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Complex peptide hormone signaling in plant stem cells

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Abstract

Peptide hormones influence diverse aspects of plant development through highly coordinated cell-cell signaling pathways. Many peptide hormone families play key roles in stem cell maintenance across land plants. In this review, we focus on recent work in two conserved peptide hormone families, *CLAVATA3/EMBRYO-SURROUNDING REGION* (*CLEs*) and *ROOT MERISTEM GROWTH FACTOR* (*RGFs*), and their roles in regulating plant stem cells. We discuss recent work establishing downstream crosstalk between peptide hormones and other conserved signaling mechanisms in meristem maintenance as well as highlight advances in peptide hormone gene identification that provide important context for *CLE*/*RGF* family evolution across diverse plant lineages. *CLE* and *RGF* gene families have greatly expanded in angiosperms, contributing to the complex genetic regulation of stem cell homeostasis observed in model systems over the last 30 years. Peptide hormone duplications have resulted in genetic compensation mechanisms that ensure robust development through the function of paralogous genes. Broad conservation of genetic compensation across angiosperms highlights the importance of these mechanisms in developmental signaling and understanding their regulation could inform broader understanding of morphological diversity and evolutionary innovation.

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Introduction

Plant development is a dynamic process where new organs form continuously throughout an individual's lifetime. To maintain a consistent body plan in a wide range of environmental conditions, plants leverage a vast array of conserved signaling mechanisms balancing stem cell homeostasis and organogenesis. Developmental regulation requires extracellular signaling to coordinate gene regulatory networks across differentiating cell types. These processes primarily converge in meristems, specialized tissues containing pools of stem cells and their derivatives, where organization of all primary tissues initiates [1]. Signaling in meristems coordinates a balance between cellular proliferation and differentiation from stem cell derived lineages impacting overall plant morphology and patterning [2–4]. Critical to this regulation are peptide hormones; small, genetically encoded, mobile proteins which, among other fundamental processes, regulate meristematic function [5]. Peptide hormones function as ligands that drive complex cell-cell communication networks via receptor-mediated signaling [6,7]. While their role in stem cell regulation has been established for almost 30 years [8], only recently have we realized the ubiquity of peptide signaling across land plants as well as the genetic consequences of large-scale gene expansions found in many peptide families. Here, we highlight recent work from two conserved peptide signaling pathways, CLAVATA3/EMBRYO-SURROUNDING REGION (CLE) peptides and ROOT MERISTEM GROWTH FACTOR (RGF)/GLOVEN (GLV)/CLE-LIKE (CLEL) peptides (hereafter RGFs), which are shaping our understanding of peptide hormone function in meristematic tissues.

The first peptide hormone identified in stem cell regulation was CLAVATA3 (CLV3) in *Arabidopsis thaliana* (Arabidopsis), a founding member of the CLE peptide family [8]. *CLEs* are small genes encoding prepropeptides (unprocessed precursors of mature peptide hormones) ∼100 amino acids in length containing a small N-terminal secretory signal and a highly conserved 12–13 amino acid CLE domain at the C-terminus. The CLE domain is cleaved and post-translationally modified to produce a mature peptide hormone that diffuses through the apoplast to mediate cell-cell signaling [9,10]. CLV3 functions to restrict the size of the stem cell pool in shoot meristems via a suite of leucine-rich repeat receptor-like kinases (LRR-RLKs), most notably through the CLAVATA1 (CLV1) and BARELY ANY MERSITEM (BAM) subclade which additionally require function of the CLAVATA3 INSENSITIVE RECEPTOR KINASE

(CIK) co-receptors [11–13]. CLE-mediated stem cell regulation has been described in all meristematic tissues studied to date in most land plant lineages; including shoot/root apical meristems, vascular development, and stomatal patterning [10,12,14].

The RGF peptide family also have conserved roles in meristem signaling, specifically during early root development. RGFs are a family of secreted, tyrosine-sulfated peptides that redundantly function to promote stem cell activity in Arabidopsis roots [15]. RGFs, like CLEs, have a conserved C-terminal domain of 13 amino acids that is cleaved and secreted for function in the root apoplast. RGFs are perceived by the RGF1-INSENSITIVE (RGI) LRR-RLK subclade which function along with the SOMATIC EMBRYOGENESIS RECEPTOR KINASE (SERK) co-receptors [16,17]. In angiosperms, both CLE and RGF stem cell signaling pathways regulate the expression of critical downstream morphogenic transcription factors, including the *WUSCHEL-RELATED HOMEOBOX* (*WOX*) family transcription factors (most notably the shoot stem cell identity gene *WUSCHEL*) and the *PLETHORA* (*PLT*) AP2-class transcription factors, respectively [12,18]. How downstream processes are coordinated for any CLE/RGF peptide hormone pathway is still a major question in the field. But recent work across model systems suggests that crosstalk with additional essential developmental signaling pathways, such as the phytohormone auxin or distribution of reactive oxygen species (ROS), may provide insight into mechanisms that connect downstream components.

In this review, we discuss how recent studies on CLE/RGF peptide hormones are shaping our understanding of peptide hormone signaling in stem cell regulation. We discuss the potential for conserved downstream signal crosstalk in both peptide families that could provide key clues for understanding the earliest mechanisms each pathways utilized. Next, we highlight advances in peptide gene identification that have aided in our understanding of both *CLE* and *RGF* gene family distributions across land plants. Moreover, we focus on recent work in *CLE*-regulation of shoot meristems in order to highlight how gene family expansions have led to robust developmental regulation in angiosperms.

Peptide hormone signaling: downstream crosstalk

In the moss *Physcomitrium patens*, the PpCLV1a, PpCLV1b, and RECEPTOR-LIKE PROTEIN KINASE (PpRPK, another CLE mediating LRR-RLK) receptors bind CLE peptides to regulate cell division and identity through complex interactions with cytokinin and auxin signaling [19–21]. CLAVATA signaling pathways in the liverwort *Marchantia polymorpha* promote rather than suppress stem cell proliferation [22–24]. MpCLE2 signals through the MpCLV1/MpCIK receptor/co-receptor complex to regulate stem cell division; however, this process is independent of the *WUS* ortholog *MpWOX* [23,24]. Coupled with an apparent lack of any stem cell regulating *WUS*-related domains in *P. patens,* these studies illustrate an ancient relationship between peptide hormones and phytohormone signaling in shoot meristem regulation that predates the *CLV-WUS* module found in angiosperms [20]. Recent work in Arabidopsis highlights this *CLAVATA* and phytohormone crosstalk further, as an auxin-dependent role for the atypical receptor pair CLAVATA2 (CLV2, a receptor-like protein) and CORYNE (CRN, a transmembrane pseudokinase) in regulating flower outgrowth was identified [25]. CLV2/CRN positively regulate cell proliferation during early flower development in a *WUS*-independent process, echoing CLAVATA signaling in *M. polymorpha*. This pathway was originally thought to be independent of CLV3-CLV1 signaling in the inflorescence; however, a closer look at *clv3* and *clv1* null mutants identified similar auxin-dependent cell proliferation phenotypes that are predominately masked by varying degrees of stem fasciation (unregulated stem-width expansion) and temperature-dependent environments [26]. These contradictory outputs from CLAVATA signaling in the shoot meristem (negative vs. positive regulation of cell proliferation) could primarily be a product of different functional meristematic domains, the central zone (where stem cell initials reside) vs. the peripheral zone (where new organs initiate through rapid division) [27]. In fact, antagonistic CLAVATA signaling domains in the shoot meristem is further supported by the function of *CLE40* in Arabidopsis inflorescence development, where *CLE40* positively regulates cell proliferation in the peripheral zone and is excluded from the center of the meristem through canonical CLV3 signaling [28]. A recent perspective on meristem zonation in CLE function highlights these opposing outputs of CLAVATA signaling further by proposing a model in which non-flowering plant lineages only have the stem cell promoting CLE-BAM related signaling modules and lack the stem cell repressive CLV3-CLV1 module [29]. More functional studies in non-flowering plant lineages are necessary to test this model; however, it is important to note that recent work uncovering a role for CLV3-CLV1 in promoting cell proliferation in the peripheral zone suggests that this signaling module regulates more than stem cell repression via crosstalk with additional developmental regulators [26].

Similar to CLEs, RGFs have some conserved activity in bryophytes compared to angiosperms. In a heterologous system, expression of *MpRGF* from *M. polymorpha* in Arabidopsis had similar effects on root development as *AtRGF1/6*, especially in the inhibition of root elongation and reduction in lateral root emergence, demonstrating broad conservation of RGF perception and downstream signaling components [30]. While no *RGFs* have been identified in *P. patens*, they are present in the transcriptomes of older moss lineages, suggesting that this was an independent loss and the most recent common ancestor of land plants possessed RGF family peptides [30]. Interestingly, *RGF* family expansion coincides with the appearance of true roots in vascular plants. But RGF-RGI signaling is present in the liverwort *M. polymorpha* which lacks true roots, suggesting this co-emergence may be coincidental and the ancestral function of *RGFs* lay outside root apical meristem development [30,31]. Recently, a study in Arabidopsis identified a relationship between *RGF1* and ROS concentrations in the root apical meristem that could point toward a conserved regulatory mechanism in need of testing in bryophyte models [32]. ROS is an integral signal in regulating root meristematic zones (proliferation vs. differentiation), and the RGF1 peptide induces shifts in O_2^- and H_2O_2 in the root apical meristem upon peptide treatments [33]. *RGF1-INDUCIBLE TRANSCRIPTION FACTOR 1* (*RITF1*) expression was induced by RGF1 treatments and found to directly impact ROS levels in the root tip. The downstream stem cell regulating transcription factor *PLT2* was stabilized by this RGF1-RITF1 induced ROS, providing a mechanism by which RGF signaling directly influences meristem size in Arabidopsis [32]. It is unclear yet how conserved RGF-mediated ROS regulation is; however, ROS levels have been linked to developmental defects in *M. polymorpha*, making this a prime target for future functional work [34].

Peptide hormone identification and gene expansions

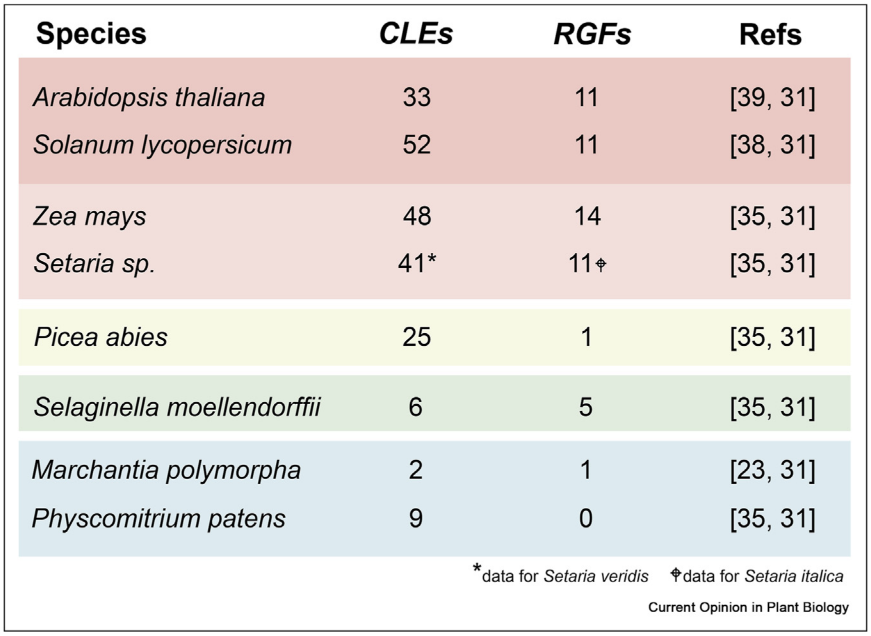
Gene-family expansions of peptide hormones are common across plant lineages, with angiosperms typically having a greater number of peptide encoding genes compared to non-vascular plants [35,36]. But the small size and sequence variability of peptide hormones can make identification difficult, limiting the evolutionary context of these important signaling genes. This problem is shared by the *CLE* and *RGF* families and has only recently been overcome through advances in computationally informed gene identification. Newer methods utilizing hidden Markov modeling (HMM) have proven effective in the detection of previously unidentified orthologs/paralogs across *CLE* and *RGF* peptide families [35,37,30,31,38]. This includes newly identified genes in established model systems. A recent study in tomato (*Solanum lycopersicum*), identified 37 new *CLEs*, alongside the known 15, using a combination of iterative tBLASTn with a HMM targeting the conserved CLE domain [38]. These new SlCLEs are active in exogenous root assays, where they inhibit cell divisions in the root meristem. Work from this same group also identified a novel *CLE* in Arabidopsis, *CLE33*, that functions in protophloem specification/restriction in the root meristem [39]. These newer bioinformatic tools in combination with the growing number of available genomic data across land plants has led to a more comprehensive understanding of *CLE* and *RGF* family diversity, with orthologs now identified in all major plant lineages. Over 600 previously unknown *CLE* sequences were detected in 69 species of plants [37]. Similarly, one study identified over 400 *RGFs* in 149 species using transcriptomic data, while a second study identified 203 candidate *RGFs* from 24 species using genome assemblies; finding only a complete absence of *RGFs* in hornworts and algae along with a secondary loss in some moss lineages [30,31].

What is abundantly clear about CLEs and RGFs in non-vascular plants, is that there are far fewer of these peptide hormones in bryophytes compared to angiosperms [29,31,35], an evolutionary trend echoed by the peptides' receptors [36,40]. There are four distinct peptides encoded by nine *CLE* genes (*PpCLE1-9*) and only three known CLE-mediating LRR-RLKs (PpCLV1a/b and PpRPK2) in *P. patens* [19]. In *M. polymorpha*, there are as few as two *CLE* genes (*MpCLE1/2*) and two CLE-mediating receptors (MpTDR and MpCLV1) [24]. Moreover, *RGFs* are absent from *P. patens* and there is only one *RGF* gene and two RGI-like receptors in *M. polymorpha* [30,31]. Fewer genes involved in peptide hormone signaling leave less room for genetic complexity in signaling pathways and, in general, a trend of increasing *CLE* and *RGF* gene family members can be seen throughout vascular plant lineages (Figure 1). Gene family expansions across these key developmental pathways has directly resulted in the complex genetic coordination observed in the regulation of stem cell homeostasis in angiosperms (Figure 2).

Genetic compensation in peptide signaling

In angiosperms, expansion of peptide hormone gene families has increased rates of gene functionalization and genetic redundancy among peptide signaling pathways, influencing the evolution of their vast morphological diversity [10,29,30]. Recent work has given us insight into how plants deal with gene-family expansions of developmentally critical pathways in the form of genetic compensation. Two forms of genetic compensation have now been described in regulating stem cell homeostasis in shoot meristems, active and passive [41]. Active compensation occurs when a paralog, or closely related gene, has altered expression levels and/or expression domains that mask phenotypes in the absence of a gene. Passive compensation occurs when mutant phenotypes are masked by the aggregate native function of paralogs, or closely related genes, with limited changes in their expression patterns [42,43]. Instances of both active and passive compensation have now been described in CLE signaling (Figure 2). Examples of active compensation have been described in the Solanaceae as a result of a lineage specific *CLV3* gene duplication. In tomato, *SlCLV3* along with its paralog *SlCLE9* function to restrict shoot meristem size throughout development [41]. Interestingly, *slclv3*

Figure 1



Species	*CLEs*	*RGFs*	Refs
Arabidopsis thaliana	33	11	[39, 31]
Solanum lycopersicum	52	11	[38, 31]
Zea mays	48	14	[35, 31]
Setaria sp.	41*	11⌽	[35, 31]
Picea abies	25	1	[35, 31]
Selaginella moellendorffii	6	5	[35, 31]
Marchantia polymorpha	2	1	[23, 31]
Physcomitrium patens	9	0	[35, 31]

*data for *Setaria veridis* ⌽data for *Setaria italica*

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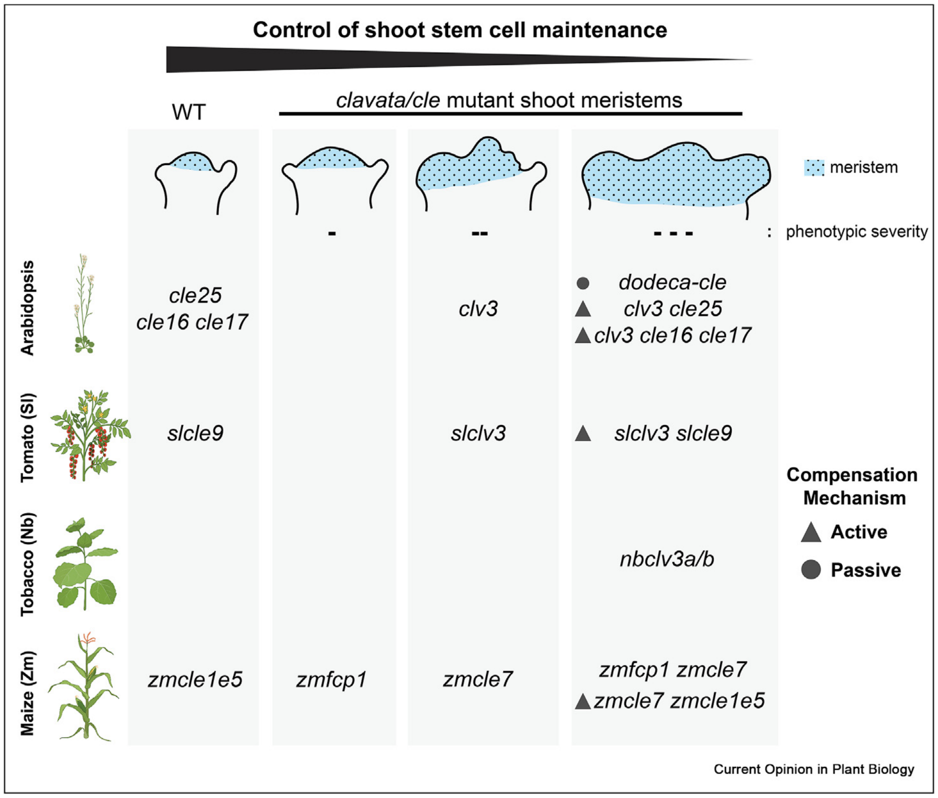
CLE/RGF gene families in species representing diverse land plant lineages. Colors represent the following groups (also in order from top to bottom): angiosperms-eudicots (dark red/top), angiosperms-monocots (light red/bottom), gymnosperms (yellow), ferns and lycophytes (green), and bryophytes (blue). Color scheme adapted from Hirakawa 2022 [29].

mutants have enlarged meristems while single mutants of *slcle9* have no phenotype. In contrast *slclv3 slcle9* double mutants show a synergistic increase in meristem size, indicating that both paralogs regulate shoot stem cell homeostasis. In *slclv3* mutant meristems, *SlCLE9* expression increases dramatically and thus compensates for the loss of *SlCLV3* [41]. This *CLV3/CLE9* duplication occurred ∼30 million years ago in the Solanaceae and has been differentially retained across the family [41,44]. *CLE9* can be found in *Physalis grisea* (groundcherry), *Petunia hybrida* (petunia), and tomato but has been partially or completely lost in *Nicotiana benthamiana* (tobacco relative), *Capsicum annuum* (pepper), *S. melongena* (eggplant) and *S. tuberosum* (potato). In species where *CLE9* is retained, active compensation persists. *clv3* mutants in both groundcherry and petunia have less severe meristem defects, with each respective *CLE9* ortholog presumably capable of compensating for the loss of *CLV3*. In contrast, *N. benthamiana* (which has lost a functional *CLE9* ortholog) has a severe phenotype in *clv3* mutants suggesting that species without *CLE9* have no direct mechanism for compensation [44].

In Arabidopsis, both active and passive compensation of CLV3 signaling has been recently described. *CLE16* and *CLE17* compensate for loss of *CLV3* in shoot meristem regulation, with higher order mutant combinations of *clv3 cle16 cle17*, all having enlarged meristems compared to *clv3* single mutants [45]. *cle16* and *cle17* single mutants have no observable meristem phenotypes, and neither gene is expressed in wildtype shoot meristems; however, both *CLE16* and *CLE17* are expressed in the meristem of *clv3* mutants. In addition to their cooperative function in the shoot, *CLE16* and *CLE17* also regulate lateral root initiation through similar compensatory mechanisms but whether this is active compensation is yet to be determined [46]. Similar to *CLE16/17*, *CLE25* compensates for the loss of *CLV3* during inflorescence development [26]. *clv3 cle25* double mutants have a severe synergistic phenotype affecting both the size and the overall shape of the inflorescence meristem, producing an enlarged disk-like meristem with an increased delay in floral development (akin to *clv2/crn* flower outgrowth defects discussed earlier). *CLE25* is not expressed in wildtype inflorescences but is upregulated in developing floral primordia in *clv3* mutants [26]. Passive compensation of CLV3 occurs via a large suite of additional *CLE* [41]. A higher order *dodeca-cle* mutant, uncovered using multiplex CRISPR gene editing targeting 11 shoot-expressed *CLEs*, has an enhanced meristem phenotype compared to *clv3* single mutants, and none of the targeted *CLEs* appear to have altered expression levels in *clv3* shoot meristems. It is important to note that compensation mechanisms in CLE signaling are not restricted to peptides and have also been described in the related CLE receptors *CLV1*, *BAM1* and *BAM3*; where expression domains of *BAM1* and *BAM3* shift in the *clv1* mutant background into the center of the shoot meristem to

Figure 2



Genetic compensation in *CLE* shoot stem cell signaling. CLE-mediated stem cell homeostasis is buffered through active and passive genetic compensation mechanisms. In Arabidopsis, CLV3 restricts shoot stem cell identity throughout development, but in *clv3* mutant meristems *CLE25* (active), *CLE16/17* (active) and a suite of 11 additional *CLEs* (passive) compensate for this loss at varying levels. A conserved gene duplication of *CLV3* occurred in the Solanaceae giving rise to the closely related paralog *CLE9*, but species across the family have differentially retained the *CLE9* ortholog. In tomato, *SlCLE9* (active) compensates for the loss of *slclv3* with *slclv3 slcle9* double mutants having far more severe defects than either single mutant. *Nicotiana benthamiana* (tobacco relative) is a tetraploid and has two unique copies of *CLV3* with no active compensation from any *CLE9* paralog(s). In this case, *nbclv3a/b* are phenotypically severe, similar to the *clv3 cle9* double mutants of Solanaceae species that have compensatory signaling mechanisms. In maize, one of its *CLV3* homolog *ZmCLE7* functions in regulating inflorescence meristem size and two additional closely related *CLEs* (*ZmFCP1* and *ZmCLE1E5*) also contribute to this regulation but in different ways. *zmfcp1 zmcle7* double mutant is additive compared to the single mutant phenotypes. *zmcle1e5* single mutants have only a very slight increase in meristem width compared to WT, but *zmcle7 zmcle1e5* double mutants are synergistic with a much more severe phenotype than either single mutant. This suggest *ZmCLE1E5* actively compensates for the loss of *zmcle7* while *ZmFCP1* likely functions in a parallel pathway. *CLE*, *CLAVATA3/EMBRYO-SURROUNDING REGION*; *CLV3*, *CLAVATA3*. Plant diagrams were created with BioRender.com.

compensate for the loss of *CLV1* [11,28]. The higher order receptor mutant *clv1 bam1/2/3* has a far more severe shoot meristem phenotype compared to *clv1* mutants, while *bam1/2/3* triple mutants have smaller shoot meristems than wildtype [11]. These complex genetic relationships influencing meristem function highlight the role both active and passive compensation play in regulating development following gene expansions of key peptide signaling components.

Until recently it was unclear if similar CLE signaling compensation mechanisms existed in monocot shoot meristems as it does in the eudicots described above. While maize has two *CLV3* homologs in *ZmCLE7* and *ZmCLE14*, only *ZmCLE7* and two closely related *CLEs* (*ZmFCP1* and *ZmCLE1E5)* are upregulated in *cle7* mutant inflorescence meristems (where *ZmCLE7* functions) [41,47,48]. *zmcle7 zmfcp1* double mutants are additive and both *zmcle7* and *zmfcp1* single mutants have enlarged inflorescence meristems on their own suggesting that these two *CLEs* likely function in parallel pathways and are not compensatory [41]. *zmcle1e5* has only a slight meristem defect on its own and is synergistic with *zmcle7*, as *zmcle7 zmcle1e5* double mutants have inflorescence meristems much larger than either single mutant demonstrating an active compensation for the loss of *ZmCLE7* by *ZmCLE1E5* [48]. Similar to maize, it was recently shown that the *CLV3* homolog in *Setaria viridis* (*SvFON2*) regulates inflorescence meristem size and effects inflorescence architecture; however, it is unclear if compensation plays a role in this CLE signaling pathway as the function of additional *CLEs* in the *svfon2* background have not been studied [49]. Identification of active compensation components in CLE signaling in maize demonstrates that these mechanisms are widely distributed across all angiosperms.

Currently, there are no described compensation mechanisms in RGF signaling, but all components involved have expanded in gene number across angiosperm lineages [36,50]. There are 11 *RGFs* in Arabidopsis, many of which have overlapping functions and only have phenotypes in higher order mutant combinations [51]. The higher order mutant *rgf1 rgf2 rgf3* has defects in root stem cell maintenance, whereas each respective single mutant has no noticeable phenotype [15]. The RGI receptors are also highly redundant, and recent work has linked higher order mutants to varying levels of phenotypic severity. Mutant combinations of *RGI1-RGI5* all show a range of root meristem defects, with *rgi1/2/4/5* and *rgi1/2/3/4/5* being the most severe [17]. Many RGF peptides and RGI receptors have overlapping expression domains with related genes and the patterns of each in different *rgf/rgi* genetic backgrounds will be needed to determine if genetic redundancy observed in RGF signaling pathways are a form of active or passive compensation.

Conclusions

Peptide hormones are key developmental regulators in all land plants. Recent studies in non-vascular plant species point toward highly conserved roles for multiple peptide families in stem cell signaling. Conservation of developmental mechanisms downstream of peptide signaling across plant lineages is still to be determined; however, it appears likely that at least CLE signaling in meristematic tissues intersects with key phytohormone signaling pathways (auxin) in all plant lineages studied to date. There is an evolutionary trend toward large-scale gene expansions in both *CLE* and *RGF* gene families (but certainly not limited to just these two peptide hormone families). Duplications of peptide hormone encoding genes can directly translate to an increase in the number of each peptide produced in a species, generating a remarkably complex genetic landscape for evolution to act on. These gene family expansions have contributed to the complex forms of genetic compensation and gene redundancy observed in angiosperm development. Continued work in diverse land plant lineages will provide more clues as to just how pervasive compensatory genetic mechanisms are in plant development and give insight into how novel gene regulation evolves.

CRediT author statement

Reid Selby: Conceptualization, Data curation, Writing. Daniel S. Jones: Conceptualization, Writing, Visualization, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this article.

Data availability

No data was used for the research described in the article.

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References

Papers of particular interest, published within the period of review, have been highlighted as:

* of special interest

1. Esau K: *Anatomy of seed plants*. Wiley; 1977.
2. Aichinger E, Kornet N, Friedrich T, Laux T: **Plant stem cell niches**. *Annu Rev Plant Biol* 2012, **63**:615–636.
3. Pierre-Jerome E, Drapek C, Benfey PN: **Regulation of division and differentiation of plant stem cells**. *Annu Rev Cell Dev Biol* 2018, **34**:289–310.
4. Han H, Liu X, Zhou Y: **Transcriptional circuits in control of shoot stem cell homeostasis**. *Curr Opin Plant Biol* 2020, **53**: 50–56.
5. Hirakawa Y, Sawa S: **Diverse function of plant peptide hormones in local signaling and development**. *Curr Opin Plant Biol* 2019, **51**:81–87.
6. Zhang H, Han Z, Song W, Chai J: **Structural insight into recognition of plant peptide hormones by receptors**. *Mol Plant* 2016, **9**:1454–1463.
7. Hirakawa Y, Torii KU, Uchida N: **Mechanisms and strategies shaping plant peptide hormones**. *Plant Cell Physiol* 2017, **58**: 1313–1318.
8. Fletcher JC, Brand U, Running MP, Simon R, Meyerowitz EM: **Signaling of cell fate decisions by CLAVATA3 in Arabidopsis shoot meristems**. *Science* 1999, **283**:1911–1914.
9. Fletcher JC: **Recent advances in Arabidopsis CLE peptide signaling**. *Trends Plant Sci* 2020, **25**:1005–1016.
10. Whitewoods C: **Evolution of CLE peptide signalling**. *Semin Cell Dev Biol* 2021, **109**:12–19.
11. Nimchuk ZL: **CLAVATA1 controls distinct signaling outputs that buffer shoot stem cell proliferation through a two-step transcriptional compensation loop**. *PLoS Genet* 2017, **13**, e1006681.
12. Willoughby AC, Nimchuk ZL: **WOX going on: CLE peptides in plant development**. *Curr Opin Plant Biol* 2021, **63**, 102056.
13. Hu C, Zhu Y, Cui Y, Cheng K, Liang W, Wei Z, Zhu M, Yin H, Zeng L, Xiao Y: **A group of receptor kinases are essential for CLAVATA signalling to maintain stem cell homeostasis**. *Nat Plants* 2018, **4**:205–211.
14. Hata Y, Kyozuka J: **Fundamental mechanisms of the stem cell regulation in land plants: lesson from shoot apical cells in bryophytes**. *Plant Mol Biol* 2021, **107**:213–225.
15. Matsuzaki Y, Ogawa-Ohnishi M, Mori A, Matsubayashi Y: **Secreted peptide signals required for maintenance of root stem cell niche in Arabidopsis**. *Science* 2010, **329**:1065–1067.

16. Ou Y, Lu X, Zi Q, Xun Q, Zhang J, Wu Y, Shi H, Wei Z, Zhao B, Zhang X: **RGF1 INSENSITIVE 1 to 5, a group of LRR receptor-like kinases, are essential for the perception of root meristem growth factor 1 in Arabidopsis thaliana**. *Cell Res* 2016, **26**: 686–698.

17. Ou Y, Tao B, Wu Y, Cai Z, Li H, Li M, He K, Gou X, Li J: **Essential roles of SERKs in the ROOT MERISTEM GROWTH FACTOR-mediated signaling pathway**. *Plant Physiol* 2022, **189**:165–177.

18. Shinohara H: **Root meristem growth factor RGF, a sulfated peptide hormone in plants**. *Peptides* 2021, **142**, 170556.

19. * Nemec-Venza Z, Madden C, Stewart A, Liu W, Novák O, Pěnčík A, Cuming AC, Kamisugi Y, Harrison CJ: ***CLAVATA* modulates auxin homeostasis and transport to regulate stem cell identity and plant shape in a moss**. *New Phytol* 2022, **234**: 149–163.

While CLAVATA signaling pathways in angiosperm shoots are all thought to converge on WUSCHEL, there is evidence WUSCHEL was only recently recruited to this module. Nemec-Venza et al. further elucidate the origins of CLAVATA-mediated stem cell regulation by describing the pathway's effect on auxin synthesis and transport in *P. patens*. This suggests an ancient and complex relationship between CLAVATA signaling and auxin predating the co-option of WUS-like genes.

20. Cammarata J, Morales Farfan C, Scanlon MJ, Roeder AHK: **Cytokinin–CLAVATA cross-talk is an ancient mechanism regulating shoot meristem homeostasis in land plants**. *Proc Natl Acad Sci USA* 2022, **119**, e2116860119.

21. Fouracre JP, Harrison CJ: **How was apical growth regulated in the ancestral land plant? Insights from the development of non-seed plants**. *Plant Physiol* 2022, **190**:100–112.

22. Hirakawa Y, Uchida N, Yamaguchi YL, Tabata R, Ishida S, Ishizaki K, Nishihama R, Kohchi T, Sawa S, Bowman JL: **Control of proliferation in the haploid meristem by CLE peptide signaling in Marchantia polymorpha**. *PLoS Genet* 2019, **15**, e1007997.

23. Hirakawa Y, Fujimoto T, Ishida S, Uchida N, Sawa S, Kiyosue T, Ishizaki K, Nishihama R, Kohchi T, Bowman JL: **Induction of multichotomous branching by CLAVATA peptide in Marchantia polymorpha**. *Curr Biol* 2020, **30**:3833–3840.e4.

24. Takahashi G, Betsuyaku S, Okuzumi N, Kiyosue T, Hirakawa Y: **An evolutionarily conserved coreceptor gene is essential for CLAVATA signaling in Marchantia polymorpha**. *Front Plant Sci* 2021, **12**, 657548.

25. Jones DS, John A, VanDerMolen KR, Nimchuk ZL: **CLAVATA signaling ensures reproductive development in plants across thermal environments**. *Curr Biol* 2021, **31**:220–227.e5.

26. * John A, Smith ES, Jones DS, Soyars CL, Nimchuk ZL: **A network of CLAVATA receptors buffers auxin-dependent meristem maintenance**. *Nature Plants* 2023:1–12.

John et al., 2023 is a comprehensive follow-up to Jones et al., 2021 that shows a conserved integration of auxin and CLAVATA signaling in the Arabidopsis shoot meristem. They connect *CLAVATA3-CLAVATA1* signaling to a similar temperature dependent auxin response reported for *CLAVATA2/CORYNE* (Jones et al., 2021), but do so by integrating multiple CLAVATA phenotypes into this process (classic phenotype as well as a newly described role for *CLV1* in inflorescence growth arrest). This study also demonstrates a role for *CLE25* in shoot meristem regulation in the absence of *CLV3*, providing evidence for active peptide hormone compensation in Arabidopsis.

27. Soyars CL, James SR, Nimchuk ZL: **Ready, aim, shoot: stem cell regulation of the shoot apical meristem**. *Curr Opin Plant Biol* 2016, **29**:163–168.

28. Schlegel J, Denay G, Wink R, Pinto KG, Stahl Y, Schmid J, Blümke P, Simon RG: **Control of Arabidopsis shoot stem cell homeostasis by two antagonistic CLE peptide signalling pathways**. *Elife* 2021, **10**, e70934.

29. Hirakawa Y: **Evolution of meristem zonation by CLE gene duplication in land plants**. *Nat Plants* 2022, **8**:735–740.

30. * Furumizu C, Sawa S: **The RGF/GLV/CLEL family of short peptides evolved through lineage-specific losses and diversification and yet conserves its signaling role between vascular plants and bryophytes**. *Front Plant Sci* 2021, **12**, 703012.

Until recently, little was known about the evolution of RGF peptides. Furumizu and Sawa conducted a large-scale survey utilizing genome and transcriptomic data from a wide range of plant taxa, identifying over 400 RGF-like sequences, suggesting a massive expansion of the family. Furthermore, the authors identify a single *RGF* in liverworts and demonstrate that it is functional when ectopically expressed in Arabidopsis, suggesting a conserved role for *RGF*s across all land plants despite the lack of true roots in bryophytes.

31. Fang Y, Chang J, Shi T, Luo W, Ou Y, Wan D, Li J: **Evolution of RGF/GLV/CLEL peptide hormones and their roles in land plant growth and regulation**. *Int J Math Stat* 2021, **22**, 13372.

32. Yamada M, Han X, Benfey PN: **RGF1 controls root meristem size through ROS signalling**. *Nature* 2020, **577**:85–88.

33. Tsukagoshi H, Busch W, Benfey PN: **Transcriptional regulation of ROS controls transition from proliferation to differentiation in the root**. *Cell* 2010, **143**:606–616.

34. Busch A, Deckena M, Almeida-Trapp M, Kopischke S, Kock C, Schüssler E, Tsiantis M, Mithöfer A, Zachgo S: **Mp TCP 1 controls cell proliferation and redox processes in Marchantia polymorpha**. *New Phytol* 2019, **224**:1627–1641.

35. Goad DM, Zhu C, Kellogg EA: **Comprehensive identification and clustering of CLV3/ESR-related (CLE) genes in plants finds groups with potentially shared function**. *New Phytol* 2017, **216**:605–616.

36. Furumizu C, Krabberød AK, Hammerstad M, Alling RM, Wildhagen M, Sawa S, Aalen RB: **The sequenced genomes of nonflowering land plants reveal the innovative evolutionary history of peptide signaling**. *Plant Cell* 2021, **33**:2915–2934.

37. Zhang Z, Liu L, Kucukoglu M, Tian D, Larkin RM, Shi X, Zheng B: **Predicting and clustering plant CLE genes with a new method developed specifically for short amino acid sequences**. *BMC Genom* 2020, **21**:709.

38. * Carbonnel S, Falquet L, Hazak O: **Deeper genomic insights into tomato CLE genes repertoire identify new active peptides**. *BMC Genom* 2022, **23**:756.

Carbonnel et al., 2022 utilized a combination of tBLASTn and HMM to survey the tomato genome for yet unidentified *CLE* genes. They identified 37 new *CLEs* in addition to the already annotated 15, and showed gene expression support for 28 from publicly available data. This study takes *CLE* identification a couple steps further by demonstrating the functionality of newly identified tomato *CLEs* in exogenous roots assays. Their function in root growth arrest was also *CLE*-receptor dependent (*SlCLAVATA2*).

39. Carbonnel S, Cornelis S, Hazak O: **The CLE33 peptide represses phloem differentiation via autocrine and paracrine signaling in Arabidopsis**. *Commun Biol* 2023, **6**:588.

40. Man J, Gallagher JP, Bartlett M: **Structural evolution drives diversification of the large LRR-RLK gene family**. *New Phytol* 2020, **226**:1492–1505.

41. Rodriguez-Leal D, Xu C, Kwon C-T, Soyars C, Demesa-Arevalo E, Man J, Liu L, Lemmon ZH, Jones DS, Van Eck J, *et al.*: **Evolution of buffering in a genetic circuit controlling plant stem cell proliferation**. *Nat Genet* 2019, **51**:786–792.

42. Diss G, Ascencio D, DeLuna A, Landry CR: **Molecular mechanisms of paralogous compensation and the robustness of cellular networks: mechanisms of paralogous compensation**. *J Exp Zool* 2014, **322**:488–499.

43. El-Brolosy MA, Stainier DYR: **Genetic compensation: a phenomenon in search of mechanisms**. *PLoS Genet* 2017, **13**, e1006780.

44. * Kwon C-T, Tang L, Wang X, Gentile I, Hendelman A, Robitaille G, Van Eck J, Xu C, Lippman ZB: **Dynamic evolution of small signalling peptide compensation in plant stem cell control**. *Nat Plants* 2022, **8**:346–355.

Kwon et al., 2022 takes a deep dive into the functional significance of the Solanaceae specific CLV3/CLE9 duplication and the dual role these peptides play in stem cell signaling across species. They uncover clear evolutionary divergence of this active genetic compensation mechanism, where some species (groundcherry, petunia and tomato) have functional copies of both genes while other species

(*N. benthamiana*, pepper, eggplant, and potato) have lost or are in the process of losing functional *CLE9* orthologs. In species with both CLV3/CLE9, *clv3* phenotypes are less severe due to *CLE9* function. When *CLE9* orthologs are missing (*N. benthamiana*), *clv3* phenotypes are very severe, similar to higher order mutants in other Solanaceae species.

45. * Dao TQ, Weksler N, Liu HM-H, Leiboff S, Fletcher JC: **Interactive CLV3, CLE16, and CLE17 signaling mediates stem cell homeostasis in the Arabidopsis shoot apical meristem**. *Development* 2022, https://doi.org/10.1242/dev.200787.

Dao et al., 2022 describes active genetic compensation of CLE peptides in Arabidopsis *clv3* mutants. Neither CLE16 or CLE17 are obviously expressed in wildtype shoot meristems; however, both are expressed across the meristems of *clv3* mutants demonstrating a clear change in expression pattern in this genetic context. On their own, *cle16* and *cle17* have no appreciable shoot phenotypes, but they both enhance *clv3* meristem defects across vegetative and reproductive development.

46. Zhang L, Yang Y, Mu C, Liu M, Ishida T, Sawa S, Zhu Y, Pi L: **Control of root stem cell differentiation and lateral root emergence by CLE16/17 peptides in Arabidopsis**. *Front Plant Sci* 2022, **13**.

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

48. Liu L, Gallagher J, Arevalo ED, Chen R, Skopelitis T, Wu Q, Bartlett M, Jackson D: **Enhancing grain-yield-related traits by CRISPR–Cas9 promoter editing of maize CLE genes**. *Nat Plants* 2021, **7**:287–294.

49. Zhu C, Liu L, Crowell O, Zhao H, Brutnell TP, Jackson D, Kellogg EA: **The CLV3 homolog in Setaria viridis selectively controls inflorescence meristem size**. *Front Plant Sci* 2021, **12**, 636749.

50. Furumizu C, Sawa S: **The RGF/GLV/CLEL family of short peptides evolved through lineage-specific losses and diversification and yet conserves its signaling role between vascular plants and bryophytes**. *Front Plant Sci* 2021, **12**, 703012.

51. Fernandez A, Hilson P, Beeckman T: **GOLVEN peptides as important regulatory signalling molecules of plant development**. *J Exp Bot* 2013, **64**:5263–5268.