DIVISION OF INTERCELLULAR SIGNALING BIOLOGY †

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Cell-to-cell signaling mediated by secreted signals and membrane-localized receptors is one of the critical mechanisms by which growth and development of multicellular organisms are cooperatively regulated. Signal molecules that specifically bind receptors are generally referred to as ligands. Because membrane-localized receptors act as master switches of complex intracellular signaling, identification of the ligand-receptor pair is one of the central issues of post-genome research. We are working to clarify the mechanisms by which plant development is regulated through identification of novel ligands such as small peptides and their specific receptors using *Arabidopsis* genome information, biochemical analysis and phenotypic observation.

I. Secreted peptide signals

Following complete sequencing of the *Arabidopsis* genome, a number of genes encoding small secreted peptides have been identified by *in silico* database analysis. Based on our own analysis, we identified 979 putative secreted peptide genes with an open reading frame (ORF) size between 50 and 150 amino acids in the *Arabidopsis* genome. These 979 ORFs include many functionally uncharacterized peptides. Although estimation of the total number of biologically relevant secreted peptide signals is difficult at present, the presence of many "orphan receptors" among receptor-like kinases in *Arabidopsis* suggest that a substantial number of intercellular signals remain to be identified.

One structurally characteristic group of peptide signals is "post-translationally modified small peptides". These peptides are characterized by the small size of mature peptides (less than 20 amino acids) and the presence of post-translational modifications. In these peptide signals, peptide chain length and post-translational modifications are generally very important for their receptor binding activity and physiological functions.

1-1 C-terminally encoded peptide (CEP)

C-terminally encoded peptide (CEP) is a 15-amino-acid peptide involved in mediating long-distance nitrogen (N)-demand signaling. The CEP family was identified by *in silico* screening for a family of secreted peptides that share short, conserved domains near the C-terminus, a feature that is common to several posttranslationally modified small peptide signals in plants. CEP1 is secreted as a 15-amino acid peptide originating from a C-terminal conserved domain (the CEP domain) through posttranslational proline hydroxylation and proteolytic processing. A total of 15 *CEP* family genes (*CEP1* through *CEP15*) have been found in the *Arabidopsis* genome.

When external N availability is lowered, CEP expression is promptly upregulated in the portion of the root system directly experiencing N starvation. CEP acts as a root-derived ascending N-demand signal to the shoot, where its perception by CEP receptors leads to the production of a putative shoot-derived descending signal that upregulates nitrate transporter genes in the roots. This mechanism supports N acquisition when nitrate is unevenly distributed within the soil (Figure 1). CEP family peptides induced on one side of the roots by local N starvation mediate upregulation of nitrate transporter genes in the distant part of the roots exposed to N-rich conditions to compensate for N deficiency.

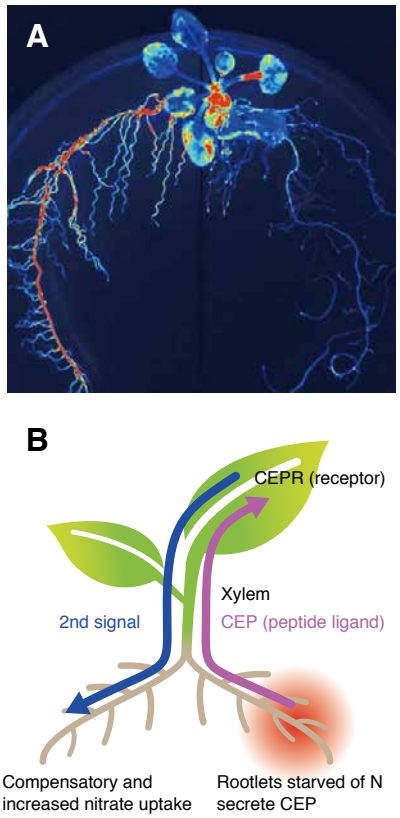

Figure 1. CEP mediates systemic N-demand signaling. (A) Systemic N-demand signaling visualized by *pNRT2.1:LUC*. N starvation on one side (right) of the root system leads to an upregulation of nitrate uptake on the other side of the root system (left). (B) Mode of action of CEP family peptides in systemic N-demand signaling. N-starvation induces CEP expression in roots. CEP acts as a root-derived ascending N-demand signal to the shoot, where its perception by CEPR leads to the production of a putative shoot-derived descending signal that upregulates nitrate transporter genes in the roots.

1-2 Other novel peptide signal candidates

The common feature of known small post-translationally modified peptide signals is that they are encoded by multiple paralogous genes whose primary products are approximately 70- to 110-amino-acid cysteine-poor secreted polypeptides

†: This laboratory was closed on 31 March, 2014.

that share short conserved domains near the C-terminus. We have identified several novel polypeptide families that fulfill the above criteria by *in silico* screening and determined their mature structures by analyzing apoplastic peptide fractions by nano LC-MS/MS. Functional analysis of these peptides is now going on.

II. Receptors for secreted peptide signals

The receptors or putative receptors for peptide signals identified to date belong to the receptor kinase (RK) or receptor-like protein (RLP) families. Among RKs, the largest subfamily is the leucine-rich repeat RK (LRR-RK) family, which consists of 216 members in *Arabidopsis*. The majority of receptors for small post-translationally modified peptide signals belong to this family. Especially, an increasing number of LRR X and LRR XI members are now being confirmed as receptors for several endogenous small peptide ligands, suggesting that these subgroups are an attractive target for binding analysis with novel peptide signals.

Although both genetic and biochemical methods have been used to identify ligand-receptor pairs in plants, genetic redundancy often interferes with the former approach, and the low levels at which ligand and receptor molecules are often present in tissues can make the latter approach very difficult. As described above, an increasing number of ligand candidates are being identified. If individual receptor kinases could be functionally overexpressed in certain cells at sufficiently high levels and sufficiently high quality for biochemical binding analysis, such a receptor library would facilitate identification of ligand-receptor pairs in plants. To this end, we established a functional and efficient expression system of plant receptor kinases in tobacco BY-2 cells and prepared an expression library of all the potential receptor candidates. This approach was successfully used for identification of CEP receptors.

Publication List

〔Original paper〕

- Bidadi, H., Matsuoka, K., Sage-Ono, K., Fukushima, J., Pitaksaringkarn, W., Asahina, M., Yamaguchi, S., Sawa, S., Fukuda, H., Matsubayashi, Y., Ono, M., and Satoh, S. (2014). CLE6 expression recovers gibberellin deficiency to promote shoot growth in *Arabidopsis*. Plant J. *78*, 241-252.
- Tabata, R., Sumida, K., Yoshii, T., Ohyama, K., Shinohara, H., and Matsubayashi, Y. (2014). Perception of root-derived peptides by shoot LRR-RKs mediates systemic N-demand signaling. Science *346*, 343-346.

〔Review article〕

- Matsubayashi, Y. (2014). Posttranslationally modified small-peptide signals in plants. Annu. Rev. Plant Biol. *65*, 385–413.