**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 (FY2021) and plan (until Mar. 2025)**

**1. 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 element 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. 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, HDA15 dominantly regulates the levels in histone H2B acetylation, which suggests that class I HDAC (HDA19) and class II HDAC control stress response through different substrates. To confirm whether H2B acetylation is involved in response to abiotic stress, we have generated plants expressing K/Q (mimicking constitutive acetylation) or K/R (mimicking constitutive deacetylation) mutations at lysine residues of histone H2B that are deacetylated in HDA15-dependent manner.

Until now, we have already revealed that the H2B K/R mutant showed tolerance to salinity stress. In this fiscal year, transcriptome analysis revealed the expression of H2B K/R resulted in the suppression of gene expression of a senescence-related gene whose overexpression induces stress-sensitive phenotype. However, chromatin immunoprecipitation (ChIP)-seq analysis showed there is no correlation between the genome-wide distribution of histone H2B acetylation and where changes in gene expression occurred in K/R mutant, which leads to the conclusion that histone H2B acetylation fine-tunes response to environmental stress through a non-transcriptional regulatory process (Ueda *et al*., in preparation).

Transcriptome analysis in H2B K/R mutant detected the suppression of gene expression for cell-cycle related genes. Cell-cycle arrest is one of key processes for surviving under salinity stress conditions. We plan to analyze the role of histone H2B acetylation in cell-cycle progression for response to environmental stress.

**2. Biochemical evaluation of the impact of histone H2B acetylation on nucleosome stability through nucleosome reconstruction**

Transcriptome and ChIP-seq analyses mentioned above suggest that histone H2B acetylation may be involved in response to stress via a non-transcriptional regulatory process. To reveal the molecular mechanism underlying response to environmental stress through histone H2B acetylation, the impact of histone H2B acetylation on nucleosome stability was surveyed by using reconstructed nucleosome. Micrococcal nuclease digestion of the reconstituted nucleosomes with acetylated or non-acetylated H2B showed different digested pattern between them, suggesting that H2B acetylation destabilized the interaction between DNA and the nucleosome itself (Ueda *et al*., in preparation).

Other core histones (H2A, H3, H4) are also subjected to acetylation at several lysine residues. We plan to analyze the effect of acetylation of core histones on the interaction among DNA and core histones by nucleosome reconstruction.

**3. Manipulation of response to environmental stress using small molecules function as epigenetic modifiers.**

The application of small molecules is useful for the improvement of plant traits responsible for stress tolerance, particularly for crops in which it is difficult to introduce traits by transformation or crossing. Furthermore, constitutive expression of stress responsive genes often induces growth inhibition. Treatment of plants with small molecules during the optimized period may have the advantage of minimizing the growth inhibition, because stopping small-molecules application can release from the growth inhibition. Under these circumstances, screening small-molecules for regulating response to environmental stress and uncovering molecular mechanism underlying the application of small-molecules to plants have been conducted. In this fiscal year, we revealed that acetic acid, which functions as a biostimulant to increase drought tolerance via changes in levels of histone acetylation etc., activates root-to-shoot jasmonate signals in rice. The application of acetic acid partially overlaps with those induced by drought, thereby conferring an acclimated state on shoots prior to subsequent drought (Ogawa et al., 2021). In addition to acetic acid, we revealed that the exogenous application of nicotinic acid enhanced tolerance to drought stress. The overexpression of *nicotinamidase3* (*NIC3*) gene, which is involved in the biochemical conversion of nicotinamide to nicotinic acid, showed a similar phenotype as the exogenous application of nicotinic acid (Ahmad et al., 2021). The data suggest that nicotinamide, which has an inhibitory effect on sirtuin-type HDAC proteins, has a potential to fine-tune response to drought stress in Arabidopsis through alteration in levels of histone or non-histone protein acetylation.

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 do further screening of small molecules, in particular, which alter HDAC activity of HDA15 and HDA19 proteins.

*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**

**(Chief Scientist)**

Motoaki Seki

**(Research scientist)**

Minoru Ueda

**(Technical Staff)**

Junko Ishida, Satoshi Takahashi, Maho Tanaka

**(4) Representative research achievements**


1. Ogawa, D., Suzuki, Y., Yokoo, T.,Katoh, E., Teruya, M., Muramatsu, M.,Ma, J.F., Yoshida, Y., Isaji, S., Ogo, Y., Miyao, M., Kim, J.M., Kojima, M., Takebayashi, Y., Sakakibara, H., Takeda, S., Okada, K., Mori, N., Seki, M. and Habu, Y. (2021) Acetic-acid-induced jasmonate signaling in root enhances drought avoidance in rice. Scientific Rep. 11: 6280.
2. Ahmad Z., Bashir, K., Matsui, A., Tanaka, M., Sasaki, R., Oikawa, A., Hirai, M.Y., Chaomurilege, Zu, Y., Kawai-Yamada M., Rashid, B., Husnain, T. and Seki, M. (2021) Overexpression of nicotinamidase 3 (NIC3) gene and the exogenous application of nicotinic acid (NA) enhance drought tolerance and increase biomass in Arabidopsis. Plant Mol. Biol. 107:63-84.


**Laboratory Homepage**

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