RESEARCH HIGHLIGHT

Cell Research (2016) 26:755-756.

www.nature.com/cr

Peptide signaling in plants: finding partners is the key

Cell Research (2016) **26**:755-756. doi:10.1038/cr.2016.73; published online 14 June 2016

Root meristem growth factors (RGFs), a family of "orphan" peptides, control root growth by altering the expression and gradient of transcription factors PLETHORAS (PLTs) that maintain stem cell niche. However, the receptors for RGFs remain unknown until recently when three groups independently reported the identification of a group of receptor-like kinases (RLKs) as cell surface receptors for RGFs.

Plants, as sessile organisms, have evolved sophisticated signaling networks to cope with the constantly changing environments. In many cases, these signaling networks are tied to the mechanisms that respond to the internal signals (such as hormones) to control the growth and development of plant organs. For example, growth of roots is controlled by a number of small-molecule hormones including auxin and cytokinin [1]. More recent work identified peptides as regulatory molecules for maintaining root stem cell niche. These include CLAVATA3/ESR (CLE) family peptides and root meristem growth factors (RGFs). Several CLEs have been shown to control root growth and development and some parallel signaling pathways maintain stem cell niche in both shoot and root meristems [2]. Through the work on CLE family and earlier work on systemin [2], it has been widely accepted that plants, like animals, also produce a number of peptides and use them to regulate plant growth and response to environments. Concerning the receptors for these peptides, researchers have locked their targets on RLK superfamily that consists of several hundreds of members in *Arabidopsis* (with one of the smallest genomes in flowering plants). In the past decade or so, a handful of RLKs have been identified as transmembrane receptors for extracellular signals, including both small-molecule hormones and peptides that facilitate developmental control and pathogen responses among other functions [3].

RGF family consists of 11 members and each of the active mature peptide has 13 amino acids. Mutation of RGFs leads to short primary root with reduced meristematic cortex cells, suggesting that RGFs are essential for maintenance of stem cell niche [4]. Overexpression of RGF caused an irregular wavy growth pattern in roots, and RGF has been proposed to play a role in gravitropic response [5-6]. A concentration gradient has been found for RGF peptides extending away from the stem cell area [4], and such gradient may further define the gradient of PLETHORAS (PLTs), transcription factors that control meristem development [7]. Concerning RGF signaling mechanism, identifying the receptor(s) for these regulatory peptides certainly represents a critical step. When studying BR signaling with *serk1 serk2 bak1* and *serk1 bak1 bkk1* triple null mutants, Du *et al*. [8] has noticed in these mutants a short-root phenotype that is BR independent. Through a yeast two-hybrid screen, Ou *et al*. [9] identified five closely related LRR-RLKs that interacted with BAK1. Because mutations in these RLKs (belonging to LRR-RLK subfamily XI), like the triple mutants of BAK1-related kinases, also resulted in short-root phenotype, they hypothesized that these RLKs may serve as receptors for signaling molecules that control root growth, such as RGFs. Indeed, in the RGF1 response assay, these RLK mutants became insensitive, and they named the five LRR-RLKs as RGIs for RGF-INSENSITIVES [9]. Ou *et al*. performed exhaustive genetic analysis of these five *RGIs* by generating a series of double, triple, quadruple, and quintuple mutants and thoroughly recorded the root growth defects. The most severe phenotype is associated with the two independent quintuple mutants in which the primary roots are only about 20% of the length in WT. The meristem size is reduced in the quintuple mutants through the PLTs-dependent pathway: both PLT promoter activity and protein levels are significantly downregulated in the quintuple mutants. In addition, ectopic expression of PLT2 completely and partially rescues the meristem size of the *rgi1,2,3,4* quadruple and *rgi1,2,3,4,5* quintuple mutants, respectively. These data strongly suggest that RGIs mediate the action of RGFs to activate PLT pathway to maintain the meristem size. Ou *et al*. [9] further demonstrated a direct interaction between RGF peptide and the extracellular domain of RGI1 protein using pull-down and dot blotting assays. More interestingly, they observed that exogenous application of RGF1 rapidly induces phosphorylation and ubiquitination of RGI1 in *planta*, typical consequences of ligand-receptor interactions [9].

Different from the genetic strategy used by Ou *et al*. [9], Song *et al*. [10] identified the same LRR-RLKs as the RGF receptors (called RGFRs) by taking a "signature motif-guided" structure approach [10]. Their previous study

identified the asparagine residue of AtPep1 as crucial for the recognition by its receptor PEPRs that belong to LRR-RLK subfamily XI [11]. The asparagine residue at the C-terminal end of AtPep1 forms salt bridges with two arginines in the subfamily XI of RLKs (RxR motif). Such RxR motif appears to be a conserved feature of all members in this subfamily of RLKs. They reasoned that the RLKs with RxR motif may recognize peptide ligands with the last amino acid as asparagine or histidine. Some regulatory peptides identified thus far (including RGFs) indeed have histidine or asparagine as the last residue. Therefore, they began to test the hypothesis using purified extracellular domains of the LRR subfamily XI and mixing each of them with a pool of peptides that have a free C-terminal histidine or asparagine. After gel filtration, they identified the LRR-RLKs that co-migrate with a particular peptide. Among the peptides are RGF1 that interacts with one LRR-RLK (named RGFR1). Structure and sequence alignments suggest that other four LRR-RLKs (RGFR2-RGFR5) may also recognize RGF1 peptide. Further analysis identified two structural features in the RLKs critical for interaction with RGF peptides: the first one is the RxR motif, together with two other residues (RGFR1^{Asp412} and RGFR1^{Leu436}) in RGFR1, which interacts with the C-terminal residue RGF1^{Asn13}; the second feature of RGFR1 is the RxGG motif that interacts with the sulfate group located at the RGF1 N-terminus. The sulfate group of RGF1 has been shown to be required for its *in vivo* function [4]. While RxR motif is highly conserved in subfamily XI of LRR-RLKs, the RxGG motif is unique to the RGFRs and appears to determine the specificity of their interaction with RGF peptides. Furthermore, Song *et al*. [10] have shown that RGF1 recruits a SERK co-receptor kinase to form a receptor complex to perceive RGF1. Consistent with this finding, the genetic analyses indicate that *serk1 serk2 bak1* mutants, like *rgfr* mutants, have smaller meristem size and short roots [10].

Just before the pair of papers were published in *Cell Research*, another group [12], using RLK expression library and photoaffinity-labeled RGF1, identified three RLKs of the same family to function as RGF receptors. Following the identification of the RGF peptides [4], Matsubayashi group began to explore the possibility of RLK family members as RGF receptors. To test this idea, they selected 95 RLKs as candidates and expressed these RLKs in tobacco BY-2 cells. Using photoaffinity-labeled RGF1, they performed binding assays with individual RLKs expressed in the BY-2 cell lines. They reported that three RLKs (RGFRs 1-3, equivalent to RGIs 1-3 in [9], and RGFRs 4, 3 and 1 in [10]) interact with RGF1 and genetic mutant lacking all three RGFRs shows a short-root phenotype with reduced meristem size and lower expression levels of PLT1 and PLT2 [12].

These three independent studies, using different strategies, have well complemented each other and identified the receptors for regulatory peptides RGFs. While linking RGF-RGFR/RGI interaction to PLT expression, these studies raise a number of important questions for future research. For example, the detailed analysis of composition and structure of the receptor complex, the downstream components and steps leading to the regulation of PLTs are among the next milestones. At the same time, strategies used in these studies, including loss-of-function genetic approaches, the "signature motif-guided' structural analysis, and RLK expression library, will be invaluable to facilitate the identification of more peptide-receptor pairs not only in plants but also in animal kingdom.

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