Plant Epigenome Regulation Laboratory
Chief Scientist: Motoaki Seki (Ph.D.)

(0) Research fields

CPR Subcommittee**:** Biology

Keywords: epigenome regulation, histone modification, histone variant, non-coding RNA, plant

(1) Long-term goal of laboratory and research background

Our laboratory aims to elucidate the molecular mechanisms of epigenome regulation networks in plant life cycle. To understand this topic involving histone modification and non-coding RNAs, we perform cutting-edge multidisciplinary approaches using model plants, including molecular biology, chemical biology and integrated omics analysis.

(2) Current research activities (FY2020) and plan (until Mar. 2025)

1. The identification of histone deacetylase involved in histone H2B deacetylation and the functional analysis of histone H2B acetylation in abiotic stress response in Arabidopsis.

Acetylation in histone and non-histone proteins is balanced by histone acetyltransferase and histone deacetylase (HDAC) enzymatic activity, an essential aspect of fine-tuning plant response to environmental stresses. A previous study indicated that class I (HDA19) and class II (HDA5/14/15/18) RPD3-like family HDACs control positive and negative responses to salinity stress, respectively. Furthermore, quintuple *hda5/14/15/18/19* mutants (*quint*) exhibit salinity stress tolerance, suggesting that *hda19* suppresses the sensitivity to salinity stress present in quadruple *hda5/14/15/18* mutants (*quad*). In the present study, the differences in acetylation sites among HDACs have been surveyed to elucidate the antagonistic and hierarchical control of salinity stress response operated by RPD3-like family HDACs (HDA5/14/15/18/19). As a result, a class II HDAC whose deficiency showed sensitive to drought stress regulates the levels in histone H2B acetylation in independent manner, suggesting that class I HDAC (HDA19) and class II HDAC control stress response through different substrates. To confirm whether H2B acetylation mediates abiotic stress response, K/Q or K/R mutations were introduced into lysine residues of histone H2B, which were acetylated in *quad*. Until now, we have revealed that K/R mutant showed tolerance to salinity stress. Transcriptome analysis and ChIP-seq are now in progress to reveal molecular mechanism through which histone H2B acetylation regulates response to salinity stress.

To date, we have identified histone deacetylases (HDA19 and a class II HDAC) involved in stress response in Arabidopsis. For the purpose of the manipulation of stress response, we will develop the screening system to find chemical compounds which alter these HDACs activity. The transcriptome data from these HDAC deficient mutants and acetylation sites recognized by them would be useful for the evaluation for the effect of newly identified compounds having HDAC inhibitory or activating effect. The methods would be applied for HDAC enzymes in land plants to understand the role of HDACs in not only stress adaptation but also development and so on.

2. Genome-wide analysis of epigenetic marks including H3K4me3, H3K36me3, and DNA methylation in Chinese cabbage inbred lines (*B. rapa* var. *pekinensis*)

Chinese cabbage is a crop closely related to *Arabidopsis thaliana*. In Chinese cabbage, the roles of epigenetic marks largely remain unknown (Mehraj et al., 2021a, Mehraj et al., 2021b). In this study, we analyzed the distribution of H3K4me3, H3K36me3, and DNA methylation, and their roles in transcription, tissue-specific gene expression, subfunctionalization of paralogous genes, and species conservation and response to biotic and abiotic stresses. Regarding H3K36me3, the presence of the active marks was associated with different gene expression levels or tissue specificity between paralogous paired genes. Our study raised the possibility that H3K36me3 might be involved in subfunctionalization of the subgenomes among the genus *B. rapa* plants (Mehraj et al., 2021b). Further study for other epigenetic marks such as acetylation and ubiquitination will uncover unique roles of epigenetic marks and contribute to improving agricultural traits in Chinese cabbage.

Future plan

Besides the above, we are elucidating novel epigenome regulation factor networks and their functions in plant life cycles. These factors will include histone modification enzymes, histone variants, DNA

methyltransferases, and non-coding RNAs. We will use integrated omics, protein-protein and protein-RNA interaction analysis, nucleosome reconstruction, and imaging analysis. Antagonistic or synergistic interactions that fine-tune biological processes, such as between histone modifications (e.g. acetylation vs methylation), between histone variants (e.g. H2B vs H3), and between histone modifications and non-coding RNAs, will be elucidated.

(3) Members as of March, 2021

(Chief Scientist)
Motoaki Seki

(Research scientist)
Minoru Ueda

(Technical Staff)
Junko Ishida, Satoshi Takahashi, Maho Tanaka

(4) Representative research achievements

1. Mehraj, H., Shea, D.J., Takahashi, S., Miyaji, N., Akter, A., Seki, M., Dennis, E.S., Fujimoto R. and Osabe, K. (2021a) Genome-wide analysis of long noncoding RNAs, 24-nt siRNAs, DNA methylation and H3K27me3 marks in *Brassica rapa*. PLOS ONE 16: e0242530.
2. Mehraj, H., Takahashi, S., Miyaji, N., Akter, A., Suzuki, Y., Seki, M., Dennis, E.S. and Fujimoto, R. (2021b) Characterization of histone H3 lysine 4 and 36 tri-methylation in *Brassica rapa* L. Front. Plant Sci. 12:659634.

Laboratory Homepage
https://www.riken.jp/en/research/labs/chief/plant_epigen_reg/index.html
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