論文内容の要旨

論文題目

"A study of factors regulating development and maintenance of plant stem cells."

(植物における幹細胞の維持と分化の制御因子の研究)

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Abstract

Stem cells could be defined as cells having ability to replenish themselves through self-renewal as well as the potential to generate differentiated cells. Recent advance on analysis of stem cells revealed common gene sets as well as regulatory mechanisms underlying stem cell regulation in plants and animals. However, most plant cells are considered to have totipotency, and thus it is difficult to precisely define stem cells in plants. It may be suitable to understand stem cell in plants as a transient cellular state to achieve activity to act as stem cells. In plants, specialized tissues containing undifferentiated stem cells are commonly referred to as meristems. Analysis on molecular mechanisms regulating development and maintenance of meristems advanced our understanding of regulatory mechanisms underlying. However, our current knowledge is mainly limited to factors involved in transcriptional regulation, and regulatory mechanisms directly involved in activity of cell metabolism that may affect meristematic activity remain to be elucidated. In order to identify genes involved in regulating meristematic activity of vascular cambium, attempts using the gene trap strategy were performed to obtain procambium genes.

The procambium stage of vascular development, which precedes the specification of each mature vascular cell, remains relatively uncharacterized. Anatomical analysis of early vascular development including procambium development has been done extensively. The primary vascular tissues are produced

from procambial initials in the apical meristems of shoots and roots. Cytologically, procambium cells become apparent as cytoplasm-dense, elongate, and narrow cells, with the long axis parallel to the axis of the procambium strand. Although these morphological characteristics are useful for recognizing procambium cells from ground cells, they do not provide the developmental cue of the procambium. In addition, how cell fate is specified to vascular development, which precedes morphological changes, is unknown. The gene trap strategy aims to visualize expression patterns and functional domains of random genes in the genome by tagging endogenous genes to produce chimeric proteins with endogenous proteins and reporter proteins. The strategy is quite useful for identifying specific genes expressed temporally and/or spatially. Using this strategy, five gene trap lines showing β-glucuronidase (GUS) staining in procambium cells were isolated and the causal genes for this staining in four of the five lines were identified. Procambium develops through undifferentiated meristematic, preprocambium, and procambium stages. The specificity of staining pattern of those procambium staining lines have varieties, and it could be subdivided into two groups (Figure 1). (1) KG09306, KG12346 and KG12419 showed GUS staining from undifferentiated GM cells near elongating higher order vein in leaves. In contrast, (2) KG11997 and KG14159 were GUS stained from fully elongated procambium cells. From these results, the *PINHEAD* gene (KG09306), the *Gamma-Glutamyl Hydrolase 1 (GGH1)* gene (KG12346) and the *KATANIN* gene (KG12419) are suggested to be expressed through the three stages, whereas the *At3g09070* gene (KG11997) and the causal gene of KG14159 are expressed from the procambium stage. Thus, these genes are useful for future analysis as markers for different stages of procambium development.

Next, the *GGH1* gene, which was tagged by reporter gene in KG12346, and orthologous *GGH2* and *GGH3* were characterized in detail. GGH cleaves the polyglutamate chain of folates. Folates are essential cofactors involved in one-carbon transfer reactions for amino acids and nucleic acid metabolism and metabolic regulation. Synthesis of purines, thymidylate, pantothenate, and formylmethionyl-tRNA, as well as interconversion of glycine and serine is mediated by tetrahydrofolate and its derivatives. Folates are also involved in DNA methylation reactions crucial for proper regulation of gene expression. Thus, appropriate control of activity of folates is inevitable for all organisms. Like in other organisms, plant folates exist as polyglutamyl form, having γ-liked chain of glutamyl residues attached to the first glutamate. Anionic nature appended by glutamate residues increases binding ability of folates to folate binding proteins. Thus, proper control of glutamate chain lengths of folates should be achieved in cells. *GGH1* and *GGH2* were expressed in cells having meristematic activity (Figure 2A - D). Overexpression of each three *GGH* genes, tandemly placed in *Arabidopsis* genome, caused similar developmental abnormalities indicating reduction of meristematic activity (fusion of cotyledons or adult leaves, reduction of meristematic cells in SAM;

Figure 2E - G). Conversely, suppression of all *GGH* genes by RNAi caused developmental abnormalities indicating enhancement of meristematic activity (overproduction of floral organs, ectopic formation of bracts, ectopic secondary lamina formation; Figure 2H - J). These results indicate that GGH is a negative regulator of meristematic activity expressed in meristematic tissues. Thus, I propose a novel molecular mechanism regulating meristematic activity via controlling glutamate chain lengths of folates. In this model, by catalyzing cleavage of glutamate residue of poly-glutamated folates that is essential to retain meristematic activity, GGH induce meristematic cells to differentiate into mature tissues (Figure 3). Although the biological significance of folates is obvious, the novelty of this model is suggesting significance of controlling glutamate chain lengths of folates. The fact that poly-glutamated folates are preferred form for most enzymes supports the model.

To elucidate the significance of controlling glutamate chain length of folates in plant development, effects of folate biosynthesis inhibitors and folates with different lengths of glutamate chain on plant development were examined. Consumption of the SAM region and reduction of root length were observed in plants treated with folate biosynthesis inhibitor. Both defects could be overcome by supplying pteroyl pentaglutamate but could not by supplying pteroyl monoglutamete (Figure 4A,B). These results indicate the role of poly-glutamated folate in maintaining proliferative activity of meristematic cells in both root and shoot meristematic region. Then, to elucidate the molecular basis of developmental defects of folate biosynthesis inhibitor treated plants, expression patterns of several genes implicated in development or maintenance of SAM were examined. Folate biosynthesis inhibitor treatment to plants induced down-regulation of genes involved in SAM development and/or maintenance. On the contrary, inhibitor treatment induced ectopic expression of genes act as negative regulators of meristematic activity and a gene related to xylem differentiation. The effect of folate supplementation on in vitro tracheary element (TE) differentiation system was examined subsequently. Pteroyl penta- glutamates inhibited TE differentiation of cultured *Zinnia* cells, while mono- glutamated folates did not have inhibitory effect (Figure 4C). In TE differentiation inhibited cells by pteroyl pentaglutamate treatment, expression of marker genes up-regulated in late stage1 and early stage 2 were sustained and expression of marker genes up-regulated in stage 2 were delayed (Figure 4D). Thus, TE differentiation inhibited cells were seem to be sustained in the late stage 1 or early stage 2, when cells are about to acquire identity as meristematic procambium-like cells. These results indicate that poly-glutamated folates are inevitable for plants to retain meristematic activity, and match well with the proposed model.

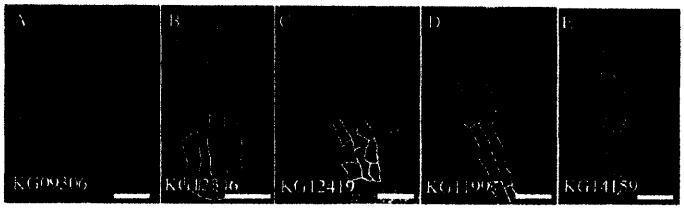

Figure 1. Gene trap lines exhibiting GUS staining in the procambium

GUS staining observed near the terminal site of elongating procambium tissues of higher order vein in young leaves of KG09306 (A), KG12346 (B), KG12419 (C), KG11997 (D) and KG14159 (E). Images are DIC images. To facilitate distinguishing GUS stained cells, color of images are reversed. GUS stains are observed as red staining. Isodiametric, polygonal GM cells are blue outlined, unelongated preprocambium cells are red outlined and elongated procambium cells are yellow outlined. Bars: 20 μm

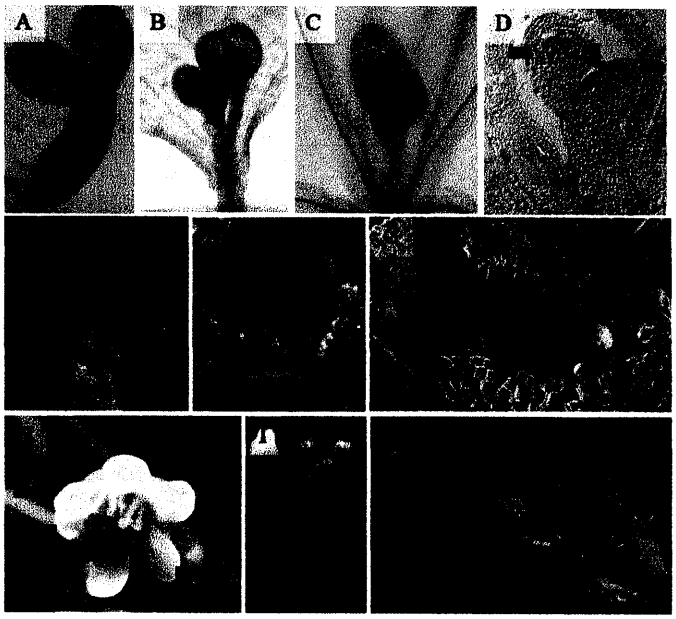

Figure 2. Analysis of *GGH* genes in Arabidopsis

(A - C) GUS staining pattern of stage 18 embryo of KG12346 plant (A), inflorescence of *pGGH1::GUS* plant (B) and inflorescence of *pGGH2::GUS* plant (C).

(D) In situ localization of *GGH1* mRNA in wild-type Arabidopsis plants. *GGH1* is expressed in SAM (blue arrow) and young floral organs (red arrow).

(E) Fused cotyledons of *35S::GGH1* plant.

(F) Fused rosette leaves of *35S::GGH1* plant.

(G) Complete loss of SAM observed in *35S::GGH1* plant.

(H) Flower of *GGH RNAi* plant with 6 petals.

(I) Flower of *GGH RNAi* plant with ectopic bract (red arrow).

(J) Cauline leaf of *GGH RNAi* plant with ectopic secondary lamina (red arrow).

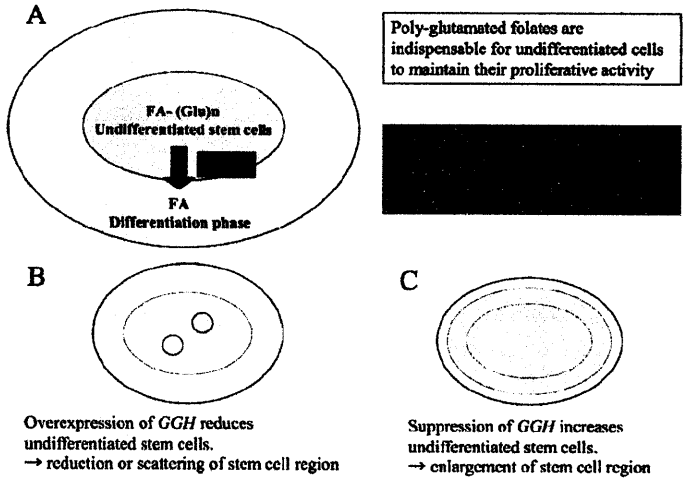

Figure 3. A model of folate-mediated regulation of meristematic activity by GGH

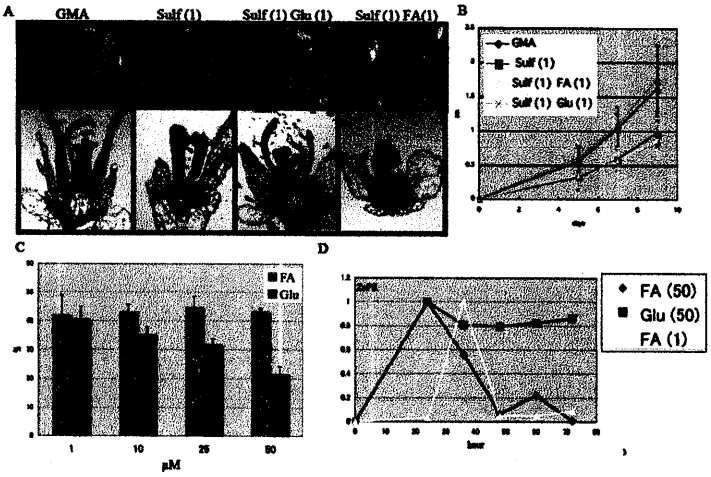

Figure 4. Physiological analysis of differential activities of mono- or penta- glutamated folates

(A) Effect of sulfanilamide treatment on SAM and recovery by penta-glutamated folates.

(B) Effect of sulfanilamide treatment on root lengths and recovery by penta-glutamated folates.

(C) Effect of folate treatment on *in vitro* TE differentiation.

(D) Effect of folate treatment on expression pattern of developmental stage1 marker gene *ZePR*.

Sulf = sulfanilamide, Glu = pteroyl pentaglutamate, FA = pteroyl monoglutamate, (1) = 1 μM, (50) = 50 μM