論文の内容の要旨

Study on Positional Signaling in Epidermis Differentiation in Arabidopsis thaliana.

(シロイヌナズナ表皮細胞分化における位置情報シグナルの分子的研究)

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The development of multicellular organisms requires orderly differentiation of specialized cell types in the correct location (Scheres, B., 2001). In the model plant Arabidopsis thaliana, epidermis, specialized tissue as a physical barrier against hostile environments, differentiates at the outermost position. Thus, the process of epidermis differentiation involves positional signaling to transmit appropriate information on cell location. However, little is known about the molecular basis of the positional signal that achieves the correct location of the epidermis. Here I show that very-long-chain fatty

acid-containing ceramides (VLCFA-Cers) are an essential component of the radial positional signaling pathway that mediates the positional information of the outermost cells.

In this thesis, I first demonstrated that the expression of ATML1, a master regulator of protoderm/epidermis differentiation (Abe et al., 2003;Lu et al., 1996;Ogawa et al., 2015) , requires cell lineage and cell position. This cell lineage- and position- dependence of ATML1 expression is suggested to be attributed to the inheritance of the autoregulatory expression within clonal cell lineage and the post-translational regulation of ATML1 protein in a position-dependent manner.

Secondary, I demonstrated outermost cell lineage and position confer stability to ATML1 protein but not to ATML1 protein carrying W471L mutation ($ATML1^{W471L}$). In vitro protein-lipid binding assay revealed that W471L mutation specifically disrupts the protein-lipid interaction between ATML1 and VLCFA-Cer. VLCFA depletion from sphingolipid pool by specific inhibitor treatment caused the outermost cells to lose the ability to stabilize ATML1 protein and significantly decreases the transcription of ATML1 and PDF2. Collectively, the interaction between VLCFA-Cers and ATML1 is crucial for maintaining ATML1 stability and confining ATML1 expression to clonal outermost cells.

Lastly, I investigated the regulatory mechanism of VLCFA-Cers biosynthesis and

trafficking. I found that PAS2, an essential and limiting enzyme for VLCFA biosynthesis (Bach et al., 2008), is expressed predominantly in outermost cells. Furthermore, VLCFAs were significantly depleted from the sphingolipid pool in an epidermis-defective mutant. These results indicate that VLCFA-Cers biosynthesis is directly regulated by ATML1 and PDF2 and confined to outermost cells. I also found that VLCFA-Cers seem to be constituents of the apical membrane of outermost cells, and thus passed on to the outermost daughter cells after cell division.

These findings indicate that VLCFA-Cers may function as an essential molecular component that mediates the positional memory of outermost cells in epidermis differentiation. Collectively, I provide a novel model for the spatial regulation of epidermis differentiation in Arabidopsis thaliana.