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研究課題名（和文）A DOF-CLE circuit that regulates phloem pattern in the Arabidopsis root

研究課題名（英文）A DOF-CLE circuit that regulates phloem pattern in the Arabidopsis root

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交付決定額（研究期間全体）：（直接経費）　　2,200,000円

研究成果の概要（和文）：本研究では、篩部領域で優先的に発現しているDofタイプの転写調節因子(Phloem-Dof)が篩部細胞形成に必要かつ十分であることをしめした。Phloem-Dofは、篩部形成過程で働く遺伝子群のみならず、篩部形成を阻害するペプチド性シグナル分子CLE25, 26, 45を誘導することがわかった。cle25/26/45変異体では篩部領域が広がっていた。また、CLE25はPhloem-Dofを転写後調節によって抑制していることを見出した。Phloem-Dofは篩部細胞を誘導すると同時に分泌性ペプチド分子CLE25/26/45を用いて周辺細胞が篩部になることを抑えていると言える

研究成果の学術的意義や社会的意義
Our results close a major gap in our understanding of phloem development in root primary growth.It is a good start for further studying the molecular mechanism of whole process of root vascular development and patterning.It also has strong theoretical guiding significance for future crop breeding.

研究成果の概要（英文）：The regulatory mechanisms of phloem development are being uncovered. We find that a series of phloem-enriched DOF transcription factors (P-Dofs) not only regulate the number of procambium cell files, but also are necessary and sufficient for phloem differentiation. Overexpression of phloem-Dofs induced cells that expressed either sieve element or companion cell marker genes, which are mutually exclusive. Conversely, disruption of phloem-Dofs caused loss of phloem. Phloem-Dofs induce CLE25/26/45 peptides, which in turn inhibit expression of phloem-Dofs and phloem formation, forming a negative feedback loop. Disruption of multiple genes for either phloem-expressed CLEs, BAM-class receptors, or their coreceptors, CIKs, caused excess formation of phloem cell files. We further show that P-Dofs are under positive self and mutual regulation. These positive and negative feedback loops create the proper phloem pattern. There are more redundant DOFs, CLEs, and BAMs/CIKs involved in this pathway.

研究分野：plant science

キーワード：DOF transcription factor　CLE peptide　LRR-RLK receptor　BAM　CIK　vasculature　phloem　cell patterning

１．研究開始当初の背景

Plant vascular tissues, phloem and xylem, differentiate in intricate patterns as an integral transport system for water, nutrients and signaling molecules. Currently, the regulatory mechanisms of vascular development are being uncovered. I have revised the developmental patterning model of the primary growth of root vasculature, which is different from that of secondary growth. In root tip, all xylem cell files were derived from xylem initial cells, but most of all phloem, companion and procambium cells are originated from a single cell at phloem poles. They divide through three orientations to produce phloem, companion and procambium cells. The different cell division domains in primary and secondary growth implicate different regulatory mechanisms.

In this study, we focus on a series of Dof-type transcription factors (named Phloem-Dofs, P-DOFs) that are specifically expressed in the phloem precursor cells positively regulate phloem development Similarly, there were two papers published in the year of 2019 about 7 members of DOF transcription factors (total 36) regulating vascular development in Arabidopsis (Miyashima et al. 2019, Nature; Smet et al. 2019, Curr Biol). However, the detail functions of these phloem-DOF and other DOFs, and also their direct downstream targets are still unclear.

Through transcriptome analyses after induction of P-DOF genes, we found that CLE25 and CLE26 (CLEs are CLAVATA/ESR peptide hormones) genes were quickly induced by P-DOFs, suggesting that these two genes are good candidates as targets of P-DOFs in periclinal cell division of vascular development. We found that *CLE25* and *CLE26* are specifically expressed in phloem region and the peptides repress phloem development. It was reported that 17 members of CLE peptides (total 27) can repress xylem and/or phloem development in Arabidopsis vasculature (Kondo et al. 2010, PCP). These CLEs are mainly perceived by BAM receptors from LRR-LRK XI family and CIK co-receptors from LRR-LRK II family in 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 detail mechanism of this signaling pathway is still unclear, because all the reported cle mutants and their receptor/coreceptor mutants (bams and ciks) don’t show obvious defects in vascular development.

２．研究の目的

Based on our primary data, I propose a working hypothesis that there is a negative feedback loop of CLE25/26-BAMs-Dofs signaling cascade in vascular development, and this signaling loop is necessary for lateral inhibition to make the proper phloem patterning. My aim is to prove this working hypothesis.

３．研究の方法

We created all marker lines of DOF transcription factors, CLE peptides and BAMs/CIKs receptor/co-receptors, some inducible overexpression lines of DOFs and CLEs, and different combination mutant lines of DOFs, CLEs, BAMs and CIKs by using transgenic methods and CRISPR methods. Then, through microscope analyses, we verified the expression patterns and mutant phenotypes of these genes. To make sure the signaling pathway, we perform the genetic

analyses by creating different multiple mutants among *dof*, *cle*, *bam/cik*. Also, we test the ligand-receptor binding by using gel-filtration assay and ITC analysis.

４．研究成果

In this study, we first found that a series of phoem-enriched DOF transcription factors (phloem-Dofs) not only regulate the number of procambium cell files as previously reported, but also are necessary and sufficient for phloem differentiation. Overexpression of phloem-Dofs induced cells that expressed either sieve element or companion cell marker genes, which are mutually exclusive. Conversely, disruption of phloem-Dofs caused loss of phloem. These results clearly indicate that Phloem-Dofs are master regulators in whole phloem development.

Phloem-Dofs induce CLE25/26/45 peptides, which in turn inhibit expression of phloem-Dofs and phloem formation through a posttranscriptional regulation, forming a negative feedback loop. CLE25/26/45 directly bind to BAM receptors, and CIKs should be the coreceptors in this perception system. Disruption of multiple genes for either phloem-expressed CLEs, or their BAM receptors and CIKs coreceptors, caused excess formation of phloem cell files, indicating the lateral inhibition of CLE signalling in phloem patterning. Genetic analyses also show that DOF-CLE-BAM/CIK act in the same pathway.

We further find that phloem-Dofs are under positive self and mutual regulation. These positive and negative feedback loops create the proper phloem pattern (Fig.1).

Furthermore, we find that there are more redundant DOFs, CLEs, and BAMs/CIKs involved in this pathway. Now these results are being further examined.

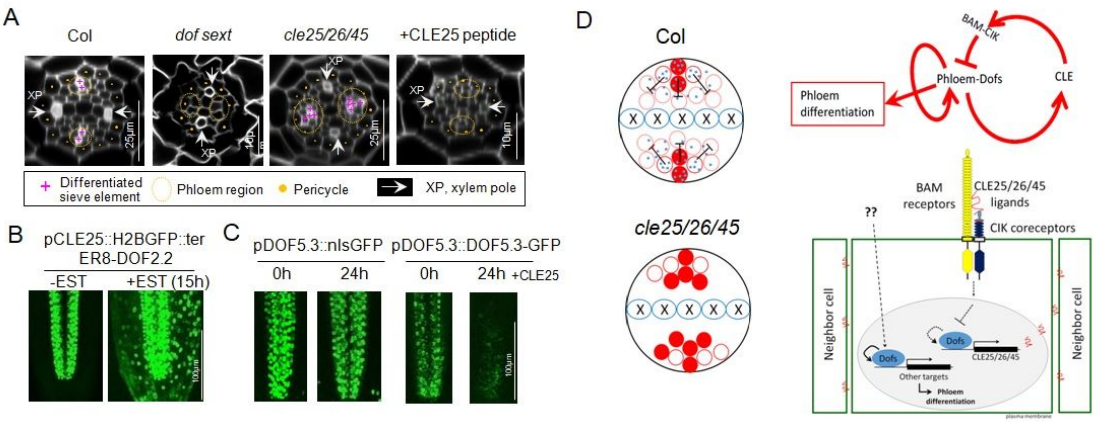

Fig. 1 The DOF-CLE circuit creates the phloem pattern.
(A) *dof-sext* mutants and CLE peptide treatment show decreased phloem files and sieve element differentiation, and *cle* mutant show increased phloem files and abnormal phloem pattern. (B) DOF2.2 induces ectopic CLE25 expression. (C) CLE25 peptide represses DOF5.3 translational- but not transcriptional- expression. (D) Model for the lateral inhibition of CLE25/26/45 during phloem development and patterning. The regulatory mechanisms for both a CLE-DOF negative feedback loop and a positive feedback one (DOF mutual- and self-active regulation) in coordinating phloem development and patterning in root tip.

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　1件）

１．著者名 Pingping Qian, Wen Song, Miki Zaizen-Iida, Sawa Kume, Guodong Wang, Ye Zhang, Jijie Chai, and Tatsuo Kakimoto	４．巻 -
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 -
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 オープンアクセスとしている（また、その予定である）	国際共著 該当する

〔学会発表〕　計3件（うち招待講演　3件／うち国際学会　1件）

１．発表者名 QIAN PINGPING
２．発表標題 Peptide signaling regulates plant stomatal and vascular development
３．学会等名 2019 First National Congress of Crop Science (Haikou)（招待講演）（国際学会）
４．発表年 2019年

１．発表者名 QIAN PINGPING
２．発表標題 Dual control: A single plant peptide regulates two distinct cell development processes
３．学会等名 Forum for International Young Scholars of Shanghai Jiaotong University（招待講演）
４．発表年 2019年

１．発表者名 QIAN PINGPING
２．発表標題 Peptide signaling regulates plant stomatal and vascular development
３．学会等名 Forum for International Young Scholars of SUN YAT-SEN UNIVERSITY（招待講演）
４．発表年 2019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