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| **No.** | K22-2057 | |
| **研究課題名** | Regulation and Function of DNA methylation | |
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(Form 2-2)

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**IMSUT International Joint Usage/Research Center Project <International>**

**Joint Research Report (Annual/Project Completion)**

**Annual Report**

**Report**

- **Elucidation of the mechanism of UHRF1-PHD recognition by DPPA3**

UHRF1-dependent maintenance of DNA methylation plays an essential role in maintaining cell fates during cell proliferation, and DPPA3 is an intrinsically disordered protein that specifically interacts with UHRF1 and promotes DNA demethylation by inhibiting UHRF1 chromatin localization. However, the molecular basis of how DPPA3 interacts with and inhibits UHRF1 remains poorly understood. We determined the solution NMR structure of mouse UHRF1 PHD domain in complex with DPPA3. Induced α-helices in DPPA3 upon binding of the UHRF1 PHD contribute to stable complex formation with multifaceted interaction unlike canonical ligand proteins of a PHD domain. Mutations on the binding interface and unfolding of helical structure of DPPA3 inhibited binding to UHRF1 and the chromatin localization of UHRF1. Our results provide structural insight into the mechanism underlying the inhibition of nucleocytoplasmic translocation of UHRF1 by DPPA3. In further studies, we found that the C-terminal region of the DPPA3 protein plays an essential role in the inhibition of UHRF1. These results indicate that DPPA3 inhibits DNA maintenance methylation by targeting two distinct regions of UHRF1, the PHD and SRA domains.

**Termination of the UHRF1-dependent ubiquitin signaling by USP7 and ATAD5**

We have identified the deubiquitinating enzyme USP7 as a PAF15-binding protein; PAF15 exhibits direct deubiquitinating activity on USP7, and inhibition of USP7 leads to significant delay in PAF15 deubiquitination and chromatin dissociation. We also found that the non-ubiquitinated form of PAF15 is removed from chromatin in ATAD5-RLC-dependent manner. This finding provides a molecular understanding of how the maintenance DNA methylation machinery is disassembled at the end of the S phase.

・Research Results from the Project during FY2022

<Publications>

1. Miyashita R, *Nishiyama A, Qin W, Chiba Y, Kori S, Kato N, Konishi C, Kumamoto S, Kozuka-Hata H, Oyama M, Kawasoe Y, Tsurimoto T, Takahashi TS, Leonhardt H, Arita K, *Nakanishi M. The termination of UHRF1-dependent PAF15 ubiquitin signaling is regulated by USP7 and ATAD5 eLife. 2023 April.

2. Hata K, Kobayashi N, Sugimura K, Qin W, Haxholli D, Chiba Y, Yoshimi S, Hayashi G, Onoda H, Ikegami T, Mulholland CB, Nishiyama A, Nakanishi M, Leonhardt H, Konuma T, Arita K. Structural basis for the unique multifaceted interaction of DPPA3 with the UHRF1 PHD finger. Nucleic Acids Res. 2022 Nov 28;50(21):12527-12542.