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研究課題名（和文）Role of a novel DNA demethylation enzyme in cellular memory

研究課題名（英文）Role of a novel DNA demethylation enzyme in cellular memory

研究代表者
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研究成果の概要（和文）：I regret that my japanese ability does not allow me to prepare an abstract in japanese. Text to 200 characters.Text to 200 characters.Text to 200 characters.Text to 200 characters.Text to 200 characters.

研究成果の概要（英文）：Some gene expression programs induced by transient signals remain stable across cell divisions. Such cellular memory may replay on the stable yet reversible DNA modification 5 cytosine methylation (5mC). We identified DRE2 as novel component involved in removal of 5mC from a set of genes manifesting memory of parent of origin in endosperm in Arabidopsis (maternal allele becomes active via 5mC removal while paternal allele remains methylated and silent). DRE2 is a highly conserved and essential protein in eukaryotes, best known for its role in biogenesis of iron sulfur cluster cofactors in the cytosol.
Here we obtained the first viable dre2 mutant in plants and characterized novel mutant phenotypes both in the reproductive and vegetative phases. The availability of viable dre2 mutants will help uncover new processes with cellular memory dependent on iron containing factors all the way to uncovering underlying molecular mechanisms.

研究分野：　Epigenetics

キーワード：　DNA demethylation

１．研究開始当初の背景

Some gene expression programs induced by transient signals remain stable across cell divisions. Such cellular memory may rely on the stable yet reversible DNA modification 5 cytosine methylation (5mC).

In Arabidopsis, the endosperm, an embryo- nourishing tissue developing after fertilization of the central cell maternal gamete, is an experimental system to study parent of origin memory. The maternal allele becomes active via 5mC removal in the central cell while paternal allele remains methylated and silent at a set of genes expressed maternally in the endosperm. The DNA glycosilase DEMETER (DME) excises 5mC at these genes via the base excision repair pathway. DNA demethylation also takes place in vegetative phase where three DME homologues are expressed. These four DNA glycosilase are dependent on a type of cofactors termed Iron-Sulfur (Fe-S) clusters.

In eukaryotes, the Fe-S clusters assembly machineries and the Fe-S proteins are distributed across different cellular compartments. Nuclear enzymes, including DME, obtain Fe-S cluster via the cytosolic Fe-S biogenesis pathway (CIA). CIA contains highly conserved proteins including DRE2, a protein that we demonstrated to be involved in parent of origin memory in the endosperm (Buzas et al 2014). However, CIA components other than DRE2 do not appear to influence the DME-dependent processes, although they share a zygotic function. DRE2 remained unstudied in plants mainly because it causes lethality when mutated. Also, it is difficult to perform biochemical studies when the process is confined to singles cells embedded in a mass of tissue, as is the case of the central cell gamete.

２．研究の目的

The role of DRE2 in DNA demethylation in the endosperm lineage could be revealed using a heterozygote allele due to its maternal gamethophytic function. However, because DRE2 has potential to regulate a myriad of other processes outside gametes via its putative role in FeS cluster biogenesis, it is of interest to also obtain viable tissues where both alleles are mutated, i.e. homozygote *dre*. Also, DRE2 is broadly expressed in Arabidopsis outside the reproductive phase, where it may have uncharacterized functions. The main aim of the study is to further characterize the DRE2 function in plants.

３．研究の方法

We previously demonstrated that expression of a DRE2 transgene driven by a central cell specific promoter can restore the defect in activation of four maternally expressed genes in *dre2-2* +/- both in the central cell and the endosperm, but not the other zygotic mutant seed phenotypes. We hypothesized that i) the expression of other DRE2-regulated genes important for viability may be similarly restored in the endosperm lineage and ii) because the mutant seed phenotype is not fully penetrant we may be able to recover *dre2-2* homozygote progeny once the

gamethophytic defect is restored. Indeed, a partial genetic complementation approach allowed us to isolate the first homozygote *dre2* mutants in plants.

４．研究成果

Here we obtained the first viable *dre2* mutant in plants and characterized novel mutant phenotypes both in the reproductive and vegetative phases.

To obtain information on reproductive development, we cleared siliques in different stages of development from *dre2-2* viable plants. While examining seeds from early globular to heart stage, we also found a rare twin embryo phenotype (approximately 1:500). This is an interesting, unexpected phenotype. Twin embryos can form in many taxa in higher plants yet always infrequently. In Arabidopsis, some suspensor cells in *twin* mutant develop into embryos. It remains possible that viable *dre2* also initiate embryos from suspensor cells because twin embryos were always located in the vicinity of suspensor cells. Unfortunately, the very low frequency of this phenotype makes it difficult to study this phenotype in more detail.

In addition to the *DRE2* expression during reproductive phase, DRE2 is also present in vegetative cells. To understand if the activity of the FeS-dependent DNA glycosylates active in vegetative cells is also reduced when *DRE2* function is abolished, we analyzed DNA methylation levels at three target demethylation genes in our viable *dre2* mutant. An increase in DNA methylation levels was especially clear in the CG context in the *dre2* viable comparing to the wild type at two out of the three genes investigated. We found that cytosine residues hypermethylated in DNA glycosilase triple mutants can also be hypermethylated in the viable *dre2* mutant, albeit the DNA methylation changes were less pronounced in *dre2* at most residues. These results indicate that DRE2 is required for activity of the DNA demethylase operating in vegetative cells, as is the case for the central cell maternal gamete, but this requirement may not be absolute.

The availability of viable *dre2* mutants will help uncover new processes with cellular memory dependent on iron containing factors all the way to uncovering underlying molecular mechanisms.

５．主な発表論文等

〔雑誌論文〕（計　2 件）

Peer reviewed publications related to the project

1. Mutants of the Fe-S biogenesis component AtDRE2 develop twin embryos and are defective in DNA demethylation in the vegetative phase. **Buzas Diana Mihaela**, Tetsu Kinoshita. *Molecular Life*, in press, 2018.

2. Emerging links between iron-sulfur clusters and 5-methylcytosine base excision repair in plants. **Buzas Diana Mihaela** *Genes & Genetic Systems* 91(2):51-62, 2016.

〔学会発表〕（計　　４件）

Conference Presentations related to the project

1. Uncovering biological roles of AtDRE2 as a cytosolic iron-sulfur biogenesis component and beyond. **Buzas Diana Mihaela**, Tetsu Kinoshita. *Latest Advances in Plant Development and Environmental response, Cold Spring Harbour Asia, Awaji, Japan. November 29-December 2, 2016 (Poster)*

2. Emerging links between iron sulphur clusters and 5 methyl cytosine base excision repair in plants ； **Buzas Diana Mihaela** *Plant genome stability and change Shonan Village Center Kanagawa, Japan. July 7-10, 2016 (Invited talk)*

3. Requirements of Iron-Sulfur clusters cofactors in DNA demethylation. **Buzas Diana Mihaela** *A consortium of Plant Epigenetisists in Japan- First meeting, National Institute of Genetics, Mishima, Japan. July 29-10, 2015 (Invited talk)*

4. The epigenetic role for the conserved Fe-S cluster biogenesis protein AtDRE2 in Arabidopsis thaliana. **Buzas Diana Mihaela**, Miyuki Nakamura, Tetsu Kinoshita *Gordon Conference series Boston, USA. August 2-7, 2015 (Poster)*

〔図書〕（計　　０件）

〔産業財産権〕

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〔その他〕
ホームページ等

https://www.gene.tsukuba.ac.jp/en/research/epigenetics.html

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