

## 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 “small post-translationally modified 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 Root meristem growth factor (RGF)

Root meristem growth factor (RGF) is a 13-amino-acid tyrosine-sulfated peptide involved in maintenance of the root stem cell niche in *Arabidopsis* identified by our group in 2010. RGF was identified in a search for sulfated peptides that recover root meristem defects of the loss-of-function mutant of tyrosylprotein sulfotransferase (*tpst-1*). TPST is a post-translational modification enzyme that catalyzes tyrosine sulfation of secreted peptides and proteins. This approach is based on the assumption that the severe short

root phenotype of the *tpst-1* mutant reflects deficiencies in the biosynthesis of all the functional tyrosine-sulfated peptides, including undiscovered peptide signals. RGFs are produced from ≈100-amino-acid precursor peptides via post-translational sulfation and proteolytic processing. RGF family peptides are expressed mainly in the stem cell area and the innermost layer of central columella cells, and diffuse into the meristematic region. RGF peptides regulate root development by stabilizing PLETHORA transcription factor proteins which are specifically expressed in root meristem and mediate patterning of the root stem cell niche (Figure 1).

To gain more insight into RGF signaling, we have developed a positive screening system to identify *Arabidopsis* mutants with altered response to RGF peptides. Several mutants that are less sensitive to RGF have been identified and are currently being further analyzed.

#### A Asp Tyr (SO<sub>3</sub>H) Ser Asn Pro Gly His His Pro Hyp Arg His Asn

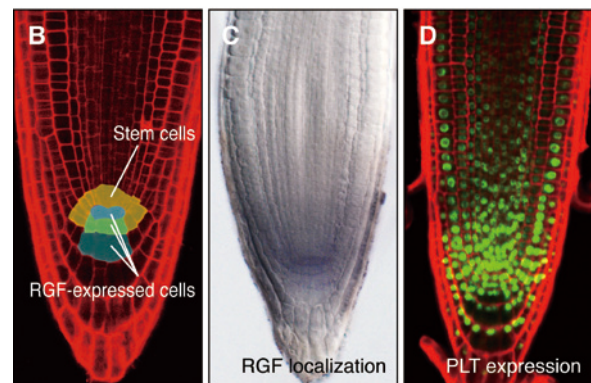


Figure 1. Mode of action of RGF peptide. (A) Structure of RGF1. (B) Expression domain of *RGF* genes. (C) Localization of RGF peptides visualized by anti-RGF1 antibody. (D) Expression pattern of PLETHORA (PLT2) transcription factor, a downstream target of RGF signaling.

#### 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 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. Post-translational modification mechanisms

Post-translational modifications are known to affect peptide conformation through steric interactions with the peptide backbone, thereby modulating the binding ability and specificity of peptides for target receptor proteins. To date, the following types of post-translational modification have been identified in secreted peptide signals in plants: tyrosine sulfation and hydroxyproline arabinosylation (Figure 2).

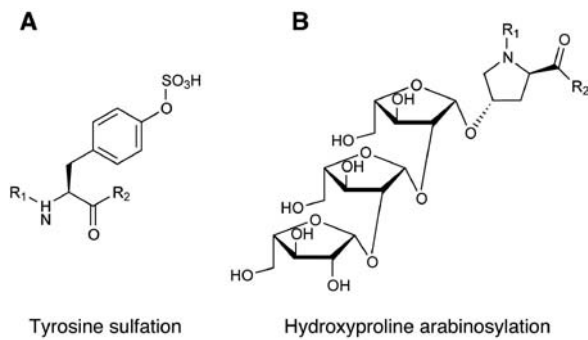


Figure 2. Post-translational modifications in secreted peptide signals in plants

### 2-1 Tyrosine sulfation

*Arabidopsis* tyrosylprotein sulfotransferase (AtTPST) is a Golgi-localized 62-kDa transmembrane protein identified by our group in 2009. AtTPST is expressed throughout the plant body, and the highest levels of expression are observed in the root apical meristem. A loss-of-function mutant of *AtTPST* (*tpst-1*) displayed a marked dwarf phenotype accompanied by stunted roots, loss of root stem cells, pale green leaves and early senescence, indicating the important roles of sulfated peptides in plant growth and development. Three known sulfated peptide signals, PSK, PSY and RGF, can almost fully restore root defects of *tpst-1* when added to the culture medium, but can not fully restore phenotypes in the above-ground parts of the plant. This observation suggests that as yet undiscovered sulfated peptides may regulate plant development. A search for novel sulfated peptide signals is now in progress.

### 2-2 Hydroxyproline arabinosylation

Hyp residues in several secreted peptide signals, such as CLV3 and CLE2 are further modified with an *O*-linked L-arabinose chain. This modification is physiologically important for these peptide signals. Biosynthesis of Hyp-bound  $\beta$ -1-2-linked triarabinoside involves two distinct arabinosyltransferases. The first is responsible for the formation of a  $\beta$ -linkage with the hydroxyproline (hydroxyproline arabinosyltransferase), and the second forms a  $\beta$ -1-2-linkage between arabinofuranose residues (arabinosyltransferase). Arabinosyltransferase has already been reported, but there have been no reports on hydroxyproline arabinosyltransferase (HPAT). We have established an *in vitro* assay system to detect HPAT activity and are currently attempting to purify this enzyme by affinity chromatography.

### 2-3 Chemical synthesis of arabinosylated peptides

Although extensive efforts have been devoted to the synthesis of various glycopeptides, a general and stereoselective synthetic route for arabinosylated peptides with  $\beta$ -1-2-linkage has yet to be developed. To address this issue we are attempting to synthesize  $\beta$ -1-2-linked tri-arabinosylated glycopeptides in a stereoselective manner.

## III. 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.

### 3-1 Receptor expression library

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 in the above section, 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.

### 3-2 Structural basis for ligand recognition

The identification of ligand-binding sites within receptor ectodomains provides valuable structural information important for understanding the basis of ligand recognition and signal transduction. We are analyzing how large consecutive LRRs specifically recognize small peptide ligands by focusing on CLV1/BAM family receptor kinases as a model.

## Publication List

### [Review articles]

- Matsubayashi, Y. (2011). Post-translational modifications in secreted peptide hormones in plants. *Plant Cell Physiol.* 52, 5-13.
- Matsubayashi, Y. (2011). Small post-translationally modified peptide signals in *Arabidopsis*. *The Arabidopsis Book* 9, e0150.