Mini review

Mechanisms and strategies shaping plant peptide hormones

Running head: Shaping peptide hormones

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Mechanisms and strategies shaping plant peptide hormones

Running head: shaping peptide hormones

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Abbreviations: CEP, C-terminally encoded peptide; CIF, Casparian strip integrity factor; CLE, CLAVATA3/EMBRYO SURROUNDING REGION-related; CRP, cysteine-rich peptide; IDA, INFLORESCENCE DEFICIENT IN ABSCISSION; LRR-RK, Leucine-rich repeat receptor kinase; PIP, PAMP-INDUCED PEPTIDE; PSK, Phytosulfokine; RGF, Root meristem growth factor; SERK, SOMATIC EMBRYOGENESIS RECEPTOR KINASE; TDIF, Tracheary element differentiation inhibitory factor

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Abstract

Plant genomes encode a variety of short peptides acting as signaling molecules. Since the discovery of tomato systemin, a myriad of peptide signals, ranging in size, structure, and modifications, have been found in plants. Moreover, new peptides are still being identified. Surprisingly, non-plant organisms, especially pathogens, also produce peptides which exert hormonal activities against host plants by hijacking their endogenous reception systems. In this review, we focus on short secretory peptides ranging from 5 to 20 amino acids. We first summarize recent advances in understanding relationships between the bioactivities and structures of plant peptide hormones. Subsequently, we introduce the topic of peptides produced by non-plant organisms. Lastly, we describe artificial peptides synthesized in laboratories, which possess intriguing bioactive properties beyond those of natural peptide hormones.

Keywords: CLE; Ligand; LRR-RK; Pathogens; Peptide hormone; Receptor

Introduction

In this mini-review, we aim to discuss how molecular structures of plant peptide hormones have been shaped and how one can design artificial peptide hormones with novel biological functions. Since the discovery of tomato systemin (Pearce et al., 1991), a number of different classes of peptide signals have been identified. They act extracellularly through recognition by their receptors on the plasma membrane of target cells. Based on 1) mature peptide structures and 2) modes of trafficking into the extracellular space, these peptide signals, or plant peptide hormones, are classified into three groups: secreted small peptides, non-secreted small peptides and secreted cysteine-rich peptides (CRP) (Matsubayashi 2014). The first two are both approximately 5-20 amino acid in length and do not undergo intramolecular disulfide bonding while the CRPs are consisted of 50-100 amino acid and have relatively fixed structure due to intracellular disulfide bridges (Ohki et al. 2011). In this mini-review, we focus on the short peptides, all of which are perceived by leucine-rich repeat receptor kinases (LRR-RK). Interestingly, such peptide signals are also made by phytopathogens to hijack functions of host plants. In the first and second sections, we will describe variations found in plants and parasitic phytopathogens, respectively. Since this paper focuses on molecular structures of peptides, especially in dicots, please refer to other literature for latest information and discussion on biological functions of peptide signals in diverse plant species including monocots (Grienenberger and Fletcher 2015, Higashiyama and Takeuchi 2015, Je et al. 2016, Okamoto et al. 2016, Somssich et al. 2016, Zipfel and Oldroyd 2017, Stegmann et al. 2017). In addition to the naturally occurring mechanisms shaping these peptide hormones, we will introduce synthetic approaches to design novel bio-active peptides in the last section.

1. Made in plants

1-1. Peptide classes, receptors and structural insights

Small peptide signals of plants include systemin, PSK (phytosulfokine), HypSys (hydroxyproline-rich glycopeptide systemin), Pep1, CLE (CLAVATA3/EMBRYO SURROUNDING REGION-related)/TDIF (tracheary element differentiation inhibitory factor), PSY (plant peptide containing sulfated tyrosine), CEP (C-terminally encoded peptide), RGF/CLEL/GLV (root meristem growth factor/CLE-like/GOLVEN), PIP (PAMP-INDUCED PEPTIDE), IDA (INFLORESCENCE DEFICIENT IN ABSCISSION), CIF (Casparian strip integrity factor) subclasses (Pearce et al. 1991, Matsubayashi and Sakagami 1996, Pearce et al. 2001, Huffaker et al. 2006, Ito et al. 2006, Ohyama et al. 2008, Ohyama et al. 2009, Matsuzaki et al. 2010, Okamoto et al. 2013, Hou et al. 2014, Schardon et al. 2016, Doblas et al. 2017, Nakayama et al. 2017). They are encoded in the genome as precursor proteins and mature into active forms via post-translational processing including proteolytic cleavage by proteases (Tamaki et al. 2011, Engineer et al. 2014, Schardon et al. 2016) and modifications of specific residues by modifying enzymes (Hieta and Myllyharju 2002, Tiainen et al. 2005, Yuasa et al. 2005, Komori et al. 2009, Ogawa-Ohnishi et al. 2013) such as tyrosine sulfation, proline hydroxylation and hydroxyproline arabinosylation.

Receptors for these peptides have been identified genetically and biochemically (Butenko et al. 2014). The major receptor class is the leucine-rich repeat receptor kinases (LRR-RKs, Shiu and Bleecker 2001). LRR-RKs are single transmembrane-domain kinases containing extracellular LRRs which can participate in versatile molecular recognition. The receptors for CLE/TDIF (Ogawa et al. 2008,

Hirakawa et al. 2008), IDA (Santiago et al. 2016), CEP (Tabata et al., 2014), Pep1 (Yamaguchi et al. 2006), RGF (Shinohara et al. 2016), PIP1 (Hou et al. 2014) and CIF (Doblas et al. 2017, Nakayama et al. 2017) are in the subclass XI of the LRR-RK family, while the PSK receptor PSKR is in the subclass X (Matsubayashi et al. 2002). Binding of the peptide hormones to their receptors is thought to recruit additional co-receptor for the activation of downstream signaling events by trans-phosphorylation between kinase domains in proximity (Fig. 1A). Co-crystal structures of several peptide-receptor pairs have been solved recently (Song et al. 2016a). The extracellular region of the receptors contains LRR, which forms a superhelix structure providing the structural backbone to form an interaction surface to a corresponding peptide ligand. In each peptide-receptor complex, a peptide molecule is stretched along the inner surface of the superhelix (Fig. 1B). Generally, peptide ligands act as a molecular glue to stabilize the interaction between each corresponding receptor and its co-receptor (Fig. 1A and 1B) (Santiago et al. 2016, Morita et al. 2016, Zhang et al. 2016, Song et al. 2016b). Interestingly, in the case of the PSK perception by PSKR, the peptide binding to the receptor triggers its allosteric change, which allows the binding of the co-receptor SERK (SOMATIC EMBRYOGENESIS RECEPTOR KINASE) to PSKR (Wang et al. 2015). In some cases, LRR-RKs may work with other classes of proteins to perceive peptide signals and/or trigger intracellular signaling, such as single-transmembrane LRR proteins harboring only extracellular LRRs without intracellular kinase domains (Jeong et al 1999, Nadeau and Sack 2002) and transmembrane kinase proteins containing only intracellular kinase domains without extracellular LRRs (Müller et al 2008) (Fig. 1C). However, the mechanisms of direct peptide recognition and signal transduction by these proteins remain to be understood precisely (Bleckmann et al 2010, Zhu et al 2010,

Kinoshita et al 2010, Nimchuk et al. 2011, Bommert et al 2013, Stahl et al 2013, Ishida et al 2014).

At the binding surface of peptide hormone and receptor, both the main and side chains of the peptide form multiple hydrogen bonds and/or hydrophobic contacts with the receptor. In some cases, side chain modifications of peptide ligands directly interact with receptor residues like the PSK-PSKR pair that involves two sulfate groups of PSK in the interaction surface (Wang et al. 2015). The sulfate group of RGF1 is recognized by the RxGG motif that is conserved among RGF receptors (Song et al. 2016b). The hydroxyproline residue of IDA peptide forms hydrogen bonds with the receptor (Santiago et al. 2016). By contrast, hydroxyprolines of CLE peptides do not directly interact with their receptors (Morita et al. 2016, Zhang et al. 2016). Further arabinosylation of hydroxyprolines is found in some CLEs, and the arabinosylation is important for bioactivity (Ohyama et al. 2009, Okamoto et al. 2013, Xu et al. 2015). A proposed role for the arabniosylation is to force a conformational distortion on the peptide backbone in a highly directional manner, conferring a significant increase in affinity with the corresponding receptors (Shinohara and Matsubayashi 2013).

The reported co-crystal structures of subclass XI LRR-RKs and their peptide ligands also show that the conserved RxR motif of the receptors are involved in the interaction with the free carboxyl group of the last residue of TDIF/CLE41, Pep1, RGF1 or IDA (Song et al. 2016a), which is in agreement with the report on the SOL1 (SUPPRESSOR OF LLP1 1) protease required for the maturation of functional CLE19 peptide by cleaving off the C-terminal extension in its precursor (Tamaki et al. 2011).

Collectively, shapes of peptide hormones and their recognition by corresponding receptors have been coordinately elaborated during their molecular

evolution.

1-2. Diversification under evolutionary constraints

Thus far, all identified plant peptide hormones are encoded in a gene family. Each family contains small variations in the mature ligand sequences. Such minor variations could be formed under certain evolutionary pressures in their molecular evolution. Contribution of each amino acid residue in peptide hormones can be examined by structure-activity relationship analysis using mutated peptides. A typical method is alanine-scanning in which every residue of a peptide hormone is substituted one by one with alanine. If the alanine substitution of a certain residue affects the bioactivity, the side chain of the residue must play an important role in exerting the specific bioactivity. For example, the 6th glycine of TDIF/CLE41 peptide is important for its kinked structure that is recognized by its receptor TDR/PXY, and indeed the substitution of the glycine into alanine abolishes the bioactivity (Ito et al. 2006, Morita et al. 2016).

On the other hand, some residues can play a role to avoid activating unwanted signaling. Very recently, an intriguing example of this case was reported (Hirakawa et al. 2017). The CLE-family peptides are classified into two subfamilies; one group (A-type) that can affect the shoot and root meristems and the other (B-type) that affects the vascular meristem (Cock and McCormick 2001, Ito et al. 2006, Whitford et al. 2008). B-type CLEs have the characteristic serine residue at the 11th position in the mature form (Fig. 2) that is conserved only among the B-type peptides within the CLE family (Oelkers et al. 2008, Hirakawa and Bowman 2015). Surprisingly, the mutation of the 11th serine into histidine results in the acquisition of the A-type activity without losing the original B-type activity (Fig. 2). Such a striking property has been overlooked

184 by the previous alanine scanning, which classified the 11th serine as a "dispensable"
185 residue for bioactivity (Ito et al. 2006). These suggest that the 11th serine may be kept
186 unchanged to avoid unwanted signaling which disrupts well-organized signaling
187 network for growth and development.
188
189 **2. Made in pathogens**
190 Homologs of plant peptide hormones are found in phytopathogen genomes, which may
191 be acquired either via convergent evolution or horizontal gene transfer from host plants
192 (Olsen and Skriver 2003). Parasitic nematodes enter the plant root and alter its tissue
193 structure to form feeding cells/tissues such as syncytia and giant cells (Mitchum et al.
194 2012). For this purpose, parasitic nematodes secrete effector proteins to hijack
195 developmental systems of host plants. The first example of secretory peptide mimics
196 produced by parasitic nematodes is HgCLE1/syv46 of soybean cyst nematode
197 *Heterodera glycines*, which shows similarity to the A-type CLE peptides of host plants
198 (Wang et al. 2001, Olsen and Skriver 2003). HgCLE1 is expressed mainly in the
199 esophageal gland and released into plant cells via stylet (Wang et al. 2005). The
200 precursor protein of HgCLE1 peptide contains a domain essential for its subcellular
201 trafficking into apoplast, allowing the nematode-derived CLE peptide to interact with
202 the extracellular domains of target receptors in host plants (Wang et al. 2010, Replogle
203 et al. 2011). In addition to the A-type CLE peptides, B-type CLE homologs were also
204 reported recently in *Heterodera schachtii* (Guo et al. 2017). Since A-type and B-type
205 CLEs synergistically promote vascular thickening in plants (Whitford et al. 2008), the
206 nematodes may have exploited this synergistic effects for maximizing their successful
207 parasitism. Interestingly, the CLE peptide sequences in nematodes are slightly different

208 from plant CLE peptides (Fig. 2, Yamaguchi et al. 2016), which may reflect differences
209 in maturation processes between nematode and plant CLE peptides. In addition to short
210 CLE peptides, functional homologues of the CRP-type peptide hormone RALF (rapid
211 alkalinization factor), are also found in fungal pathogens (Masachis et al. 2016, Thynne
212 et al. 2016). Significance of differences in peptide sequences between homologs derived
213 from plants and pathogens has not been well understood. As-yet-uncovered constraints
214 may have existed in the evolution of the plant-pathogen interaction.
215
216 **3. Made in laboratories**
217 As mentioned above, natural peptide hormones are made in living organisms and have
218 been optimally shaped under evolutionary pressures. By contrast, chemical synthesis in
219 laboratories does not have such restrictions and thus could enable new design principles
220 for functional peptides. In theory, engineering of artificial bioactive molecules could be
221 accomplished for any type of hormones. For example, one may imagine a molecule
222 which exerts both auxin and cytokinin activities by simply coupling indole-3-acetic acid
223 and kinetin. However, considering the structural information on the ligand binding
224 pockets of the auxin and cytokinin receptors (Tan et al. 2007, Hothorn et al. 2011), this
225 imaginary bi-functional molecule is difficult to be designed. On the other hand,
226 synthesis of bi-functional peptides that bind and activate two distinct CLE receptors was
227 reported recently (Hirakawa et al. 2017). CLV3 and CLE25 both belong to A-type CLE
228 peptides and affect the shoot and root meristems. They have 4 amino acid substitutions
229 to each other (Fig. 2). Surprisingly, systematic swapping of these residues led to the
230 discovery of a synthetic peptide that exerts the B-type activity in addition to the original
231 A-type activity (Fig. 2: CLV3-KIN that has the CLV3 backbone with K, I and N

232 substitutions derived from CLE25) by interacting with both A-type and B-type CLE
233 receptors. As mentioned above in the section 1-2, TDIF/CLE41 can also acquire
234 bi-functionality by an amino acid substitution (Fig. 2: CLE41-H). These studies suggest
235 that building blocks for designing unnatural bi-functional peptides (such as CLV3-KIN
236 and CLE41-H) exist in the natural diversity in the genome. Further identification of
237 such cryptic bio-activities will be a future challenge toward engineering cell-cell
238 signaling in plants.

239 Peptides are chains of amino acids linked by amide bonds (peptide bonds).
240 Peptide-like molecules with different main chain structures are collectively called
241 peptidomimetics. Peptidomimetics have been developed especially in the field of drug
242 discovery pursuing enhanced *in vivo* stability and activity (Vagner et al. 2008). A
243 previous study adopted this approach to understand the structure-activity relationship of
244 CLE peptides, and the 9th proline residue was substituted with a series of *N*-modified
245 peptoids, such as sarcosine (*N*-methylglycine), to control the bioactivity (Kondo et al.
246 2011). Besides peptoids, synthetic routes for new molecular designs of peptidomimetics
247 have been explored not only for pure chemistry but also for development of
248 bio-engineering approaches. By harnessing diversity in molecular structures of
249 peptides/peptidomimetics, which may also include unnatural side chains, we may be
250 able to expand toolkits for peptide hormone studies toward creating unprecedented
251 bioactivities.

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Disclosures

The authors have no conflicts of interest to declare.

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524 **Figure Legend**

525

526 **Fig. 1. A proposed action of a peptide hormone and its receptor/co-receptor pair in**
527 **the 'molecular glue' model.**

528 (A) A receptor (red) interacts with its co-receptor (blue) only in the presence of a
529 peptide hormone (brown). Upon the peptide binding, it is considered that the receptor
530 and co-receptor can phosphorylate each other, triggering the intracellular signaling.
531 (B) Co-crystal structure of the IDA peptide (yellow) and LRR domains of its receptor
532 HAESA (red) and co-receptor SERK1 (blue) (PBD accession number: 5IYX) (Santiago
533 et al. 2016) is shown as an example of the peptide/receptor/co-receptor complex. The
534 structure was illustrated using the NGL viewer in the Protein Data Bank website.
535 (C) LRR proteins without kinase domains (green) may participate in direst recognition
536 of ligands with LRR-RK receptors (red). Also, transmembrane kinases without
537 extracellular domains (purple) may act to trigger intracellular signal transduction
538 coordinately with LRR-RK receptors (red).

539

540 **Fig. 2. CLE peptide hormones made in plants, pathogens and laboratories.**

541 Amino acid sequences of representative CLE peptides are shown. CLE peptides
542 produced by plants and pathogens are classified into A and B types depending on their
543 activities. CLE peptides in one group do not exert the activity of the other group,
544 indicating that there exists a strict specificity barrier (green) between the two groups.
545 However, it was recently reported that some artificial CLE peptides synthesized in
546 laboratories by human beings show both activities beyond the specificity barrier
547 (Hirakawa et al. 2017) as indicated by open pink arrows. Solid pink arrows indicate the

548 flows to design the synthetic bi-functional peptides. Black bold fonts indicate
549 characteristic 11th residues. Pink bold fonts indicate swapped residues to create the
550 bi-functional peptides. See the main text for the detailed explanation.

Fig. 1

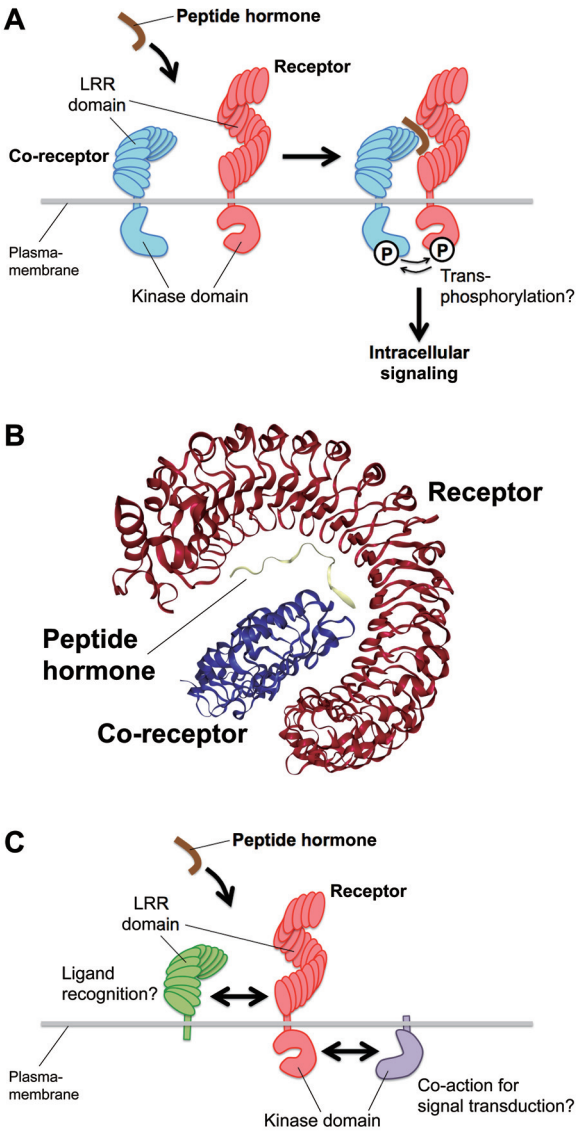
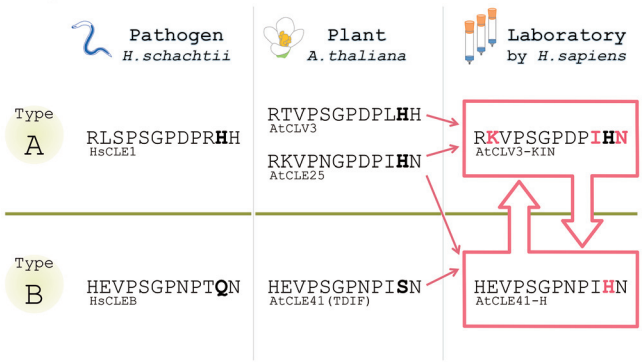

A
Peptide hormone
Receptor
LRR
domain
Co-receptor
Plasma-
membrane
Kinase domain
P
P
Trans-
phosphorylation?
Intracellular
signaling
B
Receptor
Peptide
hormone
Co-receptor
C
Peptide hormone
Receptor
LRR
domain
Ligand
recognition?
Plasma-
membrane
Kinase domain
Co-action for
signal transduction?

Fig. 2


Pathogen
H.schachtii
Plant
A.thaliana
Laboratory
by H.sapiens
Type
A
RLSPSGPDDPRHH
HsCLE1
RTVPSGPDPLHH
AtCLV3
RKVPNGPDPIHN
AtCLE25
RKVPSGPDPIHN
AtCLV3-KIN
Type
B
HEVPSGPNPTQN
HsCLEB
HEVPSGPNPISN
AtCLE41(TDIF)
HEVPSGPNPIHN
AtCLE41-H