氏　　名　森下 美生

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学位論文題目　Functional analysis of an AP2/ERF transcription factor STEMIN1 that induces reprogramming of gametophore leaf cells to chloronema apical stem cells in the moss *Physcomitrella patens*

論文審査委員　主　査　教授 吉田 松生
教授 長谷部 光泰
准教授 坪内 知美
教授 藤田 知道
北海道大学大学院理学研究院

（様式３）

博士論文の要旨

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論文題目

Functional analysis of an AP2/ERF transcription factor STEMIN1 that induces reprogramming of gametophore leaf cells to chloronema apical stem cells in the moss *Physcomitrella patens*

Epigenetic modifications including histone modifications, stabilize cell-specific gene expression programs to maintain cell identities in both land plants and metazoans. Notwithstanding the existence of these stable cell states, in land plants, differentiated cells are changed to stem cells during post-embryonic development and regeneration, indicating that land plants have an intrinsic ability to regulate epigenetic memory to initiate a new gene regulatory network. A repressive chromatin mark, trimethylation at lysine 27 of histone H3 (H3K27me3) at specific loci, contributes to determination of tissue-specific gene expression and maintenance of differentiated states. Therefore, locus-specific reprogramming of H3K27me3 should be required for the rewriting of gene regulatory networks during plant development and regeneration, which could be one of the intrinsic mechanisms underlying stem cell formation. However, it remains to be elucidated what factors regulate chromatin modifications at specific loci to induce stem cell formation.

In this study, I have addressed this question using the moss *Physcomitrella patens* (Physcomitrella). After germination from spores, hypha-like bodies, called protonemata, and subsequently shoot-like bodies, called gametophores are formed. Protonemata comprise two cell types: chloronema and caulonema cells. A single stem cell is situated at the tips of chloronema and caulonema filaments, which is named chloronema and caulonema apical stem cell, respectively. Since protonemata are filamentous tissues and gametophore leaves are formed as a single cell layer, cellular

changes are easily observed. In particular, when a gametophore leaf is cut and incubated on culture medium without exogenous phytohormones, leaf cells facing the cut edge are reprogrammed to chloronema apical stem cells. Moreover, it had been found that ectopic induction of an AP2/ERF transcription factor, *STEM CELL-INDUCING FACTOR 1* (*STEMIN1*), in gametophores induces cellular changes from leaf cells to chloronema apical stem cells without leaf excision. This finding provided a tractable system to investigate the mechanisms underlying reprogramming of differentiated cells to stem cells.

The phylogenetic analysis of STEMIN-related proteins had identified two other genes closely related to *STEMIN1*, designated *STEMIN2* and *STEMIN3*. First, I examined whether these genes also function in initiation of stem cell formation in gametophores. However, I found that STEMIN2 and STEMIN3 lack the ability of STEMIN1 to change intact leaf cells into stem cells, although they have functions overlapping those of *STEMIN1* during stem cell formation in cut leaves. Therefore, I focused on elucidation of molecular mechanisms of reprogramming by the STEMIN1 induction in intact gametophores.

In this thesis, STEMIN1 targeted genes were identified using a combination of RNA-sequencing (RNA-seq) and chromatin immunoprecipitation-sequencing (ChIP-seq). I generated a transgenic line in which STEMIN1-Myc fusion protein is inducible by β-estradiol treatment (GX6:STEMIN1-Myc), and cultivated gametophores of GX6:STEMIN1-Myc with or without β-estradiol for 24 hours. The RNA-seq analysis demonstrated that transcript levels of 2,871 and 3,890 genes increased and decreased, respectively. The ChIP-seq analyses using anti-Myc antibody identified 6,733 putative directly targeted genes, in which STEMIN1-Myc proteins were significantly enriched in their promoter regions. In the up- and down-regulated genes, 1,416 and 716 genes were directly targeted by STEMIN1-Myc, respectively.

I next examined the changes in the H3K27me3 levels after STEMIN1 induction

in gametophores with ChIP-seq using anti-histone H3 and H3K27me3 antibodies. Among the 2,871 upregulated genes, H3K27me3 levels of 1,416 targeted genes were higher than those of 1,455 non-targeted genes before the STEMIN1 induction. After *STEMIN1* induction, H3K27me3 levels decreased more conspicuously in STEMIN1-targeted genes than in non-targeted ones. Transcript levels of 1,416 upregulated targeted genes were more highly correlated to the decrease of H3K27me3 levels than those of 1,455 upregulated non-targeted genes were. *CYCD;1* and *EXPANSIN* genes that have previously shown to function in stem cell formation was found in the 1,416 genes. Therefore, *STEMIN1* induction functions to decrease H3K27me3 levels at certain target genes and thereby increase the abundance of their transcripts, at least some of which are involved in stem cell formation.

When differentiated cells are reprogrammed, levels of chromatin modifications are decreased via cell divisions. In *Arabidopsis thaliana*, H3K27me3 levels are diluted by repeated cell divisions during the change from vegetative meristem to reproductive meristem. On the other hand, ectopic induction of STEMIN1 decreased H3K27me3 on its direct target genes before a cell division, indicating that chromatin modifications by STEMIN1 induction is independent of a cell division. Thus, STEMIN1 functions to induce a part of its targeted genes with reduction of H3K27me3 and triggers stem cell formation including tip growth and cell cycle reentry. Since other land plants have orthologs of the *STEMIN* genes, further studies of this gene family should provide insights into whether this is a general mechanism for reprogramming in land plants.

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Results of the doctoral thesis screening

博士論文審査結果

Name in Full
氏　名　森下　美生

Title
論文題目　Functional analysis of an AP2/ERF transcription factor STEMIN1 that induces reprogramming of gametophore leaf cells to chloronema apical stem cells in the moss *Physcomitrella patens*

森下美生氏の博士論文審査として、提出された博士論文を検討するとともに、森下氏に約30分間博士論文の内容を発表させ、その後、提出された博士論文と発表内容について1時間程度質疑応答を行った。

ヒストン修飾を含むエピジェネティック修飾は個々の分化細胞に特有の遺伝子発現を安定的に維持している。一方、陸上植物では、発生過程や再生時に分化細胞から幹細胞への転換が観察されることから、なんらかの幹細胞化誘導機構を持っていると考えられており、その分子機構解明が進行している。森下美生氏は異所的過剰発現することによってヒメツリガネゴケの葉細胞を原糸体頂端幹細胞に変えることができるSTEM CELL INDUCING FACTOR (STEMIN) 1がどのような仕組みによって幹細胞化を誘導できるかの分子機構の解明を目指した。クロマチン免疫沈降法によってSTEMIN1の直接ターゲットを推定するとともに、ゲノムワイドにH3K27me3とH3K4me3のクロマチン修飾を解析した。その結果、STEMIN1の直接ターゲットとなっている遺伝子は、そうでない遺伝子と比較して、分化状態においてH3K27me3が蓄積していることを発見した。さらに、STEMIN1を発現誘導すると、直接ターゲット遺伝子のH3K27me3が減少することを発見した。以上から森下氏は、STEMIN1は配列から転写因子と推定されるが、ターゲットのクロマチン修飾を変化させることで幹細胞化を誘導しているのではないかと考察した。

森下氏の発見は、幹細胞化過程において、ゲノムの中の特定の遺伝子のクロマチン修飾をどのように変化させるかという幹細胞化の分子機構の一端を明らかにしたものであり、幹細胞研究に新しい局面を切り拓くものである。従って、本審査委員会では本博士論文について博士学位授与に十分値し合格であると判定した。

(備考)

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Note: