A**), consistent with FEA2 and CT2 localization (Bommert et al. 2013a). We then tested pairwise interactions using co-IP experiments following transient expression. ZmCRN-mCherry

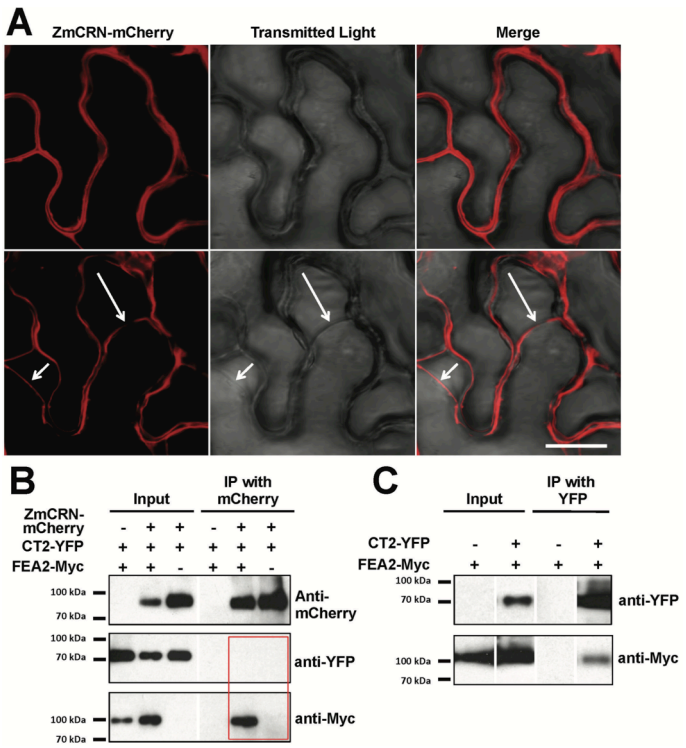

Figure 4. FEA2 is present in 2 different complexes. (A) ZmCRN-mCherry was localized at the plasmamembrane following tobacco transient expression (top), and in subsequent plasmolysis (bottom). In transient expression followed by immunoprecipitation (IP) assay, ZmCRN-mCherry could IP FEA2-Myc, but not CT2-YFP (B), however CT2-YFP was able to IP FEA2-Myc, as expected (C). Scale bar: 20 µm in A.

was able to pull down FEA2-Myc, but not CT2-YFP, even when FEA2-YFP was also co-expressed (**Figure 4B**). We confirmed that CT2-YFP was properly expressed, because it could pull down FEA2-Myc (**Figure 4C**), as previously demonstrated in *in vivo* co-IPs (Bommert et al. 2013a). In summary, the FEA2 receptor-like protein interacted with both candidate signaling molecules, ZmCRN and CT2, but these interactions appeared to be in different protein complexes, rather than in a common complex, because ZmCRN was not able to immunoprecipitate CT2.

ct2 and *Zmcrn* show differential sensitivity to ZmCLE7 and ZmFCP1 peptides

Next, we tested the sensitivity of each mutant to different CLE peptides, using embryo culture, as previously described (Bommert et al. 2013a; Je et al. 2016). *ct2* or *Zmcrn* segregating populations were grown in the presence of different peptides, and shoots fixed and cleared for SAM measurements after 12 days. We found that *ct2* mutants were partially resistant to ZmCLE7, but not to ZmFCP1 peptide (**Figure 5A and B**), suggesting that CT2 functions specifically in signaling by ZmCLE7, the maize CLV3 ortholog. In contrast, we found that *Zmcrn* mutants were partially resistant to ZmFCP1, but not to ZmCLE7 (**Figure 5C and D**), suggesting that ZmCRN functions specifically in a

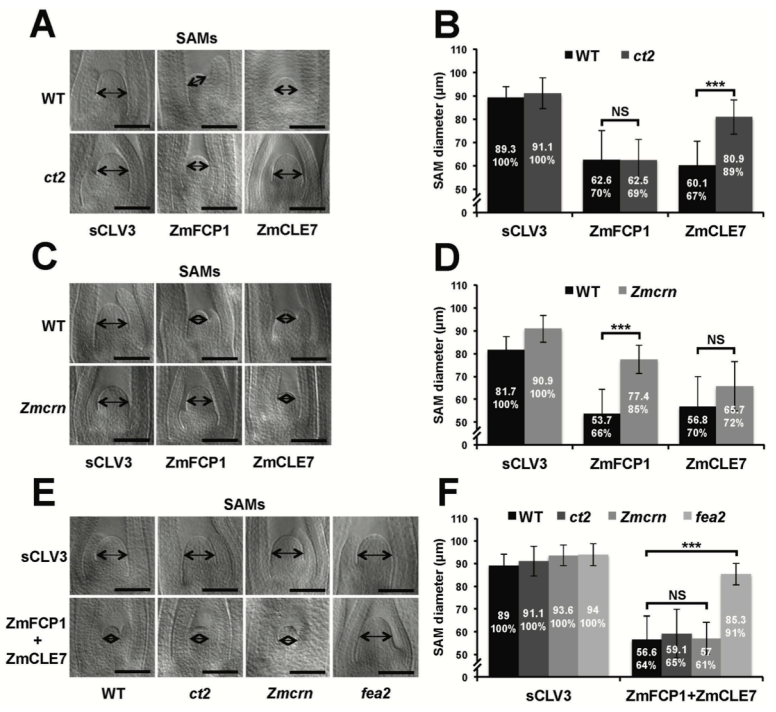

Figure 5. *ct2* and *Zmcrn* show different sensitivity to ZmCLE7 and ZmFCP1 peptides. Embryos of each genotype were cultured with control, scrambled peptide (sCLV3) or with ZmFCP1 or ZmCLE7. Wild type SAM growth (double-headed arrows) was strongly inhibited by all peptides except sCLV3, and *ct2* growth was insensitive only to ZmCLE7 peptide (A), whereas *Zmcrn* was insensitive only to ZmFCP1 peptide (C); SAM diameter was quantified (B, D). In treatments with both ZmFCP1 and ZmCLE7, only *fea2* showed resistance, but *Zmcrn* or *ct2* did not. Scale bars: 100 µm in A, C and E. N = 25 (C) plants for each genotype. Data in B, D and F are shown as means ± s.d.: ***P value < 0.0001, two-tailed, two-sample t test, NS, not significant

ZmFCP1 signaling pathway. To confirm these results, we treated each mutant with both ZmCLE7 and ZmFCP1 together. We found that only *fea2,* but not *ct2 or Zmcrn* mutants, showed resistance to the double peptide treatment (**Figure 5E and F**). Together, these results suggest that FEA2 functions in both ZmCLE7 and ZmFCP1 signaling pathways, but CT2 and ZmCRN function specifically in ZmCLE7 or in ZmFCP1 signaling, respectively.

In summary, through identification of maize *crn* mutants, we were able to show that signaling through FEA2 by two different CLE peptides is differentiated using different candidate downstream signaling components; with the ZmCLE7 signal passing through CT2 and the ZmFCP1 signal passing through ZmCRN (**Figure 6**).

Discussion

A major question in signal transduction is how multiple inputs can be translated into distinct outputs. CLV-WUS feedback signaling is the central regulatory pathway in shoot meristem development, and perception of CLV3 peptide involves the CLV1 receptor-like kinase and the CLV2 receptor-like protein together with the CRN pseudokinase (Brand et al. 2000; Schoof et al. 2000; Miwa et al. 2008; Muller et al. 2008; Bleckmann et al. 2010; Zhu et al. 2010; Nimchuk et al. 2011a). However, genetic evidence in both maize and *Arabidopsis* suggests these receptors function independently, and CLV2, and its maize ortholog FEA2, respond to multiple CLE peptides (Bommert et al. 2005; Fiers et al. 2005; Muller et al.

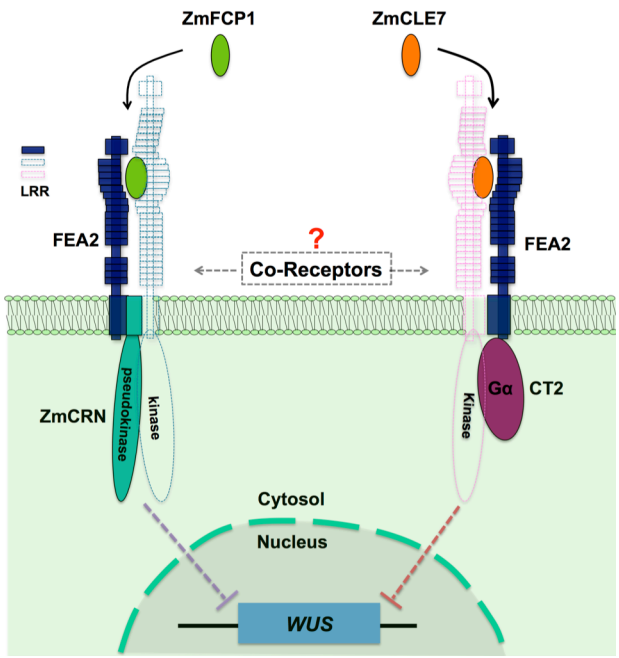

Figure 6. Hypothetical model for FEA2 signaling through two different pathways. Two different peptides, ZmFCP1 and ZmCLE7, are proposed to bind to two separate FEA2 receptor complexes, and the two signals are differentially transmitted to downstream components; with the ZmCLE7 signal passing through CT2, and the ZmFCP1 signal passing through ZmCRN.

2008; Guo et al. 2010; Meng and Feldman 2010; Je et al. 2016; Hazak et al. 2017). So how is the information conferred by these different signals kept separate during transmission through a common receptor?

To address this question and further decipher the FEA2 signaling pathway, we isolated mutants in the maize *CRN* ortholog, *ZmCRN,* by reverse genetics and by cloning a newly identified fasciated ear mutant *fea*148*. *ZmCRN* was predicted to encode a membrane localize pseudokinase, like *CRN* in *Arabidopsis* (Nimchuk et al. 2011a), and characterization of the mutants indicated that *ZmCRN* similarly functions as a negative regulator of stem cell proliferation. We found that *fea2* was epistatic to *Zmcrn*, and FEA2 and ZmCRN interacted physically, suggesting that ZmCRN is a signaling component in the FEA2 pathway. Natural variation in the CLV-WUS pathway underlies yield improvements in different crop species including tomato, maize and mustard (Bommert et al. 2013b; Fan et al. 2014; Xu et al. 2015; Je et al. 2016), and FEA2 is a quantitative trait locus (QTL) for kernel row number (KRN) (Bommert et al. 2013b). In this study, we used a maize association panel of 368 diverse inbred lines to show that *ZmCRN* also has significant association with KRN under multiple environments (Li et al. 2013; Liu et al. 2015), suggesting that *ZmCRN* contributes to quantitative variation in this trait. Therefore, ZmCRN could be manipulated for maize yield enhancement.

Previously, we identified the alpha subunit of the heterotrimeric G protein, CT2, as an additional interactor of FEA2. *fea2* is epistatic to *ct2* in meristem regulation, similar to its genetic interaction with *Zmcrn*, and FEA2 interacts with CT2 *in vivo*, revealing that *CT2*, like *ZmCRN*, is a candidate downstream signaling component of *FEA2* (Bommert et al. 2013a). Although *fea2* is epistatic both to *ct2* and to *Zmcrn*, we found that *ct2; Zmcrn* double mutants have an additive interaction, suggesting they function in parallel, and that the FEA2 signaling pathway branches into these 2 different downstream components. This idea was supported by peptide assays in different mutants, which suggested that ZmCRN and CT2 function specifically in ZmFCP1 or ZmCLE7 signaling, respectively, while FEA2 is involved in both. These data suggest a novel mechanism in plant receptor signaling, where a single receptor, FEA2, can transmit signals from two different CLE peptides, ZmFCP1 and ZmCLE7, through two different downstream

components, ZmCRN and CT2. We thereby shed light on how distinct signaling by different peptides can be achieved through a common receptor. Our results are largely consistent with findings in Arabidopsis, that CRN is dispensable for CLV3 perception and signaling (Nimchuk 2017), and that CLV2/CRN can function with other CLE ligand-receptor complexes (Hazak et al. 2017). However, in Arabidopsis CRN is required for CLV2 trafficking to the plasmamembrane (Bleckmann et al. 2010). Our results suggest that the maize CLV2 ortholog FEA2 still functions (with CT2) in a *crn* mutant, so is presumably on the plasmamembrane even in the absence of ZmCRN.

How then can a single receptor recognize different signals and transmit them differentially? The most obvious answer depends on the idea that FEA2 and CLV2 are co-receptors that function with LRR RLKs, which binds CLE peptides directly (**Figure 6**). This idea is supported by the finding that CLV1 binds CLV3 with high affinity, but CLV2 is unable to bind CLE peptides (Shinohara and Matsubayashi 2015), and that CLV2/CRN can function with different CLE ligand-receptor complexes (Hazak et al. 2017). There are conflicting results surrounding the interaction between CLV2 and CLV1; some experiments detect their physical interaction, but many of them use over-expression and are prone to false positive results, and in double mutant combinations *clv2* and *clv1* mutants act additively (Kayes and Clark 1998; Muller et al. 2008). This genetic result suggests they act separately, and the same is true for the orthologs *FEA2* and *TD1* in maize (Bommert et al. 2005). A possible explanation for these conflicting findings is that CLV2 may act with multiple CLE receptor RLKs. This model is supported by the observation that CLV1 homologs, the BAMs, function redundantly with CLV1, so multiple LRR RLKs do indeed function in meristem size control. Why then are *clv2* phenotypes weaker than *clv1*? Maybe *clv2*, like *clv1*, has functional homologs. The use of CRISPR to make simultaneous multiplex gene knockouts should help solve these mysteries.

Despite not knowing the details of the CLE-receptor interactions, our data show that FEA2 can specifically transmit different peptide signals through two distinct downstream components that most likely converge on the regulation of ZmWUS expression to regulate stem cell proliferation in meristem development (**Figure 6**). This suggests a new working model for meristem size regulation, in which

ligand binding can be transmitted by a common co-receptor working with different RLKs coupled to distinct signaling proteins. Our model differs from most well-studied ligand-receptor signaling pathways, in which the signaling pathways usually converge (Couto and Zipfel 2016). For instance, different microbial ligands such as flagellin and Elongation Factor Thermo unstable (EF-Tu) are specifically recognized by the FLAGELLIN-SENSITIVE 2 (FLS2)-BRI1 ASSOCIATED RECEPTOR KINASE (BAK1) or EF-Tu RECEPTOR (EFR)-BAK1 RLK complexes, respectively, while signal transduction requires a shared set of cytosolic kinases, including BOTRYTIS-INDUCED KINASE 1 (BIK1) (Aarts et al. 1998; Lu et al. 2010). Nevertheless, a similar principle can be drawn from the different signaling pathways mediated by BAK1, which functions as a co-receptor for the brassinosteroid (BR) receptor, BR INSENSITIVE 1 (BRI1) or for FLS2. After ligand perception, BR signaling through the BAK1-BRI1 complex is transmitted through the receptor-like cytoplasmic kinase (RLCK) BRASSINOSTEROID-SIGNALING KINASE 1 (BSK1), and flagellin signaling through the BAK1-FLS2 complex is transmitted through a different RLCK, BIK1 (Li et al. 2002; Nam and Li 2002; Chinchilla et al. 2007; Lu et al. 2010; Wang 2012; Sun et al. 2013).

Our study also highlights another source of variation in meristem receptor signaling by highlighting the role of an additional CLE peptide, ZmFCP1. The role of FCP1 in meristem maintenance has been characterized in both maize and rice (Suzaki et al. 2008; Je et al. 2016).

In summary, multiple receptor signaling pathways appear to be required to for the perception of different CLE peptide signals to fine tune meristem development. This complex system of multiple peptides, receptors and downstream components presumably confers robustness on the meristem structure, as well as providing flexibility to control meristem development according to different physiological or developmental cues. For example, meristem size responds to stress and developmental transitions, such as floral induction, and different signaling pathways may confer such responsiveness. Our results help explain how meristem size regulation is orchestrated by multiple CLE peptides and receptors, as observed in many species including Arabidopsis, rice, maize and tomato (Ito et al. 2006; Strabala et al. 2006; Suzaki et al. 2009; Nimchuk et al. 2015; Xu et al. 2015). They also support the idea that meristem

signaling components are highly conserved between diverse plant species, and a major challenge is to understand how differential regulation of these common components leads to diversity in meristem organization and size across diverse plant taxa.

Materials and methods

Plant growth and map based cloning.

Maize plants were grown in the field or in the greenhouse. The *Zmcrn Mu* insertion allele was isolated from TUSC lines and was backcrossed 3 generations to the standard B73 inbred line. The *fea*148* allele was isolated in an EMS mutagenesis screen using F2 seed stocks prepared by Prof. Gerald Neuffer, derived from a cross of mutagenized B73 pollen onto A619 ears. One fasciated plant from the segregating *fea*148* M2 population from the maize GDB stock center was crossed to the A619 inbred, then selfed to make an F2 segregating population. Pooled DNAs from ~50 mutants or the same number of normal ear plants screened from the segregating F2 population were used for bulked segregant analysis (BSA) using a maize SNP50 chip (Illumina, Inc.). The BSA analysis revealed a clear linkage of the mutation on Chromosome 3 at 153-158 Mbp. As *ZmCRN* was an obvious candidate gene within the region, we sequenced the locus of *ZmCRN* using the mutant pool DNA and found a C to T mutation in the pseudokinase domain, which led to an early stop codon.

To measure meristem size, segregating siblings were genotyped and shoot apices of 7-day-old plants (**Figure 2B**) or 21-day-old plants (**Figure 3A and D**) were dissected, cleared and measured as described previously (Taguchi-Shiobara et al. 2001). All measurements included at least 10 samples of each genotype, and two or three independent biological replicates, and mean values ± s.d. were presented, with significance calculated using two-tailed, two-sample *t* tests, and significant differences reported as *P* values.

Imaging.

Scanning electron microscopy was performed on fresh tissues of maize using a Hitachi S-3500N SEM, as described (Taguchi-Shiobara et al. 2001). For confocal microscopy, tobacco infiltrated tissues were dissected and images were taken with a Zeiss LSM 710 microscope, using 561nm laser excitation and

580–675nm emission for detection ZmCRN-mCherry. Subsequently for plasmolysis, leaf tissues were incubated for 30 min with 800mM mannitol and imaged.

Double mutant analysis.

Double mutants were constructed by crossing mutants introgressed into B73, followed by selfing or backcrossing to the F1. All plants were subsequently genotyped (primers are listed in S2 Table).

Protein expression and co-IP assays.

CT2-YFP, *ZmCRN-mCherry*, or *FEA2-Myc* expression constructs were infiltrated into 4-week-old *Nicotiana benthamiana* leaves together with a P19 plasmids to suppress posttranscriptional silencing (Mohammadzadeh et al. 2016). The protein extraction and membrane fraction enrichment were described in Bommert et al., 2013. Briefly, the infiltrated leaves were harvested 3-d post infiltration. The leaf tissues were ground in liquid nitrogen to a fine powder then suspended in twice the volume of protein extraction buffer containing 150 mM NaCl, 50 mM Tris-HCl pH 7.6, 5% glycerol, and EDTA-free Protease inhibitor cocktail (Roche). After filtration through Miracloth, and centrifugation at 4,000g for 10 min at 4°C, the extract was centrifuged at 100,000g for 1h at 4°C to enrich the microsomal membrane fraction. The resulting pellet was resuspended in the extraction buffer supplemented with 1% Triton X-100. Lysates were cleared by centrifugation at 100,000g for 30 min at 4°C to remove non-solubilized material. ZmCRN-mCherry was immunoprecipitated uisng RFP-Trap (Chromotek) in membrane solubilization buffer for 40 min followed by washing 3 times with 1 ml of the same buffer. The IP'd proteins were eluted with 50 μl 1xSDS loading buffer at 95°C, followed by standard SDS-PAGE electrophoresis and western blotting. CRN-mCherry was detected using an anti-RFP antibody (Rockland, 600-401-379), FEA2-Myc was detected using an anti-Myc antibody (Millipore, 05-724), and CT2-YFP was detected using an anti-GFP antibody (Roche, 11814460001). The co-IP experiment between ZmCRN-mCherry and FEA3-Myc was performed by the same protocol.

Peptide assays.

Maize embryos segregating for each mutant were dissected at 10 days after pollination, when the SAM was exposed, and cultured on gel media (Bommert et al. 2013a) containing scrambled peptide (30μM; Genscript) or ZmFCP1 peptide or ZmCLE7 peptide or a mixture of ZmCLE7 and ZmFCP1 peptides (Je et al. 2016). After 12 days, the tissues were harvested for genotyping and the embryos were fixed in FAA (10%, formalin, 5% acetic acid, 45% ethanol) and cleared in methyl salicylate, and SAMs measured by microscopy, as described (Je et al. 2016). Experiments used at least 10 embryos per genotype, and were replicated in triplicate.

Two-components transactivation assay.

The two-component transactivation assay was performed as described (Je et al. 2016), and the lines were backcrossed into the *fea3* mutant background. To measure meristem size, segregating siblings were genotyped and shoot apical meristems of 14-day-old plants (**Figure 1A**) were dissected, cleared and measured as described previously (Taguchi-Shiobara et al. 2001).

Association analysis of the *ZmCRN* locus.

The candidate gene association analysis of *ZmCRN* with the kernel row number (KRN) trait was conducted in a maize association panel with 368 diverse inbred lines (Li et al. 2013). 22 SNPs in the *ZmCRN* gene region were observed based on previously released genotypes in the association panel. This was combined with KRN phenotypic data from five environments and BLUP (Best Linear Unbiased Prediction) data, including in Ya'an (30°N, 103°E), Sanya (18°N, 109°E) and Kunming (25°N, 102°E) in 2009 and Wuhan (30°N, 114°E) and Kunming (25°N, 102°E) in 2010 (Liu et al. 2015). The association between *ZmCRN* and KRN was established by a mixed linear model corrected by population structure, with P-value < 0.001 as threshold (Zhang et al. 2010; Li et al. 2013).

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Byoung Il Je, Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing—original draft, Writing—review and editing; Fang Xu, Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Writing—review and editing; Qingyu Wu, Data curation, Formal analysis, Investigation, Methodology; Lei Liu, Data curation, Methodology; Robert Meeley, Conceptualization, Formal analysis, Methodology; David Jackson, Conceptualization, Data curation, Formal analysis, Supervision, Funding acquisition, Investigation, Methodology, Writing—original draft, Writing—review and editing.

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Additional files

Figure supplements

. Figure supplement 1. Phylogeny of CRN related proteins, and ZmCRN features of a pseudokinase.

. Figure supplement 2. Mapping of the *fea*148* fasciated ear mutant.

. Figure supplement 3. *Zmcrn/ Zmcrn-148* F1 plants develop fasciated ears.

. Figure supplement 4. The association of *ZmCRN* locus with kernel row number.

Table supplements

. Table supplement 1. The *P*-value of the association between ZmCRN SNPs with kernel row number in multiple environments.

. Table supplement 2. List of Primers.

References

Aarts N, Metz M, Holub E, Staskawicz BJ, Daniels MJ, Parker JE. 1998. Different requirements for EDS1 and NDR1 by disease resistance genes define at least two R gene-mediated signaling pathways in Arabidopsis. *Proc Natl Acad Sci U S A* **95**: 10306-10311.

Bleckmann A, Weidtkamp-Peters S, Seidel CA, Simon R. 2010. Stem cell signaling in Arabidopsis requires CRN to localize CLV2 to the plasma membrane. *Plant physiology* **152**: 166-176.

Bommert P, Je BI, Goldshmidt A, Jackson D. 2013a. The maize Galpha gene COMPACT PLANT2 functions in CLAVATA signalling to control shoot meristem size. *Nature* **502**: 555-558.

Bommert P, Lunde C, Nardmann J, Vollbrecht E, Running M, Jackson D, Hake S, Werr W. 2005. thick tassel dwarf1 encodes a putative maize ortholog of the Arabidopsis CLAVATA1 leucine-rich repeat receptor-like kinase. *Development* **132**: 1235-1245.

Bommert P, Nagasawa NS, Jackson D. 2013b. Quantitative variation in maize kernel row number is controlled by the FASCIATED EAR2 locus. *Nature genetics* **45**: 334-337.

Boudeau J, Miranda-Saavedra D, Barton GJ, Alessi DR. 2006. Emerging roles of pseudokinases. *Trends Cell Biol* **16**: 443-452.

Brand U, Fletcher JC, Hobe M, Meyerowitz EM, Simon R. 2000. Dependence of stem cell fate in Arabidopsis on a feedback loop regulated by CLV3 activity. *Science* **289**: 617-619.

Chinchilla D, Zipfel C, Robatzek S, Kemmerling B, Nürnberger T, Jones JD, Felix G, Boller T. 2007. A flagellin-induced complex of the receptor FLS2 and BAK1 initiates plant defence. *Nature* **448**: 497-500.

Chu H, Qian Q, Liang W, Yin C, Tan H, Yao X, Yuan Z, Yang J, Huang H, Luo D et al. 2006. The floral organ number4 gene encoding a putative ortholog of Arabidopsis CLAVATA3 regulates apical meristem size in rice. *Plant physiology* **142**: 1039-1052.

Clark SE, Running MP, Meyerowitz EM. 1993. Clavata1, a Regulator of Meristem and Flower Development in Arabidopsis. *Development* **119**: 397-418.

Clark SE, Williams RW, Meyerowitz EM. 1997. The CLAVATA1 gene encodes a putative receptor kinase that controls shoot and floral meristem size in Arabidopsis. *Cell* **89**: 575-585.

Couto D, Zipfel C. 2016. Regulation of pattern recognition receptor signalling in plants. *Nat Rev Immunol* **16**: 537-552.

Daum G, Medzihradszky A, Suzaki T, Lohmann JU. 2014. A mechanistic framework for noncell autonomous stem cell induction in Arabidopsis. *Proceedings of the National Academy of Sciences of the United States of America* **111**: 14619-14624.

DeYoung BJ, Bickle KL, Schrage KJ, Muskett P, Patel K, Clark SE. 2006. The CLAVATA1-related BAM1, BAM2 and BAM3 receptor kinase-like proteins are required for meristem function in Arabidopsis. *Plant J* **45**: 1-16.

Deyoung BJ, Clark SE. 2008. BAM receptors regulate stem cell specification and organ development through complex interactions with CLAVATA signaling. *Genetics* **180**: 895-904.

Fan C, Wu Y, Yang Q, Yang Y, Meng Q, Zhang K, Li J, Wang J, Zhou Y. 2014. A novel single-nucleotide mutation in a CLAVATA3 gene homolog controls a multilocular silique trait in Brassica rapa L. *Mol Plant* **7**: 1788-1792.

Fiers M, Golemiec E, Xu J, van der Geest L, Heidstra R, Stiekema W, Liu CM. 2005. The 14-amino acid CLV3, CLE19, and CLE40 peptides trigger consumption of the root meristem in Arabidopsis through a CLAVATA2-dependent pathway. *The Plant cell* **17**: 2542-2553.

Fletcher JC, Brand U, Running MP, Simon R, Meyerowitz EM. 1999. Signaling of cell fate decisions by CLAVATA3 in Arabidopsis shoot meristems. *Science* **283**: 1911-1914.

Guo Y, Han L, Hymes M, Denver R, Clark SE. 2010. CLAVATA2 forms a distinct CLE-binding receptor complex regulating Arabidopsis stem cell specification. *Plant J* **63**: 889-900.

Hanemian M, Barlet X, Sorin C, Yadeta KA, Keller H, Favery B, Simon R, Thomma BP, Hartmann C, Crespi M et al. 2016. Arabidopsis CLAVATA1 and CLAVATA2 receptors contribute to Ralstonia solanacearum pathogenicity through a miR169-dependent pathway. *The New phytologist* **211**: 502-515.

Hazak O, Brandt B, Cattaneo P, Santiago J, Rodriguez-Villalon A, Hothorn M, Hardtke CS. 2017. Perception of root-active CLE peptides requires CORYNE function in the phloem vasculature. *EMBO Rep*.

Ito Y, Nakanomyo I, Motose H, Iwamoto K, Sawa S, Dohmae N, Fukuda H. 2006. Dodeca-CLE peptides as suppressors of plant stem cell differentiation. *Science* **313**: 842-845.

Je BI, Gruel J, Lee YK, Bommert P, Arevalo ED, Eveland AL, Wu Q, Goldshmidt A, Meeley R, Bartlett M et al. 2016. Signaling from maize organ primordia via FASCIATED EAR3 regulates stem cell proliferation and yield traits. *Nature genetics* **48**: 785-791.

Jeong S, Trotochaud AE, Clark SE. 1999. The Arabidopsis CLAVATA2 gene encodes a receptor-like protein required for the stability of the CLAVATA1 receptor-like kinase. *The Plant cell* **11**: 1925-1934.

Kayes JM, Clark SE. 1998. CLAVATA2, a regulator of meristem and organ development in Arabidopsis. *Development* **125**: 3843-3851.

Kinoshita A, Betsuyaku S, Osakabe Y, Mizuno S, Nagawa S, Stahl Y, Simon R, Yamaguchi-Shinozaki K, Fukuda H, Sawa S. 2010. RPK2 is an essential receptor-like kinase that transmits the CLV3 signal in Arabidopsis (vol 137, pg 3911, 2010). *Development* **137**: 4327-4327.

Kondo T, Sawa S, Kinoshita A, Mizuno S, Kakimoto T, Fukuda H, Sakagami Y. 2006. A plant peptide encoded by CLV3 identified by in situ MALDI-TOF MS analysis. *Science* **313**: 845-848.

Li H, Peng Z, Yang X, Wang W, Fu J, Wang J, Han Y, Chai Y, Guo T, Yang N et al. 2013. Genome-wide association study dissects the genetic architecture of oil biosynthesis in maize kernels. *Nature genetics* **45**: 43-50.

Li J, Wen J, Lease KA, Doke JT, Tax FE, Walker JC. 2002. BAK1, an Arabidopsis LRR receptor-like protein kinase, interacts with BRI1 and modulates brassinosteroid signaling. *Cell* **110**: 213-222.

Liu L, Du Y, Huo D, Wang M, Shen X, Yue B, Qiu F, Zheng Y, Yan J, Zhang Z. 2015. Genetic architecture of maize kernel row number and whole genome prediction. *Theor Appl Genet* **128**: 2243-2254.

Lu D, Wu S, Gao X, Zhang Y, Shan L, He P. 2010. A receptor-like cytoplasmic kinase, BIK1, associates with a flagellin receptor complex to initiate plant innate immunity. *Proc Natl Acad Sci U S A* **107**: 496-501.

Mayer KF, Schoof H, Haecker A, Lenhard M, Jurgens G, Laux T. 1998. Role of WUSCHEL in regulating stem cell fate in the Arabidopsis shoot meristem. *Cell* **95**: 805-815.

McCarty DR, Meeley RB. 2009. Transposon resources for forward and reverse genetics in maize. in *Handbook of Maize: Genetics and Genomics* (eds. JL Bennetzen, SC Hake), pp. 561-584, Springer.

Meng L, Feldman LJ. 2010. CLE14/CLE20 peptides may interact with CLAVATA2/CORYNE receptor-like kinases to irreversibly inhibit cell division in the root meristem of Arabidopsis. *Planta* **232**: 1061-1074.

Miwa H, Betsuyaku S, Iwamoto K, Kinoshita A, Fukuda H, Sawa S. 2008. The receptor-like kinase SOL2 mediates CLE signaling in Arabidopsis. *Plant & cell physiology* **49**: 1752-1757.

Mizuno S, Osakabe Y, Maruyama K, Ito T, Osakabe K, Sato T, Shinozaki K, Yamaguchi-Shinozaki K. 2007. Receptor-like protein kinase 2 (RPK 2) is a novel factor controlling anther development in Arabidopsis thaliana. *Plant J* **50**: 751-766.

Mohammadzadeh S, Roohvand F, Memarnejadian A, Jafari A, Ajdary S, Salmanian AH, Ehsani P. 2016. Co-expression of hepatitis C virus polytope-HBsAg and p19-silencing suppressor protein in tobacco leaves. *Pharm Biol* **54**: 465-473.

Muller R, Bleckmann A, Simon R. 2008. The receptor kinase CORYNE of Arabidopsis transmits the stem cell-limiting signal CLAVATA3 independently of CLAVATA1. *The Plant cell* **20**: 934-946.

Nam KH, Li J. 2002. BRI1/BAK1, a receptor kinase pair mediating brassinosteroid signaling. *Cell* **110**: 203-212.

Nardmann J, Werr W. 2006. The shoot stem cell niche in angiosperms: Expression patterns of WUS orthologues in rice and maize imply major modifications in the course of mono- and dicot evolution. *Mol Biol Evol* **23**: 2492-2504.

Nimchuk ZL. 2017. CLAVATA1 controls distinct signaling outputs that buffer shoot stem cell proliferation through a two-step transcriptional compensation loop. *Plos Genet* **13**.

Nimchuk ZL, Tarr PT, Meyerowitz EM. 2011a. An evolutionarily conserved pseudokinase mediates stem cell production in plants. *The Plant cell* **23**: 851-854.

Nimchuk ZL, Tarr PT, Ohno C, Qu XA, Meyerowitz EM. 2011b. Plant Stem Cell Signaling Involves Ligand-Dependent Trafficking of the CLAVATA1 Receptor Kinase. *Curr Biol* **21**: 345-352.

Nimchuk ZL, Zhou Y, Tarr PT, Peterson BA, Meyerowitz EM. 2015. Plant stem cell maintenance by transcriptional cross-regulation of related receptor kinases. *Development* **142**: 1043-1049.

Nodine MD, Yadegari R, Tax FE. 2007. RPK1 and TOAD2 are two receptor-like kinases redundantly required for arabidopsis embryonic pattern formation. *Developmental cell* **12**: 943-956.

Ogawa M, Shinohara H, Sakagami Y, Matsubayashi Y. 2008. Arabidopsis CLV3 peptide directly binds CLV1 ectodomain. *Science* **319**: 294.

Ohyama K, Shinohara H, Ogawa-Ohnishi M, Matsubayashi Y. 2009. A glycopeptide regulating stem cell fate in Arabidopsis thaliana. *Nat Chem Biol* **5**: 578-580.

Pan L, Lv S, Yang N, Lv Y, Liu Z, Wu J, Wang G. 2016. The Multifunction of CLAVATA2 in Plant Development and Immunity. *Front Plant Sci* **7**: 1573.

Replogle A, Wang J, Bleckmann A, Hussey RS, Baum TJ, Sawa S, Davis EL, Wang X, Simon R, Mitchum MG. 2011. Nematode CLE signaling in Arabidopsis requires CLAVATA2 and CORYNE. *Plant J* **65**: 430-440.

Rojo E, Sharma VK, Kovaleva V, Raikhel NV, Fletcher JC. 2002. CLV3 is localized to the extracellular space, where it activates the Arabidopsis CLAVATA stem cell signaling pathway. *The Plant cell* **14**: 969-977.

Schoof H, Lenhard M, Haecker A, Mayer KFX, Jurgens G, Laux T. 2000. The stem cell population of Arabidopsis shoot meristems is maintained by a regulatory loop between the CLAVATA and WUSCHEL genes. *Cell* **100**: 635-644.

Shinohara H, Matsubayashi Y. 2015. Reevaluation of the CLV3-receptor interaction in the shoot apical meristem: dissection of the CLV3 signaling pathway from a direct ligand-binding point of view. *Plant J* **82**: 328-336.

Somssich M, Je BI, Simon R, Jackson D. 2016. CLAVATA-WUSCHEL signaling in the shoot meristem. *Development* **143**: 3238-3248.

Somssich M, Ma Q, Weidtkamp-Peters S, Stahl Y, Felekyan S, Bleckmann A, Seidel CA, Simon R. 2015. Real-time dynamics of peptide ligand-dependent receptor complex formation in planta. *Sci Signal* **8**: ra76.

Soyars CL, James SR, Nimchuk ZL. 2016. Ready, aim, shoot: stem cell regulation of the shoot apical meristem. *Current opinion in plant biology* **29**: 163-168.

Strabala TJ, O'donnell PJ, Smit AM, Ampomah-Dwamena C, Martin EJ, Netzler N, Nieuwenhuizen NJ, Quinn BD, Foote HC, Hudson KR. 2006. Gain-of-function phenotypes of many CLAVATA3/ESR genes, including four new family members, correlate with tandem variations in the conserved CLAVATA3/ESR domain. *Plant Physiol* **140**: 1331-1344.

Sun Y, Li L, Macho AP, Han Z, Hu Z, Zipfel C, Zhou JM, Chai J. 2013. Structural basis for flg22-induced activation of the Arabidopsis FLS2-BAK1 immune complex. *Science* **342**: 624-628.

Suzaki T, Ohneda M, Toriba T, Yoshida A, Hirano HY. 2009. FON2 SPARE1 redundantly regulates floral meristem maintenance with FLORAL ORGAN NUMBER2 in rice. *Plos Genet* **5**: e1000693.

Suzaki T, Sato M, Ashikari M, Miyoshi M, Nagato Y, Hirano HY. 2004. The gene FLORAL ORGAN NUMBER1 regulates floral meristem size in rice and encodes a leucine-rich repeat receptor kinase orthologous to Arabidopsis CLAVATA1. *Development* **131**: 5649-5657.

Suzaki T, Toriba T, Fujimoto M, Tsutsumi N, Kitano H, Hirano HY. 2006. Conservation and diversification of meristem maintenance mechanism in Oryza sativa: Function of the FLORAL ORGAN NUMBER2 gene. *Plant & cell physiology* **47**: 1591-1602.

Suzaki T, Yoshida A, Hirano HY. 2008. Functional diversification of CLAVATA3-related CLE proteins in meristem maintenance in rice. *The Plant cell* **20**: 2049-2058.

Taguchi-Shiobara F, Yuan Z, Hake S, Jackson D. 2001. The fasciated ear2 gene encodes a leucine-rich repeat receptor-like protein that regulates shoot meristem proliferation in maize. *Gene Dev* **15**: 2755-2766.

Wang ZY. 2012. Brassinosteroids modulate plant immunity at multiple levels. *Proc Natl Acad Sci U S A* **109**: 7-8.

Wu Q, Luo A, Zadrozny T, Sylvester A, Jackson D. 2013. Fluorescent protein marker lines in maize: generation and applications. *The International journal of developmental biology* **57**: 535-543.

Xu C, Liberatore KL, MacAlister CA, Huang Z, Chu YH, Jiang K, Brooks C, Ogawa-Ohnishi M, Xiong G, Pauly M et al. 2015. A cascade of arabinosyltransferases controls shoot meristem size in tomato. *Nat Genet* **47**: 784-792.

Yadav RK, Perales M, Gruel J, Girke T, Jonsson H, Reddy GV. 2011. WUSCHEL protein movement mediates stem cell homeostasis in the Arabidopsis shoot apex. *Genes Dev* **25**: 2025-2030.

Zhang Z, Ersoz E, Lai CQ, Todhunter RJ, Tiwari HK, Gore MA, Bradbury PJ, Yu J, Arnett DK, Ordovas JM et al. 2010. Mixed linear model approach adapted for genome-wide association studies. *Nature genetics* **42**: 355-360.

Zhu YF, Wang YQ, Li RL, Song XF, Wang QL, Huang SJ, Jin JB, Liu CM, Lin JX. 2010. Analysis of interactions among the CLAVATA3 receptors reveals a direct interaction between CLAVATA2 and CORYNE in Arabidopsis. *Plant J* **61**: 223-233.