**Chihiro Furumizu,[a] Anders K. Krabberød,[b] Marta Hammerstad,[c] Renate M. Alling,[b] Mari Wildhagen,[b] Shinichiro Sawa,[a,1] and Reidunn B. Aalen[b,2]**

[a] Graduate School of Science and Technology, Kumamoto University, Kumamoto, Japan.

[b] Section for Evolutionary Biology and Genetics, Department of Biosciences, University of Oslo, Norway

[c] Section for Biochemistry and Molecular Biology, Department of Biosciences, University of Oslo, Norway

Contact information:

[1] sawa@kumamoto-u.ac.jp.

[2] reidunn.aalen@ibv.uio.no

Corresponding author:

Reidunn B. Aalen

# The Sequenced Genomes of Non-Seed Land Plants Reveal the (R)Evolutionary History of Peptide Signaling

**Short title**

**(R)Evolutionary Peptide Signaling History**

The author(s) responsible for distribution of materials integral to the findings presented in this article in accordance with the policy described in the Instructions for Authors (www.plantcell.org) are: Reidunn B. Aalen (reidunn.aalen@ibv.uio.no) and Chihiro Furumizu (furumizu@kumamoto-u.ac.jp).

## Abstract

An understanding of land plant evolution is a prerequisite for in-depth knowledge of plant biology. Here we illustrate how to extract and explore information hidden in the increasing number of sequenced plant genomes, from bryophytes to angiosperms, to elucidate a specific biological question – how peptide signaling evolved. To conquer land and cope with changing environmental conditions, plants have gone through profound transformations that must have required a revolution in cell-to-cell communication. Peptides can act as signals of endogenous and exogenous changes, and interactions with leucine-rich repeat receptor-like kinases activate intracellular molecular signaling. Signaling peptides are typically active in organs like flowers and seeds, vascular tissue, root and shoot meristems, which are absent in the most primitive land plants. However, putative orthologues for several peptide-receptor pairs have been identified in non-seed land plants. These discoveries and elucidation of co-evolution of such ligands and their receptors, have profound implications for the understanding of evolution and diversity of cell-to-cell communication, as *de novo* interactions in peptide signaling pathways may have contributed to generate novel traits in land plants. Phylogenetic analyses, genomic, structural and functional data can guide us to reveal evolutionary steps that laid the foundation for a wealth of diversified terrestrial plants.

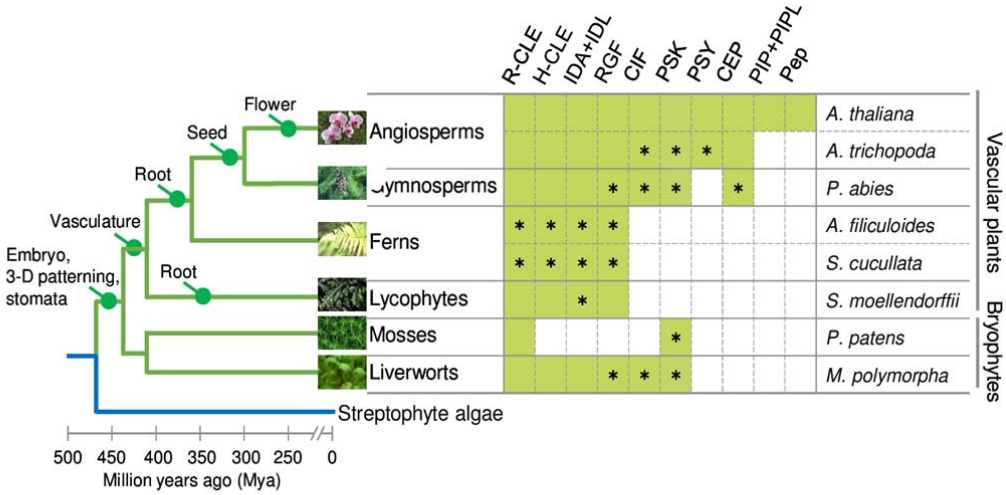
## Introduction

Land plants have evolved from a class of unicellular freshwater green algae that started to colonize land ~ 470 million years ago (Mya) (Delwiche and Cooper, 2015; Ishizaki, 2017). This successful evolution has required groundbreaking biological innovations, such as the transition from the ancestral haploid unicellular life style of the algae to multicellular plants with alternating haploid and diploid generations, and development of new specialized organs (Figure 1). While the diploid sporophyte and the haploid gametophyte initially might have employed the same genes, gene duplications followed by neofunctionalization likely facilitated the development of a multicellular shoot apical meristem (SAM) in the polysporangiate sporophytes, a transition that necessitated extensive changes in gene expression patterns (Ligrone et al., 2012). Lycophytes and ferns, which appeared ~410 Mya (Gensel, 2008; Kenrick and Crane, 1997; Steemans et al., 2009), are extant plants representing early diverging lineages with vasculature, which provided mechanical support and transport of nutrients (Ishizaki, 2017). The fossil record suggests that during the Devonian period (400 - 360 Mya) roots evolved from shoots, and provided anchorage for the plants and allowed acquisition of water and nutrients from the soil.

Evolution of novel cell types, tissue types, and organs with increasing multicellular complexity, must have put a high demand on cell-to-cell communication in land plants. Recent advances in whole genome sequencing have facilitated large-scale comparative genomic studies in bryophytes, lycophytes, ferns, gymnosperms, angiosperms, and streptophyte algae, and identified genes that were lost or acquired during land plant evolution (Amborella Genome, 2013; Banks et al., 2011; Bowman et al., 2017; Li et al., 2018; Nystedt et al., 2013; Rensing et al., 2008). The ancestry of several hormone and stress signaling pathways in land plants has for instance been traced back to algae (de Vries et al., 2018; Nishiyama et al., 2018).

Small peptides interacting with plasma-membrane bound receptor-like kinases (RLKs) have recently challenged the position of the classical hormones as the major mediators of signaling processes (Grienenberger and Fletcher, 2015). The liverwort *Marchantia polymorpha,* the moss *Physcomitrella patens*, and the lycophyte *Selaginella moellendorffii,* together representing the present-day descendants of the earliest land plants, as well as *Amborella trichopoda,* representing the earliest

**Figure 1. Phylogenetic distribution of small post-translationally modified peptides in land plants.**




Left: Simplified phylogenetic tree of major extant land plant lineages (green lines) based on (One Thousand Plant Transcriptomes, 2019). The branch lengths are roughly proportional to the estimated divergence dates (Chang et al., 2016). Gains of key morphological innovations are shown as green circles mapped on the tree while some gain events are still under discussion. Right: Colored boxes indicate the presence of homologues of the indicated small post-translationally modified signaling peptides for the model plants *Arabidopsis thaliana* (Lamesch et al., 2012), *Amborella trichopoda* (Amborella Genome, 2013), *Picea abies* (Nystedt et al., 2013), *Azolla filiculoides* and *Salvinia cucullate* (Li et al., 2018), *Selaginella moellendorffii* (Banks et al., 2011), *Physcomitrella patens* (Lang et al., 2018), and *Marchantia polymorpha* (Bowman et al., 2017). Asterisks indicate putative peptides newly identified in our sequence analyses. Blank boxes indicate that respective peptide homologues were not detected in our analyses. Except for *M. polymorpha*, images represent each taxa and are from Pixabay (https://pixabay.com).

Image source
Pixabay (https://pixabay.com)
Orchid, Sunflower, *Picea abies*: Image by Goran Horvat from Pixabay
https://pixabay.com/photos/orchid-orchidaceae-phalenopsis-3567929/
https://pixabay.com/photos/agriculture-sunflowers-bloom-flower-4351480/
https://pixabay.com/photos/picea-abies-european-spruce-cone-4396763/
Fern: Image by pasja1000 from Pixabay
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Selaginella: Image by Nika Akin from Pixabay
https://pixabay.com/photos/fern-leaves-nature-environment-4662759/
Star moss: Image by cocoparisienne from Pixabay
https://pixabay.com/photos/moss-star-moss-forest-plant-2683009/
*M. polymorpha*: Chihiro

82 flowering plants, have newly been established as models that can help us
83 understand the evolution of land plants. Recent phylogenetic analyses have

identified RLKs that likely bind peptides in these species, but not detected in the sequenced algae genomes (Liu et al., 2017). The question therefore arises as to whether the appearance of peptide ligands, their receptors and components of their signaling pathways, can be linked to cell-to-cell communication pathways that facilitated the evolution of land plants and their adaptive strategies to terrestrial environments.

A number of papers have reviewed specific peptide or receptor families involved in peptide signaling (Fernandez et al., 2013; Kaufmann and Sauter, 2019; Muschietti and Wengier, 2018; Oh et al., 2018; Segonzac and Monaghan, 2019; Shi et al., 2019; Taleski et al., 2018); and references listed for Table 1 (see Supplemental Table 1). Our perspective offers cross comparison of multiple peptide ligand-receptor pairs and argues that cell-to-cell communication through peptide signaling pathways needs to be studied in an integrative manner, combining both *in silico* and experimental approaches, if we are to understand their (r)evolutionary history and importance. With emphasis on leucine-rich repeat (LRR) RLKs interacting with small post-transcriptionally modified peptides (PTMPs) (Table 1A), we highlight emerging ideas and issues elucidating the evolution of peptide signaling systems.

## LRR-RLKs and post-transcriptionally modified peptides (PTMP) evolved with the land plants

Since the first discovery of a small signaling peptide in plants (Pearce et al., 1991), increase in the number and quality of sequenced genomes, as well as advances in methods for *in silico* annotation of small genes, have allowed identification of a substantial number of potential plant signaling peptides (Ghorbani et al., 2015; Goad et al., 2017; Gong et al., 2002; Tavormina et al., 2015). They can be divided into two major groups, the cysteine-rich peptides (CRPs) (Table 1B), which attain their 3D structure by disulfide bridges formed between pairs of cysteines, and the PTMPs, generated from prepropeptides and processed to mature peptides of 5-20 amino acids (Table 1A). The majority of both classes of peptides signal through plasma-membrane-bound receptors with cytoplasmic serine/threonine kinase domains and ligand-binding ectodomains built of leucine-rich repeats (LRRs) (Shiu and Bleecker, 2001). In *Arabidopsis thaliana* (Arabidopsis), peptides and LRR-RLKs are crucial players in developmental and physiological processes, and in adaptation to

environmental changes and challenges (de Bang et al., 2017; Olsson et al., 2019). The recently sequenced genomes of algae species, bryophytes, lycophytes, ferns, gymnosperms and basal angiosperms, and the identification of numerous bryophyte and lycophyte LRR-RLKs (Amborella Genome, 2013; Banks et al., 2011; Bowman et al., 2017; Liu et al., 2016; Nishiyama et al., 2018; Rensing et al., 2008) facilitate investigations of both the evolution of various biological processes, and the importance of peptide signaling for the overwhelming diversity of land plants. A crucial question is whether bryophytes or lycophytes have LRR-RLKs orthologues with homologous or analogous functions as in Arabidopsis and other angiosperms. To address this question, it is not only necessary to identify orthologues of peptides and receptors in the descendants of the earliest land plants, but also to substantiate that their ancestral versions could have functioned as signaling ligand-receptor pairs.

More than 200 LRR-RLKs in Arabidopsis have been assigned to subfamilies according to their unique structure, organization and number of LRRs (Shiu and Bleecker, 2001). To date, RLKs of the LRR X, XI, and XIII subfamilies have been identified as receptors for endogenous small signaling peptides. Among them, subfamily X receptors are unique in the chemical diversity of their ligands; they interact with CRPs and PTMPs as well as steroid plant hormones, the brassinosteroids (Kutschera and Wang, 2012; Yokota et al., 2017). In contrast, ERECTA (ER) and ER-LIKE (ERL) receptors are the only known peptide receptors of the LRR XIII. ER/ERL receptors bind CRPs named EPIDERMAL PATTERNING FACTOR (EPF) and EPF-LIKE (EPFL), and in angiosperms, the EPF/EPFL–ER/ERL signaling controls stomatal patterning. Stomata have been identified in fossil records of extinct plants and all extant land plant lineages except for the liverworts (Peterson et al., 2010). Evolution of stomata was a major step in the adaptation of plants to a terrestrial life, as stomata enable the plant to control the water balance and thereby survive in the dry atmosphere. The conserved EPF signaling components are involved in stomatal patterning in *P. patens*, which support the proposed monophyletic origin of stomata or, alternatively, independent recruitment of the same module in analogous systems (Caine et al., 2016; Chater et al., 2017; Harris et al., 2020). Either way, these experiments demonstrate that comparative studies of peptide signaling can give valuable insight into the molecular basis of cell-to-cell communication in the evolution of a complex organ.

Tracing the evolutionary trajectory of the subclasses X and XIII will be an exciting future challenge for understanding the emergence of new ligand-receptor pairs. Here, however, we will focus on the PTMPs, which - with a few exceptions - all signal through subfamily XI receptors with >20 LRRs.

Both peptide ligands and LRR-RLKs can be assigned to small gene families (Table 1). Genetic and biochemical studies suggest that members of a given peptide family bind and activate a subset of phylogenetically related receptors (Butenko et al., 2009). The necessity of molecular interaction between signaling peptides and their receptors must have set mutual constraints on mutational changes of amino acids with properties and location directly needed for binding and activation of receptors. The selective pressure on mutations in a LRR-RLK gene could potentially differ between the kinase domain, which determines the outcome of receptor activation, and the ectodomain, where a set of amino acid residues determine the ability to bind peptides. To explore this possibility, the phylogeny of the LRR ectodomains and of the kinase domains of subclass XI LRR-RLKs - from extant representatives of the earliest diverged lineages (liverworts, mosses, and lycophytes) to representatives of monocot and dicot angiosperms - were analyzed separately (see Supplemental material and methods).

The dual phylogenetic analyses also included algal sequences as outgroup (Figure 2A). Interestingly, membrane-anchored LRR proteins, however without kinase domain, were found present in the tiny freshwater algae *Micromonas* (Worden et al., 2009), and kinase domains without LRR ectodomains were identified in *Volvox* (Matt and Umen, 2016) and *Chlamydomonas reinhardtii* (Salomé and Merchant, 2019). The *Micromonas* LRR proteins have more than 20 highly similar repeats, with conserved leucine residues positioned similarly to those of angiosperm LRRs, although with a repeat-unit of 23 amino acids, not 24 as found in land plants

**Figure 2. Phylogenetic analysis of the LRR-RLK subclass XI, with independent analysis of the LRR domain and the kinase domain.**

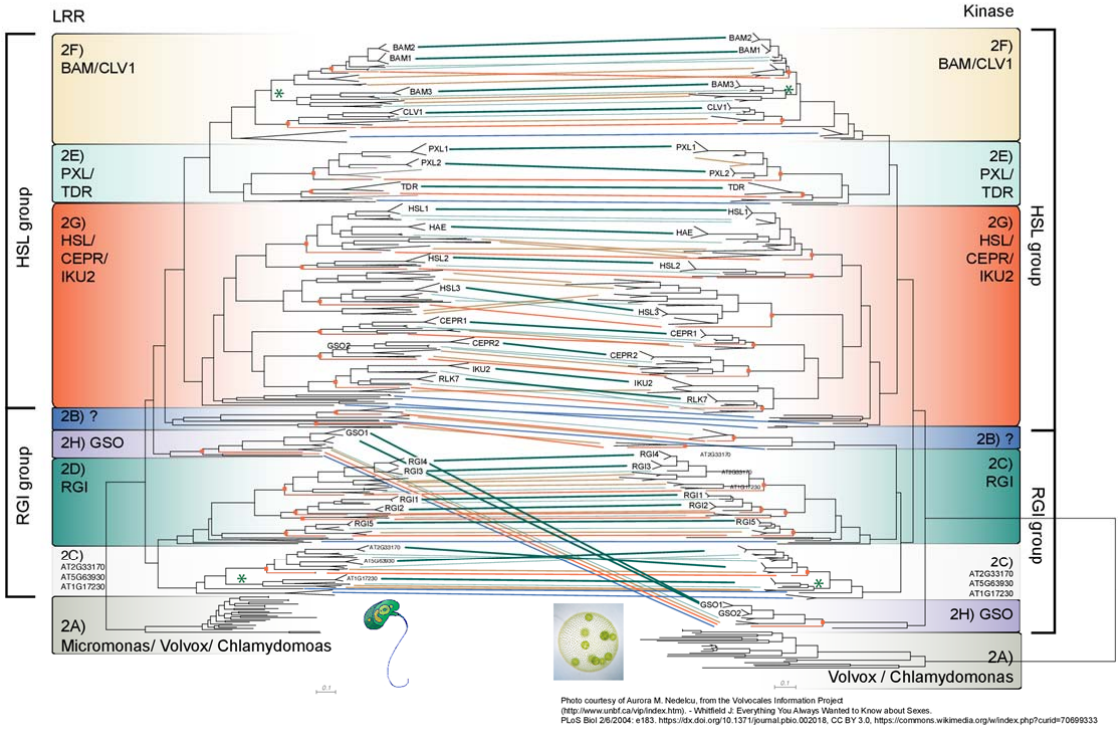



Photo courtesy of Aurora M. Nedelcu, from the Volvocales Information Project (http://www.unbf.ca/vip/index.htm). - Whitfield J: Everything You Always Wanted to Know about Sexes. PLoS Biol 2/6/2004: e183. https://dx.doi.org/10.1371/journal.pbio.0020183, CC BY 3.0, https://commons.wikimedia.org/w/index.php?curid=70699333

Tangelgram of the LRR-RLK subclass XI, with independent phylogenetic analysis of the LRR-domain (left) and the kinase domain (right) with lines connecting the domains for each gene. A) Micromonas, Volvox and Chladmydomonas sequences of LRR proteins or kinases were used as outgrous. B) These LRR-LRKs have no orthologue in Arabidopsis and are without known ligands and functions. C) These LRR-RLKs are without known ligands and functions. D) The RGI LRR-RLKs have evolved by several duplications, some specific for the eudicots. E) The TDF/POXY LRR and kinase trees differ with respect to the phylogeny of eudicot and monocot PXL1 and PXL2. F) The BAM/CLV LRR and kinase trees differ with respect to the phylogeny of eudicot and monocot BAM1 and BAM2. G) The HSL/CEPR/IKU LRR and kinase trees differ with respect to the phylogeny of eudicot and monocot HSL1, CEPR and IKU/RLK7. H) The GSO kinase domain group with the RGIs, and the LRR with the HSLs.
The LRR and kinase domain of receptors from early-diverging land plants (S. moellendorffii, P. patens and M. polymorpha) are connected with dark blue lines; the primitive angiosperm A. trichopoda with orange lines, monocots with brown lines; dicots, including Arabidopsis, with dark green lines; and other eudicots in light green. Common origins of A. trichopoda of and eudicots marked with orange squares and presumed losses in A. trichopoda are marked in green. A detailed version of this figure, with species and gene names is found as Supplemental Figure 2.

(Supplemental Figure 1). Searches for signaling peptides in algae, have so far not given any solid evidence (Goad et al., 2017; Oelkers et al., 2008), suggesting that known signaling peptides evolved after the divergence of land plants from ancestral algae. Consistent with this, we were not able to identify any gene encoding >20 LRRs coupled to a kinase domain in the recently sequenced genome of the algae *Chara braunii*, which is one of the closest relatives to land plants (Nishiyama et al., 2018).

Thus, the combination of long LRRs and kinase domains may seem to be an invention that facilitated the evolution of land plants. Additonal sequenced genomes from early-diverging land plants may be needed to unravel the early evolutionary history of LRR-RLKs.

**LRRs and the kinase domains have different evolutionary rate**

The LRR-RLKs of subclass XI represent the major receptor class that interacts with PTMPs (Bowman et al., 2017). (Figure 2, and with all details Supplemental Figure 2). The dual phylogenetic analyses (see Supplemental Methods) of members of this subclass consistently demonstrate that branches are longer for ectodomains than kinase domains (Supplemental Figure 2), implying that the LRRs have evolved faster than the kinase domains. This is likely due to the differential structural and functional requirements facing the respective domains. The LRRs need a structural scaffold guaranteed by the repeated leucines, and additionally amino acid residues involved in peptide recognition. As the peptide ligands vary in sequence and length, the LRR must have sequence differences adapted to their specific ligand. The varying length of the LRR domain is contributing to the observed difference in branch lengths between the two phylogenies. The shorter branches for the kinase domain may on the other hand reflect a need for preservation of an overall structure to secure the kinase activity.

The identification of XI type LRR-RLKs receptor sequences from the descendants of early-diverging land plant lineages, *M. polymorpha, P. patens* and *S. moellendorffii,* indicate that a diversification took place very early in land plant evolution, with separation of LRR-RLK XI into seven clades already in the common ancestor of bryophytes and vascular plants (Figure 2, dark blue lines). *Amborella trichopoda,* currently accepted as the most basal flowering plant (Soltis et al., 2008) was included in the analyses as a landmark for identification of evolutionary changes that took place before and after the emergence of angiosperms. The positions of the *A. trichopoda* receptors in the tree visualize that the number of LRR-RLK XI increased further in the angiosperms, and especially in the eudicot lineage (Figure 2, green lines), associated with whole genome duplication and later genome fractionation (Clark and Donoghue, 2018). The phylogenetic analysis also indicates that some of

the receptor genes originally duplicated in the ancestor of vascular plants have been lost in *A. trichopoda,* but have been retained in eudicots (Figure 2, green asterisks).

There are two major groups, here named the RGI and HSL after the ROOT GROWTH FACTOR INSENSITIVE (RGI)/RGF RECEPTOR (RGFR) and the HAESA-LIKE (HSL) receptors, respectively. These groups are further divided into clades which – with the exception for one clade without Arabidopsis homologues (Figure 2B, marked "?") – are characterized by their orthologues in *A. thaliana*, Of these, one clade (Figure 2C) in the RGI major group, encompass three Arabidopsis genes (At2g33170, At5g63930 and A1g17230) with neither known ligands nor known functions, and multivalent mutants may be needed to obtain lines with informative mutant phenotypes. *A. trichopoda*, has one copy of this gene, but the phylogenetic analysis suggest that one copy was lost early in *A. trichopoda* (Figure 2C, green asterisk). The closest sister to this clade is the RGI clade (Figure 2D). The phylogenetic relationship between the five Arabidopsis *RGI* genes, the four copies in *A. trichopoda*, and two in monocot species suggests that the RGI branch was divided in two groups prior to angiosperm evolution: one represented by RGI5 and the other encompassing RGI1-4.

This conclusion was substantiated by inclusion of a gymnosperm (Norway spruce, *Picea abies*) and ferns (duckweed, *Azolla filiculoide*, and buce plant, *Salvinia cucullata*) in the analyses, which demonstrated the presence of two presumptive RGI orthologues also in these species (Supplemental Figure 3), suggesting a gene duplication took place before the divergence of ferns and seed plants. Especially the RGI1-4 group shows expansion through gene duplications. It's not clear whether monocots lost RGI homologues in their evolution or independent gene duplication took place during the evolution of basal angiosperms.

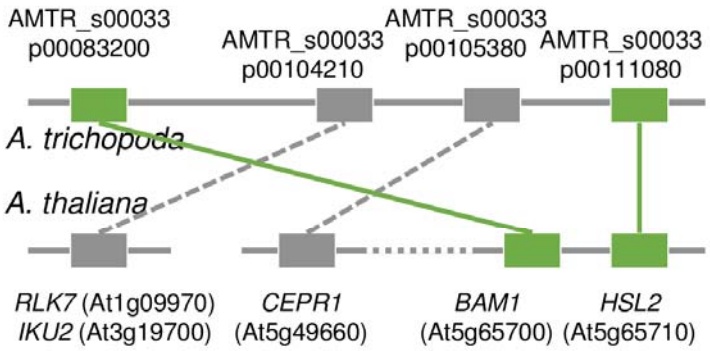
The other major group is comprised of three subclades, represented by HSL receptors, TDIF RECEPTOR (TDR)/PHLOEM INTERCALATED WITH XYLEM (PXY), and BARELY ANY MERISTEM (BAM)/CLAVATA1 (CLV1) receptors. TDR/PXY and BAM/CLV receptors form a clade distinct from that of HSL receptors. The phylogenetic analyses suggest that an ancestral *BAM* gene was duplicated first to give rise to the *TDR/PXY* sub-branch, which before the appearance of the

247 angiosperms gave rise to a *PXY-LIKE* (*PXL*) gene, which duplicated into *PXL1* and
248 *PXL2* in the eudicot lineage (Figure 2E). In parallel *BAM* gave rise to *CLV1* and
249 *BAM3* (Figure 2F), and finally during the development of the angiosperms, *BAM2*.
250 Receptors similar to CLV1 have been identified in both *P. patens* and *M. polymorpha*
251 whereas PXY/TDR-related sequences are found in *M. polymorpha* but not in P.
252 *patens.* The branch pattern suggests that the ancestor of *A. trichopoda* lost the gene
253 leading to *BAM3* in eudicots (Figure 2F, green asterisk). A similar conclusion can be
254 drawn for the dicot *PXL*. Moreover, the phylogenetic analyses of the BAM3 LRR
255 placed it closer to the other BAMs, while the kinase domain of BAM3 seems closer to
256 CLV1.

257 Within the HSL subclade (Figure 2G), gene duplications and diversification gave rise
258 to two branches, one with *HSL* genes, and the other that includes *CEP RECEPTOR*s
259 (*CEPR*s). In the latter, the eudicot lineage experienced a recent gene duplication that
260 generated *HAIKU2* (*IKU2*) and *RECEPTOR-LIKE KINASE7* (*RLK7*) in Arabidopsis.
261 While RLK7 is involved in innate immunity, *IKU2*, with its expression in pollen, during
262 early endosperm and embryo development, and involvement in regulation of seed
263 size in Arabidopsis (Luo et al., 2005), may have been recruited to regulate truly
264 angiosperm- or eudicot-specific characters (Friedman and Williams, 2004).
265 Interestingly, in the *A. trichopoda* genome there are small clusters of *LRR-RLK*
266 genes (Liu et al., 2016), one of which encodes ancestral versions of HSL clade
267 receptors, namely BAM1, CEPR1, HSL2, and a common ancestor for IKU2 and
268 RLK7 in proximity (Figure 3). Reshuffling of the *A. trichopoda* genome has moved
269 CEPR1 and IKU2/RLK7 away, leaving only BAM1 and HSL2 as neighboring genes
270 in Arabidopsis. When studying families of highly similar members it is a great
271 advantage when data on whole genome alignments and synteny are available, e.g.
272 in Ensembl plants (http://plants.ensembl.org), so that one can follow the evolution of
273 genes in a genomic context.

274 During the evolution of *HSL* genes, a gene duplication that precedes the
275 diversification of early angiosperms yielded two clades, HSL1 and HSL2 (Stø et al.,
276 2015) (Figure 2G). Additionally, *HSL1* has been duplicated twice in the angiosperms
277 and once in the monocots, giving rise to *HSL1A* and *HSL1B*, which interestingly are
278 more similar in the kinase domain than in the LRR. Early during eudicot evolution

**Figure 3. Syntenic relation between LRR-RLK genes in *A. trichopoda* and their *A. thaliana* homologues.**

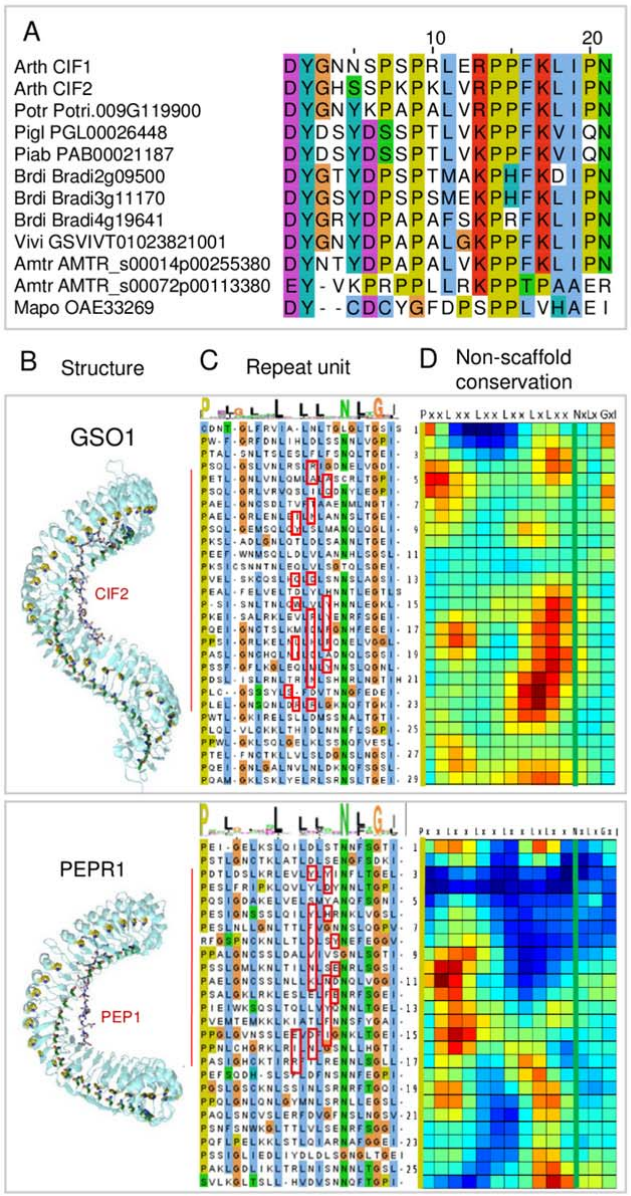



Four LRR-RLKs genes that are found in near proximity in the genome of *A. trichopoda* (SuperContig AmTr_v1.0_scaffold00033), have been reshuffled during angiosperm evolution, and have brought *BAM1* and *HSL2* together as neighboring genes.

279 *HSL1* was duplicated and gave rise to *HAESA* (*HAE*) (Stø et al., 2015). An early split
280 in the HSL clade resulted also in *At5g25930* (called *HSL3*), for which the function is
281 unknown. The HSL3 kinase domain seems to be more closely related to the CEPRs
282 while the HSL3 ectodomain is more closely related to the HSL receptors (Figure 2G).
283 This suggests that HSL3 might bind peptides resembling ligands of HSL1 or HSL2,
284 but give a CEPR-like downstream response to ligand binding. Likewise, CEPR2 is
285 more similar to CEPR1 with regards to the kinase domain, but closer to IKU2 and
286 RLK7 with regards to the LRRs.

287 A similar and more striking positional difference was observed when analyzing the
288 kinase and LRR domains of GASSHO (GSO) in the RGI major clade (Figure 2H); the
289 kinase domain forms a sister clade to the rest of the tree while the ectodomain
290 instead is sister to the other major clades, HSL/BAM. This implies that the two
291 domains of the GSO receptors have evolved with different speed, and that the
292 kinase domain has evolved more slowly, indicating a strong pressure against
293 mutational changes. Alternatively, one can speculate in a recombination event

**Figure 4. Conservation of peptides, LRR scaffold and peptide-ligand binding sites.**




A) Conservation of CIF peptides in diverse species including angiosperms (Arth – *Arabidiopsis thaliana*, Potr – *Populus thrichocarpa*, Brdi - *Brachypodium distachyon*, Vivi – *Vitus vinifera*, Amt – *Amborella trichopoda*), gymnosperms (Piac - *Picea abies*, Pigl – *Picea glauca*) and a bryophyte (Mapo – *Marchantia polymorpha*).B) Crystal structure of GSO1 (PDBid: 6S6Q) and PEPR1 (PDBid:5GR8) with their peptide ligands CIF2 and PEP1 (grey backbones), respectively, interacting along the inner side of the LRR structures. The conserved Pro (P) and Asn (N) residues of the scaffold are highlighted in mustard and green colors. C) Alignment of the 24 amino acids long leucine-rich repeat units of GSO1 and PEPR1, with coloration based on amino acid properties. Above a WebLogo (http://weblogo.berkeley.edu/) consensus sequence visualizing the conservation of the scaffold residues. Residues that interact with the respective peptides according to the crystal structures shown in B) are marked with red rectangles. The red vertical lines mark the repeats covered by the peptide as shown in B). D) Heat maps generated using Repeat Conservation Mapping (http://www.bentlab.russell.wisc.edu/main/main.php) for GSO and PEPR reflecting the degree of identify and similarity in a given position (X-axis) in a given repeat (Y-axis) of the non-scaffold residues, the most conserved are in red and least conserved in blue. The position of the conserved Ps and Ns of the scaffolds are indicated by mustard and green colored vertical lines, respectively. The higher conservation in columns 2 to 4 in some repeats coincide with conserved N-linked glycosylation sites.

between two ancient genes that resulted in a novel fusion gene made up of an ectodomain and a LRR each with different evolutionary histories. GSOs have 31 LRRs while the other subclass XI receptors have only 21-23. The crystal structure of GSO2 bound to its ligand CASPARIAN STRIP INTEGRITY FACTOR (CIFs) has recently been solved (Okuda et al., 2020), which revealed a simple explanation: CIFs interact with their receptors along the inner surface, and since the CIFs are longer (20 amino acids) this surface also needs to be longer (Figure 4A-B). It is of note that

a GSO-like sequence in *M. polymorpha* clusters together with other GSO-like sequences in phylogenetic analyses of both the kinase and LRR domains, suggesting that the two domains followed different evolutionary trajectories since the earliest period of land plant diversification. We have identified potential CIF in *A. trichopoda*, gymnosperms and *M. polymorpha* (Figure 4A), and so far it is unknown whether *M. polymorpha* GSOs recognize this peptide as a ligand.

In Arabidopsis, the two GSO receptors are redundantly involved in regulation of stem cell identity, cell proliferation, control of seedling root growth, determination of root epidermal cell fates, and formation of a functional cuticle around the developing embryo (Tsuwamoto et al., 2008). GSO1 is additionally involved in establishment of the Casparian strip, which works as a diffusion barrier in the vasculature of the Arabidopsis root (Barbosa et al., 2019). *M. polymorpha* is a seedless plant, and lacks vascular tissue, and may at first sight not be expected to have a GSO gene (Zhao et al., 2013). However, a function in the furnishing of epidermal cells with a cuticle that protect them from the dry and potential hostile surrounding (San-Bento et al., 2014), might well have been very important during land plant evolution. Orthologues of genes involved in cuticle development are present in *M. polymorpha* (Bowman et al., 2017), and it is therefore of high interest to find out where the putative GSO orthologues are expressed.

**Structural data are valuable for identification of matching ligand-receptor pairs**

When analyzing the LRR of different RLKs, one should be aware of the highly conserved amino acids making up the scaffold of the ectodomain. In the 24 residues of each repeat, certain amino acids are found in given positions in land plants (PxxLxxLxxLxxLxLxxNxLxGxI with P – Pro; L – Leu; N – Asn: G – Gly; I – Ile; x – any amino acid), and these amino acids may be as conserved as the residues involved in ligand binding (Orr and Aalen, 2017). Therefore, there is a risk that high similarity using Basic Local Alignment Search Tool (BLAST) is based primarily on the scaffold.

One example is the PEP receptors (AtPEPR1 and AtPEPR2) which in Arabidopsis by interaction with endogenous stress-induced PEP peptides are involved in amplification of biotic and abiotic stress responses (Safaeizadeh and Boller, 2019). BLAST searches suggest that there are LRR-RLKs in land plants that are

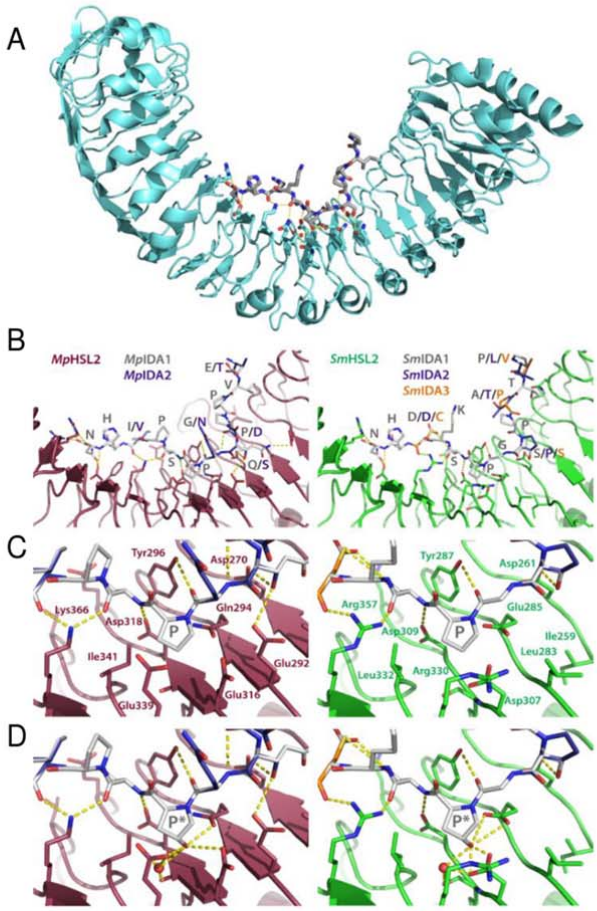
phylogenetically very close to the AtPEPR. However, the phylogenetic distribution of the known ligands, PEP peptides, is limited to angiosperms and PEP peptides show interfamily incompatibility even within flowering plants; PEPs of heterologous origin cannot be recognized as ligands due to the rapid co-evolution of PEPR LRRs with PEPs (Lori et al., 2015). The scaffold residues of the PEPR are highly conserved, but hardly any of the residues involved in ligand binding in Arabidopsis, according to the crystal structure (Tang et al., 2015), are conserved (Figure 4B-D), in strong contrast to for instance GSO receptors (Figure 4D) or HSL2 orthologues (Supplemental Figure 4).

Thus, the putative orthologues may be false positives in the sense that they are not likely to have a conserved function with respect to interaction with highly similar peptide ligands. Identification of ligands for homologues of PEPR receptors in lineages where their known ligands have not been identified, will be instrumental in understanding the evolution of ligand-recognition mechanisms. Possible ligand-receptor pairs have been identified widely across land plants through *in silico* searches for homologues of many other known peptide sequences. Tips for successful peptide hunting will be discussed later in the last section, and here, we walk through two cases and highlight two approaches to corroborating hypothetical interactions: IDA by modelling and CLE by genetics.

The members of peptide families interacting with receptors in the subclass XI LRR-RLK represent the PTMP type and are generated from prepro-precursors by posttranslational processing and amino acid modifications. Gene and genome duplications have facilitated evolutionary changes, but the amino acid sequences of C-terminal bioactive peptides have been conserved. Therefore, the variable region between the hydrophobic N-terminal secretion signal and the peptide motif, has evolved much faster whereas, for instance, are 10 out of the 12 amino acids of the proline-rich PTMP INFLORESCENCE DEFICIENT IN ABSCISSION (IDA) (Butenko et al., 2014), preserved in all orders of angiosperms (Stø et al., 2015).

Putative orthologues of IDA and HSL2 have been identified in *M. polymorpha* (Bowman et al., 2017) *and S. moellendorffii* (Figures 2 and 5). The crystal structure of synthetic AtIDA peptide bound to its receptor AtHAE has recently been solved, which revealed that the ligand is positioned along the inner surface of the LRR

**Figure 5. Homology models of MpHSL2 and SmHSL2 interacting with MpIDA and SmIDA peptides.**




A) The AtHAE-AtIDA crystal structure (PDBid:5ixq) (Santiago et al. 2016 ) the IDA peptide is lining up along the inner face of the LRR structure. B) Overall models of the interaction between *M. polymorpha* and *S. moellendorffii* putative HSL2 receptors (MpHSL2 in magenta and SmHSL2 in green) with respectively two and three *M. polymorpha* and *S. moellendorffii* superimposed putative IDA peptides (grey backbones) built on the AtHAE-AtIDA structure using SWISS-MODEL (Arnold et al., 2005). Note in particular receptor interaction with the Asn (N) at the C-terminal end of the peptides. C) Close-up view of the central parts of the respective receptor models and the surrounding hydrogen bonding network, with a central Pro (P) in the ligands. D) Close-up view as in C), however, with hydroxylation of the central Pro (P*), which facilitates formation of additional hydrogen bonds.
Central amino acids of the receptors, as well as the peptides, are shown as sticks and colored by atom type. Water molecules are shown as red spheres, and modelled based on coordinates from the AtHAE-AtIDA crystal structure. Hydrogen bonds are depicted as dotted lines (yellow). Residues involved in hydrogen bonding to the peptides are depicted with three-letter symbols in colors according to the respective structures. The peptide residues are shown in one-letter symbols. All structure figures were prepared using PyMOL (Schrödinger, LLC).

365 ectodomain of the receptor (Figure 5A) (Santiago et al., 2016). Using this structure
366 (PDBid:5ixq), a three-dimensional model has been generated for IDA-HSL2

orthologues from oil palm, thereby substantiating a likely interaction in this monocot species (Shi et al., 2019). The same modelling strategy was used for putative peptide ligands and receptors of *M. polymorpha* and *S. moellendorffii*, and showed that the potential overall hydrogen bonding interactions between the modelled MpHSL2 and SmHSL2 receptors and their corresponding IDA peptides (MpIDA1-2 and SmIDA1-3) support receptor-peptide binding, regardless of variations in IDA-peptide sequences (Figure 5B-C).

A substantial fraction of the AtHAE amino acids generating hydrogen bonds with IDA residues are identical in MpHSL2 and SmHSL2 (Figure 5A-B and Supplemental Figure 4), and crucial residues, in particular the central Pro and the C-terminal His-Asn are also conserved in the amino acid sequence of the MpIDA1-2 and SmIDA peptides (Figure 5C). Thus, the generated models are consistent with the possible function as ligand-receptor pairs in early-diverging land plant lineages. In Arabidopsis, AtIDA and its receptors AtHAE and AtHSL2 are involved in cell separation processes, like floral organ abscission, lateral root emergence and root cap sloughing (Aalen et al., 2013; Shi et al., 2018). In species that shed other organs, like fruits or leaves, the IDA-HAE/HSL2 signaling module has also been found expressed at the base of the organ to be shed (Shi et al., 2019), indicating that the molecular process of cell separation is highly conserved. This suggests that gain or loss of expression of peptide signaling components through changes in their cis-regulatory elements may have served as an important driving force for evolutionary changes in plant architecture and reproductive strategies. Cell separation process also plays a role in bryophyte development, for example in air chamber formation of *M. polymorpha*. Ishizaki et al. (2015) revealed that E3 ubiquitin ligase regulates cell separation in Marchantia air chamber formation, and it's not known whether the IDL-HAE/HSL signaling module is involved or not in this process. It will be important to find out more about biological processes in bryophytes and lycophytes that are controlled by cell separation, where the putative IDA and HSL orthologues might have any conserved roles.

It has just started to be revealed that the CLE peptide signaling has evolutionary conserved roles from angiosperms to *P. patens* and *M. polymorpha*, two models of early-appearing land plant lineages to which molecular genetic tools are readily

399 applicable (Hirakawa et al., 2019; Whitewoods et al., 2018). One of the known key
400 roles of the CLE signaling in angiosperms is to regulate cell proliferation in the
401 sporophytic (2*n*) meristems, which grow indeterminately. In the bryophytes, the
402 sporophyte shows determinate growth while indeterminate meristems are present in
403 the gametophyte body (1*n*). The CLE signaling in both *P. patens* and *M. polymorpha*
404 is involved in cell proliferation in the gametophyte (Hirakawa et al., 2019;
405 Whitewoods et al., 2018). These studies support the idea that peptide-receptor
406 modules are conserved and employed in a parallel manner although the evolutionary
407 relationship between the bryophyte gametophytic meristems and the vascular plant
408 sporophytic meristems is still debated (Bowman, 2013).

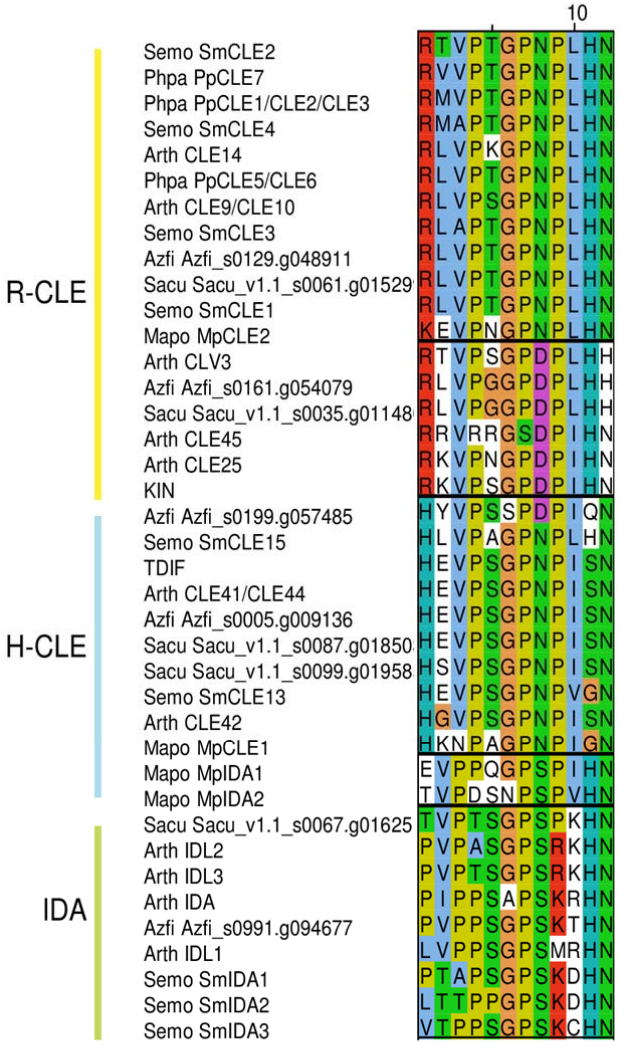
409 In both cases, structural and biochemical studies are needed to establish that these
410 peptide ligand-receptor pairs are conserved between divergent lineages.

411 **PTMPs of different families share structural features**

412 Being the largest peptide family, the CLE family is diverse, with altogether 32
413 members in Arabidopsis, some without and some with known receptors, e.g. CLV3
414 and CLE1-8 can bind and activate CLV1, BAM1 and BAM2; but not BAM3, which
415 instead has a preference for the arginine-rich CLE45 (Endo et al., 2013; Hazak et al.,
416 2017). There are three TDR/PXY-interacting CLE peptides that have an N-terminal
417 histidine (His, H) residue instead of the arginine (Arg, R) found in this position in
418 other CLE peptide (Ito et al., 2006) (Figure 6). While TDR/PXY receptors and H-type
419 CLE peptides are involved in vascular development, R-type CLE peptides are known
420 for involvement in maintenance of the root and shoot meristems (Hirakawa and
421 Sawa, 2019; Yamaguchi et al., 2016).

422 To our surprise, several Arabidopsis CLE peptides were found to interact with LRRs
423 of different clades. The AtCLE9/CLE10 peptides interact with both the HSL1 and
424 BAM1 receptors, depending on tissue type (Qian et al., 2018). AtCLE14 peptide can
425 signal through AtPEPR2 (Gutierrez-Alanis et al., 2017). While PEPs and CLEs differ
426 in length, the C-terminal 12 amino acids of mature PEPs share sequence similarity to
427 CLEs (Table 1A), offering a possible explanation for the recognition mechanism.
428 Another CLE peptide, AtCLE45, which diverge from other CLE peptides by a number
429 of arginine (Arg, R) residues near its N-terminus, is perceived by canonical CLE

**Figure 6. Conservation of CLE and IDA peptides.**




Alignments of mature CLE and IDA peptides from the bryophyte *M polymorpha* to angiosperms. Note shared core residues (SGPS) and C-terminal end (NH) between IDA and some of the CLE peptides, and the presence of almost identical R-CLE, H-CLE and IDA peptides in early-diverging plants, ferns and Arabidopsis. The amino acids are colored according to chemical properties.

430 receptors as well as an RGF receptor homologue, AtRGI4/SKM2 (Endo et al., 2013;

Hazak et al., 2017).

These “unconventional” ligand-receptor pairs raise a question of how the specificity of ligand-receptor pairs evolves. It is of note that genes encoding CLE peptides highly similar to AtCLE9/CLE10 are present in both *M. polymorpha* and *P. patens* (PpCLE5/CLE6) (Whitewoods et al., 2018) (Figure 6). We therefore speculate that during early evolution of peptide signaling, there were fewer peptides, and receptors were less specific. Accordingly, large-scale clustering analyses of land plant CLE peptides based on their entire prepropeptide sequences found a smaller number of clusters in *S. moellendorffii* and bryophyte species, indicative of the diversification of CLE peptide sequences, which could have resulted in changes in the specificity of ligand-receptor interactions, during land plant evolution (Goad et al., 2017). Hirakawa et al. (2017) found that a synthetic CLE peptide, KIN named after the K ($2^{nd}$), I ($10^{th}$), and N ($12^{th}$) residues crucial for function, exerts both R-type and H-type CLE activities and interact directly with both receptors (Figure 6) (Hirakawa et al., 2017). Intriguingly, the three critical residues in KIN are conserved in the H-type CLE of *M. polymorpha*, MpCLE1, illustrating the potential for a broader specificity in ligand-receptor interaction hidden in short mature peptide sequences (Figure 6).

We may also learn from some common structural features in known endogenous peptide ligands, reviewed and discussed in (Zhang et al., 2016). For instance, the IDA and CLE peptides are all 12-14 amino acids long, proline-rich, and need C-terminal NH or HH residues for function (Figure 6; Table 1A). Similarities are also recognized among CEPs, PIP-PIPLs, and IDA peptides with respect to the size and amino acid composition. Most of the CEPs and PIP-PIPLs, however, lack the C-terminal HN or HH residues that have been shown to interact with two closely positioned Arg (R) residues found in many receptors (Hou et al., 2014; Song et al., 2016; Vie et al., 2015) (Table 1A). Instead, CEPs and PIP-PIPLs share the C-terminal GxGH motif (Vie et al., 2015) (Table 1A). The CEPs and PIP-PIPLs have been demonstrated to signal through closely-related receptors of the HSL major clade, CEPRs and RLK7, respectively (Figure 2). This point to the possibility that a ligand recognition mechanism is conserved among recently diversified receptors, and in the case of the CEP and PIP-PIPL signaling, the GxGH motif could be crucial for peptide-receptor interactions.

## Proline hydroxylation and tyrosine sulfation may have contributed to more stringent peptide-receptor interactions

Structural variation of signaling peptides with more stringent ligand-receptor interactions can evolve not only from changes in the primary sequences, but through proteolytic processing of prepropeptides to release bioactive small peptides and post-translational modifications (PTMs) of amino acids. In fact, mass spectrometric analyses identified PTM residues in mature, biologically functional peptides, suggesting their importance (Kondo et al., 2006). The PTM processes tend to be irreversible and hence require precise regulation.

Two PTMs, proline hydroxylation and hydroxyproline arabinosylation, have been detected in planta on CLE peptides (Stührwohldt and Schaller, 2019), and synthetic peptides of the IDA/IDL family have been shown to bind and activate the HAE and HSL2 receptors more efficiently with than without hydroxylation of the central proline (Pro) (Butenko et al., 2014; Santiago et al., 2016). Prolyl 4-hydroxylases (P4Hs) and hydroxyproline O-arabinosyl transferase (HPAT), which mediate proline hydroxylation and hydroxyproline arabinosylation, respectively, are evolutionary conserved from algae to angiosperms (Myllyharju, 2003; Ogawa-Ohnishi et al., 2013). Thus, mature peptides produced from IDA and CLE orthologues may have been hydroxylated already in early land plants (Table 1A) (Bowman et al., 2017; Oelkers et al., 2008). As an initial approach to test this possibility, ligand-receptor interactions of putative *S. moellendorffii* and *M. polymorph*a IDA and HSL2 orthologues were modelled based on the crystal structure of AtIDA and AtHAE both without and with the hydroxylated central Pro (Figure 5B and C). In these models the hydroxylation postulated an increased number of hydrogen bonds, which would result in a stronger interaction.

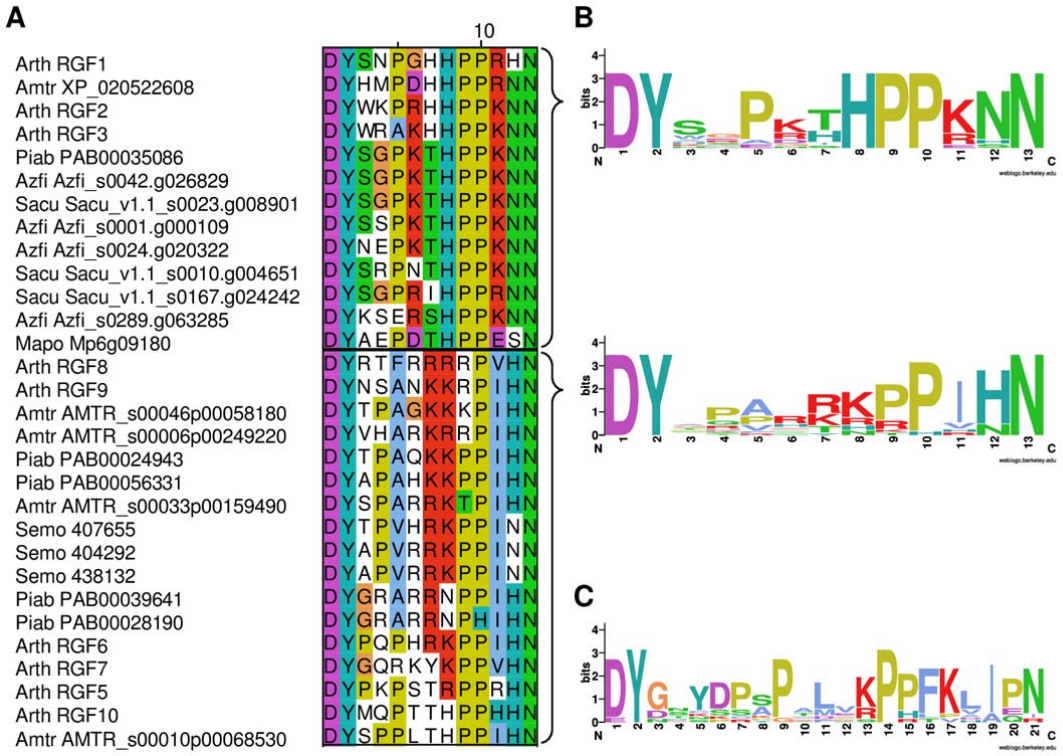
Structural analyses of bioactive forms of PTMPs in basal land plants will be critical for confirming the prediction from the models. These data, however, point to the possible contribution of PTMs in diversification of peptide ligands and in defining the specificity of ligand-receptor interaction since the early stages of land plant evolution.

We have identified putative homologues in gymnosperms, ferns, lycophytes of genes encoding RGF, CIF and phytosulfokine (PSK) peptides, which are the ligands of RGI, GSO, and PSK receptors (PSKR), respectively, and characterized by the serial arrangement of aspartic acid (Asp, D) and sulfated tyrosine (Tyr, Y) residues at the N-terminus of the mature peptide. In Arabidopsis, Tyr sulfation regulates protein-protein interactions and affects receptor binding (Kaufmann and Sauter, 2019; Matsubayashi and Sakagami, 1996; Shinohara et al., 2016). Komori et al. (2009) identified TYROSYLPROTEIN SULFOTRANSFERASE (AtTPST) as an enzyme responsible for tyrosine sulfation in Arabidopsis. TPST-encoding genes are conserved across land plants and are found in angiosperms as well as in *S. moellendorffii*, *P. patens*, and *M. polymorpha*, supporting the likely presence of sulfated signaling peptides and the plausible contribution of PTMs to the ligand diversity in early land plant evolution. The solved crystal structure of RGF1 and its receptor RGI3 (At4g26540) (Song et al., 2016); (note that the *RGFR/RGI* gene nomenclature differs among literatures) revealed that the $DY^{sulf}$ residues are recognized by the amino acid motif RxGG of the receptor. Importantly, the RGI orthologues from bryophytes to Arabidopsis, contain this motif, which distinguishes RGIs from other LRR-RLK XI members (Supplemental Figure 5).

RGF and CIF peptides may seem difficult to tell apart as they both start with $DY^{sulf}$, end with an asparagine (Asn, N), and are enriched in proline (Pro, P) residues. But they are, as mentioned, of different lengths (Figure 4A and Figure 7). Alignments of putative RGF orthologues differ from that of CIFs, and additionally suggest that RGFs fall in two groups, which can be hypothesized to correspond to the two RGI receptor groups mentioned earlier (Figure 7 and Supplemental Figure 3).

The mature active form of PSK is easily recognized, but difficult to find in *in silico* searches, as the mature signaling molecule is just a pentapeptide, YIYIQ, with two sulfated Tyr (Matsubayashi et al., 1996). In Arabidopsis, PSKs are perceived by two LRR-RLKs of subclass X, PSKRs, and bind in a small island in the LRR structure that doesn't fit in with the rest of the repeats. Amino acids involved in peptide binding in this region are conserved according to homology mapping (Amano et al., 2007; Matsubayashi et al., 2002) (Supplemental Figure 6). We have identified putative homologues of PSK and PSKR in *P. abies*. Together with previous studies that

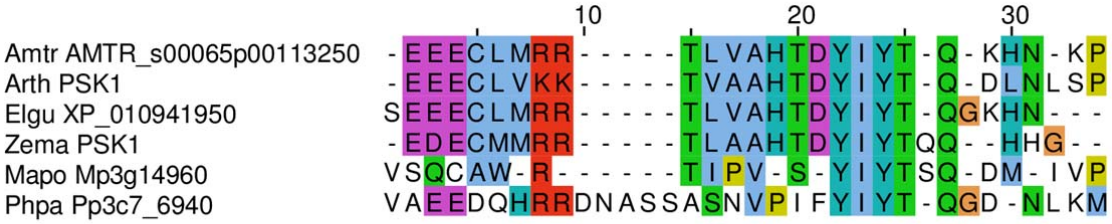
**Figure 7. Conservation of RGF peptides.**




A) Alignment of RGFs from the bryophyte *M. polymorpha* to angiosperms, with amino acids colored according to chemical properties, suggesting that there are two distinct types of RGFs. B) WEBlogo for the two RGF types. C) For comparison, WEBlogo for CIF peptides.

report the identification of *PSK*-like genes and bioactivities in *Cryptomeria japonica* and *Cunninghamia lanceolata*, the PSK signaling is likely conserved in gymnosperms (Igasaki et al., 2003; Wu et al., 2019). We have also identified putative *PSK*-like genes in the bryophyte model plants (Figure 8). In the PSK-like sequences found in *P. patens* and *M. polymorpha* the Asp residue that precedes the YIYIQ sequence is missing, but the conserved region N-terminally to the DYIYIQ motif can still be recognized. Such conserved sequences outside the assumed mature peptide, might be recognition sites for processing enzymes. This suggests that mechanisms for processing prepropeptides to mature secretable peptides must have evolved at an early stage of the evolution of peptide signaling.

Figure 8. Conservation of PSK




Alignments of a part of PSK propeptides covering the active peptide (YIYTQ) from diverse species, and the nearby conserved motif of unknown function. The amino acids are colored according to chemical properties. (Elgu – Elaeis guineensis, oil palm; Zema – Zea maize)

Our comparison of signaling peptides have revealed similarities between members of different peptide families, suggesting common origin, although the highly variable prepro sequences would rather support convergent evolution. Anyhow, an increase in number and structural variations of both peptides and receptors could have contributed to greater stringency of interactions of each peptide-receptor pair over evolutionary time.

## Perspectives

With the increasing availability of genomic data, we can now analyze the molecular inventory of peptide signaling components in all major lineages of land plants. All known signaling peptides were originally identified in angiosperms. Their homologues, however, exist in other taxa (Figure 1). Several peptide classes have their ancestry in bryophytes while no trustworthy homologues have been found to date in extant algal genomes, suggesting that peptide signaling evolved in the earliest land plants.

Most receptor families of LRR-RLK XI have their bryophyte postulated orthologues (Figure 2). Since PTMPs are short, and the rest of the prepropeptides highly variable, it is difficult to find peptide-encoding genes by conventional BLAST

searches. During annotation of genes in newly sequenced genomes, peptide genes are for the same reasons at risk of being overlooked. We have, however, successfully identified RGF, PSK and CIF peptides using Pattern Hit Initiated (PHI) BLAST searches where the residues DY should be present, and analyzed the hits for a suitable prepropeptide length, presence of a hydrophobic N-terminal export signal, and the assumed mature peptide sequence positioned near or at the C-terminal end (Figure 1; Table 1). Another useful procedure is to first identify homologues in seed plants and ferns, confirm their homology, and thereafter include these sequences in queries when setting up new searches in lycophytes and bryophytes. Limiting the query sequences to the region encompassing mature peptides works more efficiently, but the results should be examined using the entire coding sequences as in PHI-BLAST. When interpreting the sequences, it has to be kept in mind that one can collect and analyze sequence information only from extant species, which may have highly diversified from early land plant ancestors. As discussed in the previous section, *in silico* modelling based on solved crystal structures of related ligand/receptor pairs, turned out to be helpful in substantiating hypothesized interactions of putatively orthologous ligand-receptor pairs. A topic in its own right, which we have not covered here, is the importance of extensive interactions between LRR-RLK subclass XI receptors and the multi-faceted smaller co-receptors as key partners in receptor complexes in diverse peptide signaling pathways (Gou and Li, 2020; He et al., 2018; Hohmann et al., 2018; Liang and Zhou, 2018; Ma et al., 2016).

As we have seen, genes encoding potential peptide ligands and receptors are now available in a wide range of species including non-seed plants. Concurrently, land plant evolution has successfully made use of a broadened spectrum of endogenous and exogenous ligands as more complex organ evolved and interspecies interactions increased. Except for the bryophyte CLE signaling pathways, however, biological roles have not been assigned for peptide signaling outside of seed plants. Functional and biochemical studies of these sequences are critical for elucidating the (r)evolutionary history of land plant peptide signaling pathways.

A number of receptors, even some in Arabidopsis, are orphan, in the sense that their peptide ligands are unknown. Likewise, all PTMP families seem to have orphan members. These may have resulted from gene or genome duplications where some

sister genes have accumulated mutations that make it difficult to bind receptors. An alternative (and likely) explanation is that more complex genetic, molecular and biochemical factors and condition for peptide-mediated cell-to-cell communication remains to be discovered.

*Where do new peptide-encoding genes come from? Can we trace their ancestry back in ancient peptides conserved in earlier-appearing land plant lineages?* Progressive expansion of the peptide repertoire is observed during land plant evolution. Several peptide families show similarities in their mature peptide sequences. Nevertheless, there is no conclusive evidence for the origin of derived peptide families. The highly variable central part of peptide precursor sequences makes it difficult to infer interfamily relationships by phylogenetic analyses. At present it looks like the largest expansion of the peptide diversity occurred during seed plant evolution (Figure 1). This may reflect the increased need for cell-to-cell communication for development of more complex organisms with more complex organs. More genomes of gymnosperm and fern species should be sequenced to test how tight this correlation is.

Our knowledge of the biological roles of the peptide-LRR-RLK modules is limited mostly to angiosperm organs such as flowers and seeds, vascular tissue, and multicellular root and shoot meristems. These organs evolved in the vascular plant lineages, and most lack homologous structures outside of flowering plants. A question therefore arises as to whether these peptide signaling modules mainly have undergone neofunctionalization in derived lineages. This may well be the case from an organismal perspective, however, from a molecular and cellular perspective it may not be. Where and when a signaling system triggers a given outcome (e.g. cell division or cell separation) may be the crux of the matter. Thus, a very important next step in tracing the evolutionary origin and history of land plant peptide signaling pathways will be to investigate thoroughly the expression patterns of LRR-RLK receptors and their putative peptide ligands, and to study downstream molecular events.

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We apologize to authors of literature not discussed due to space constraints. Investigations by CF were supported by Nakatsuji Foresight Foundation Research Grant and JSPS KAKENHI Grant Number 20K06770.

**Author Contributions**

MW and SS initiated and drafted the work; CF, AKK, RMA and RBA identified and analyzed sequences; MH performed structure analyses and modelling; CF and RBA wrote the article.

626 **Table 1. Major families of signaling peptides in land plants.**

| **A. Small post-translationally modified peptides (PTMP)** | | | Modified amino acids:<br>P – hydroxylated Pro<br>Y – sulfated Tyr |
|---|---|---|---|
| Peptide family | Major receptor(s) | Amino acid sequence of a representative mature peptide | Amino acid sequence of mature peptide in *M. polymorpha* |
| CEP | CEPR<br>(LRR-RLK XI) | Arth CEP1:<br>DFRPTNPGNSPGVGH | |
| CIF | GSO1/SGN3, GSO2<br>(LRR-RLK XI) | Arth CIF1:<br>DYGNNSPSPRLERPPFKLIPN | Putative MpCIF:<br>DYCDCYGFDPSPPLVHAEITF |
| CLE<br>(CLV3/R class) | CLV<br>(LRR-RLK XI) | Arth CLV3:<br>RTVPSGPDPLHH | MpCLE2:<br>KEVPNGPNPLHN |
| CLE<br>(TDIF/H class) | PXY/TDR<br>(LRR-RLK XI) | Arth CLE41:<br>HEVPSGPNPISN | MpCLE1:<br>HKNPAGPNPIGN |
| IDA + IDL | HAE, HSL<br>(LRR-RLK XI) | Arth IDA:<br>PIPPSAPSKRHN | MpIDA1:<br>EVPPQGPSPIHN |
| Pep | PEPR<br>(LRR-RLK XI) | Arth Pep1:<br>ATKVKAKQRGKEKVSSGRPGQHN | |
| PIP + PIPL | RLK7<br>(LRR-RLK XI) | Arth TOLS2/PIPL3:<br>ASGPSRRGAGH | |
| PSK | PSKR<br>(LRR-RLK X) | Arth PSK1:<br>YIYTQ | Putative MpPSK:<br>YIYTSQ |
| PSY | PSYR<br>(LRR-RLK X) | Arth PSY1:<br>DYGDPSANPKHDPGVPPS | |
| RGF/GLV/ | RGFR | Arth RGF1/GLV11: | Putative MpRGF: |

| **B. Cysteine-rich peptide (CRP)** | | | Modified amino acids<br>C- conserved Cys |
|---|---|---|---|
| Peptide family | Major receptor(s) | Amino acid sequence of the conserved region in a representative peptide | *M. polymorpha* peptide sequence |
| EPF-EPFL | ER, ERL<br>(LRR-RLK XIII) | Arth EPFL5:<br>PGSVPPMCRLKCGKCEPCKAVHVPIQPGLIMPLEYYP EAWRCKCGNKLFMP | MpEPFL2:<br>MGSKPPNCEDKCHYCNPCEAVEV PISNREVQKVKALETDAAAGTSY QKVSTALADPHDTNYMPESWRCA CGDEYFNP |
| LURE | PRK (LRR-RLK III),<br>MIK-MDIS<br>(LRR-RLK XI/XII-VI) | Arth LURE1.1:<br>ILIKESSEEERIYPFNPVASPFDPRSLNQILKIGKIG YCFDCARACMRRDRYIRTCSFERKLCRCSYSHIHHTH G | |
| RALF-RALFL | CrRLK1L family | Arth RALF1:<br>ATTKYISYQSLKRNSVPCSRRGASYYNCQNGAQANPY SRGCSKIARCRS | MpRALF:<br>ASGYYVGYGALTANRVPCPPQSG RSYYTPGCSTASGPVRPYTRGCS TIITRCARDG |
| SCR/SP11-SCRL | S-domain RLK | Brol SCR6:<br>NLKKNCVGKTRLPGPCGDSGASSCRDLYNQTEKTMPV SCRCVPTGRCFCSLCK | |
| TPD1 | EMS1<br>(LRR-RLK X) | Arth TPD1:<br>ERIGEKCKSTDIVVNQAVTEPMPNGIPGYMVEITNQC MSGCIISRIHINCGWFSSAKLINPRVF | MpTPD1:<br>QSRRLLISDQCSNKDISIIQNEE TNAGIPRYVVQIVNTCISDCAPT DIHVFCGWFASSPLVNPNYF |

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