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研究課題名（和文）Mechanisms of vascular pattern formation through multiple intercellular communication molecules

研究課題名（英文）Mechanisms of vascular pattern formation through multiple intercellular communication molecules

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研究成果の概要（和文）：植物の維管束系の機能は、師部、木部、（前）形成層を含む組織化された構造に依存しています。師部は、篩管要素（SE）と伴細胞（CC）で構成されています。本研究において、適切な師部パターン形成に不可欠な、師部(P)-DofとCLEペプチドを含むフィードバックシステムを特定しました（Qian et al. 2022, Nat Plants）。その後の研究により、師部パターンは、師部領域で作動するフィードバックループによってだけでなく、非師部細胞から発せられるシグナルによっても調節されることがわかってきました。さらに、P-Dofと協力して師部形成を促進する新しいDOF標的転写因子を見出しました。

研究成果の学術的意義や社会的意義
Our findings represent a significant advancement in understanding phloem development and patterning, marking a crucial step forward in the investigation of molecular mechanisms underlying vascular development. This research will also provide valuable guidance for future crop breeding efforts.

研究成果の概要（英文）：The function of plant vasculature relies on its organized structure, which includes phloem, xylem, (pro)cambium. The phloem consists of sieve elements (SEs) and companion cells (CCs). We here identified a feedback system involving phloem-Dofs and CLE peptides, essential for proper phloem patterning(Qian et al. 2022, Nat Plants). Recent findings suggest that phloem patterning is regulated not only by feedback loops within the phloem region but also by signals from non-phloem cells. Additionally, we identified new DOF-targeted transcription factors that collaborate with P-DOFs to promote phloem formation.

研究分野：Plant science

キーワード：vasculature　transcription factor　peptide　phloem　CLE　Dof

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１．研究開始当初の背景

Vascular plants have a sophisticated nutrient-transporting system, the phloem, which consists of sieve elements and companion cells arranged in a specific pattern. Although regulatory mechanisms of phloem development and patterning are being uncovered, they remain only partially understood.

In our study, we have found that a series of phloem-expressed Dof-type transcription factors (named P-DOFs) not only positively regulate periclinal phloem cell division but also play key roles in phloem differentiation. The DOF transcription factor family consists of 36 members. Two papers reported that seven members of the DOF transcription factors positively regulate vascular cell proliferation in Arabidopsis (Miyashima et al. 2019, Nature; Smet et al. 2019, Curr Biol). However, the number of Dof transcription factors involved in vascular development and their detailed functions are still unclear.

Our transcriptome analyses showed that P-DOFs up-regulate many vascular positive regulators, including cytokinin biosynthetic enzymes (LOG3/5), auxin transporters and response factors (PINs and ARFs), TDIF peptides (CLE41/44), transcription factors (APL and NACs), SMXLs, and MAKR kinases. However, the functions of many of these regulators are still unclear. Additionally, P-DOFs up-regulate some vascular negative regulators, including LRR-RLK receptors (BAM3, RPK2, and CIK2) and CLE25/26 peptides. It is known that several CLEs (like CLE26/45) are mainly perceived by receptors/co-receptors from the LRR-LRK XI and LRR-LRK II families, repressing vascular development and root growth (Shinohara and Matsubayashi 2015, Plant J; Hu et al. 2017, Nature Plants; Hazak et al. 2017, EMBO Rep). However, the in vivo functions of the CLE signaling pathways are still unclear because many single mutations in any of the CLEs and their receptor/co-receptor (like BAMs and CIKs) do not show obvious vascular phenotypes. Furthermore, the interactions between P-DOF targeted vascular positive regulators and negative regulators are also unknown.

２．研究の目的

Based on our preliminary data, we hypothesize that P-DOFs and their targeted vascular positive regulators mediate a positive regulation that overcomes the lateral inhibition effect of P-DOF-targeted CLE peptide signaling, ensuring proper phloem specification. The aim of this research is to clarify the detailed functions of P-DOFs and their targets and to prove this hypothesis.

３．研究の方法

To find out how many DOF transcription factors, CLE peptides and BAMs/CIKs receptor/co-receptors are involved in vascular development, we created all marker lines of 36 DOFs, 33 CLE peptides and ~40 LRR-LRK XI and II receptor/co-receptors. We also created many inducible overexpression lines of DOFs, CLEs, and other DOF-targeted genes to know their gain of functions. Different combination mutant lines of DOFs, CLEs, BAMs and CIKs by using CRISPR methods. To make sure the signaling pathway, we perform the genetic analyses by creating different combinations of multiple mutants among dof, cle, bam/cik. Also, we test the ligand-receptor binding by using gel-filtration assay and ITC analysis. To study the detailed function of DOF-targeted transcription factors (NACs), we performed transcriptome analyses on the NAC inducible overexpression lines to find out their direct targets.

４．研究成果

During primary vascular development of Arabidopsis root tip, the precursor cells of the phloem and xylem are derived from the vascular initial cells, forming a biopolar pattern. I found that phloem precursors of one side distinct one protophloem sieve element precursor and two companion precursors, which can divide ~3 rounds to produce proto- and meta- sieve elements, two companion cells, and a large portion of procambium cells, while the xylem precursors give rise to the xylem and a small portion of procambium cells. Interestingly, the procambium cells do not divide in the whole process of primary vascular development, suggesting that procambium cells, as daughter cells produced from the primary phloem and xylem precursors, just make the stem cell source (called cambium cells) for vascular secondary growth (Fig. 1).

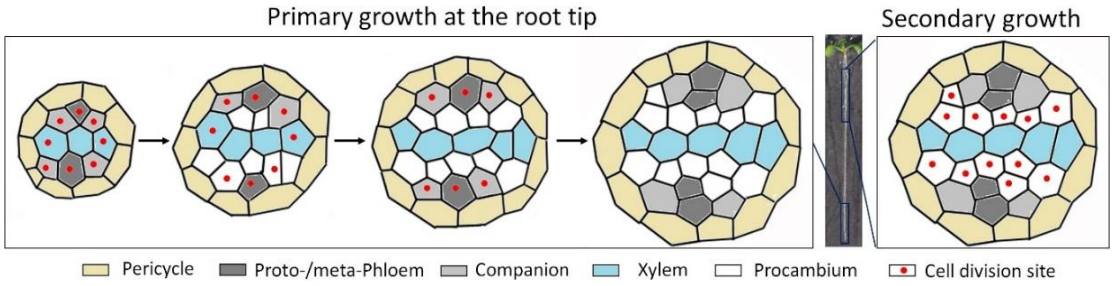

Fig 1. Vascular cell patterning of root primary growth is different from that of secondary growth.

We found that Dof-class transcription factors preferentially expressed in the phloem (phloem-Dofs) are not only necessary and sufficient for SEs and CCs differentiation, but also induce negative regulators of phloem development, CLE25, CLE26 and CLE45 secretory peptides. CLEs were perceived by BAM-class receptors and CIK co-receptors, and post-transcriptionally decreased phloem-Dof proteins and repressed SE and CC formation. Multiple mutations in CLE-, BAM- or CIK-class genes caused ectopic formation of SEs and CCs, producing an SE/CC cluster at each phloem region. We propose that while phloem-Dofs induce phloem cell formation, they inhibit excess phloem cell formation by inducing CLEs. Normal-positioned SE and CC precursor cells appear to overcome the effect of CLEs by reinforcing the production of phloem-Dofs through a positive feedback transcriptional regulation (Fig. 2; Qian et al., 2022, Nat Plants).

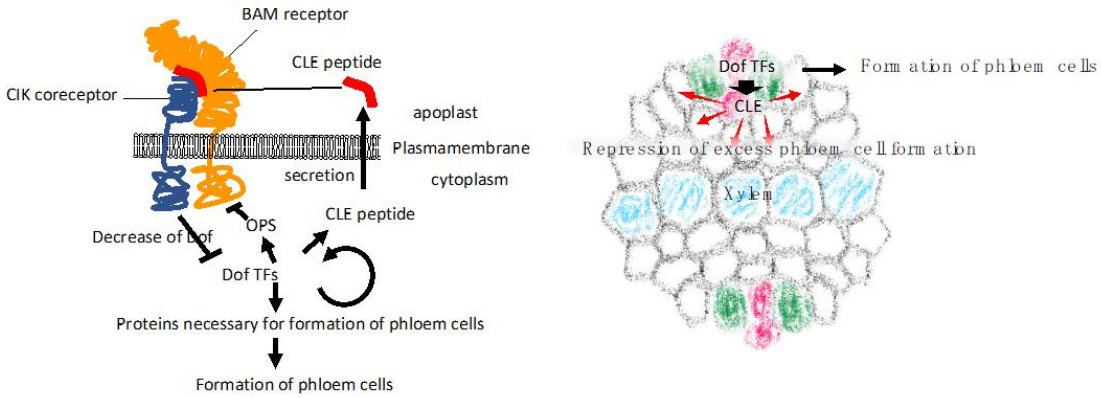

Fig 2. Phloem formation depends on interconnected positive and negative regulation.
Left, Molecular mechanisms for phloem formation. Right, Phloem pattern formation by Dof transcription factors and CLE peptides. Magenta, sieve elements. Green, companion cells.

However, these mechanisms do not fully explain how phloem differentiation is repressed in the xylem region and xylem-neighboring procambium. In addition to phloem-expressed CLEs, several xylem- and procambium-expressed CLEs have been identified from our new CLE marker lines. When we disrupt the procambium- and xylem-specific CLE genes in a cle25/26/45 triple mutant background, the higher-order multiple mutants show broader ectopic phloem cell formation compared to the cle25/26/45 peptide or their bam1/2/3 receptor mutants (our unpublished data). This suggests the involvement of novel CLE peptides and unknown receptors in controlling proper phloem formation. We are currently exploring the detailed regulatory mechanisms.

I am also investigating how phloem-Dofs enforce phloem development. I have confirmed that downstream transcription factors regulated by phloem-Dofs, including CDFs (another DOF subfamily) and NACs, are involved in phloem development. Interestingly, these NAC transcription factors can induce ectopic pAPL::nlsGFP expression similar to phloem-Dofs (our unpublished data). These target genes may play a role in continuously enforcing phloem formation step by step. Furthermore, I am studying the mutual regulation among DOF, CLEs, auxin, and cytokinin. I aim to fill the knowledge gaps in phloem development regulation in the near future.

５．主な発表論文等

〔雑誌論文〕　計1件（うち査読付論文　1件／うち国際共著　1件／うちオープンアクセス　0件）

１．著者名 Pingping Qian, Wen Song, Miki Zaizen-Iida, Sawa Kume, Guodong Wang, Ye Zhang, Kaori Kinoshita-Tsujimura, Jijie Chai and Tatsuo Kakimoto	４．巻 8
２．論文標題 A Dof-CLE circuit controls phloem organization	５．発行年 2022年
３．雑誌名 Nature Plants	６．最初と最後の頁 817;827
掲載論文のDOI（デジタルオブジェクト識別子） 10.1038/s41477-022-01176-0	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 該当する

〔学会発表〕　計3件（うち招待講演　1件／うち国際学会　2件）

１．発表者名 Qian, Pingping; Kakimoto, Tatsuo
２．発表標題 A Dof-CLE circuit controls phloem organization
３．学会等名 Second Plant Cell Atlas Symposium（国際学会）
４．発表年 2022年

１．発表者名 Qian, Pingping; Kakimoto, Tatsuo
２．発表標題 A Dof-CLE circuit controls phloem organization
３．学会等名 THE 4th WUHAN INTERNATIONAL SYMPOSIUM ON BIOLOGICAL SIGNAL TRANSDUCTION（招待講演）（国際学会）
４．発表年 2022年

１．発表者名 Qian, Pingping; Kakimoto, Tatsuo
２．発表標題 A Dof-CLE circuit controls phloem organization
３．学会等名 日本植 物学会第86回大会
４．発表年 2022年

〔図書〕　計0件

〔産業財産権〕

〔その他〕

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６．研究組織

	氏名 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （機関番号）	備考

７．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

８．本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関