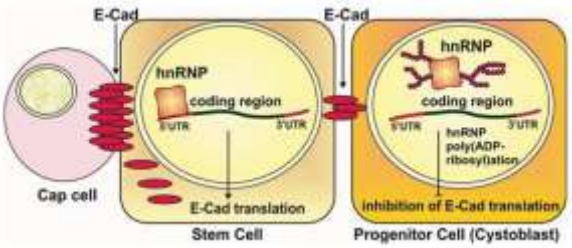
課題番号（Project number） 13

The function of hnRNPR in genome integrity and aging

［1］**組織**（Research group）
代表者（Principal Investigator (PI)）：
Zehui Hong
(Medical School of Southeast University, China)
対応者（Host researcher at IDAC）：
Akira Yasui
(IDAC, Tohoku University)
研究費（Expenditure report of research funds）： consumable goods 300,000 Yen,

［2］**研究経過**（Research setup）
Heterogeneous nuclear ribonucleoproteins (hnRNPs) are a large family of proteins, which were first described as a set of proteins binding to mRNA in the cell nucleus. The hnRNPs are multifunctional proteins involved in many cellular processes such as chromatin remodeling, transcription and telomere elongation (Fig. 1). Recent studies have shown that hnRNPs are also involved in DNA damage response and repair. We have identified that 3 hnRNPs (hnRPUL1, hnRNPG, hnRNPR) proteins are recruited to DNA damage sits induced by laser irradiation and these recruitment are regulated by PARP1.

Fig. 1 An example of poly(ADP-ribosyl)ation in hnRNP function (Y. Ji and A. V. Tulin, Int. J. Mol. Sci. 2013).



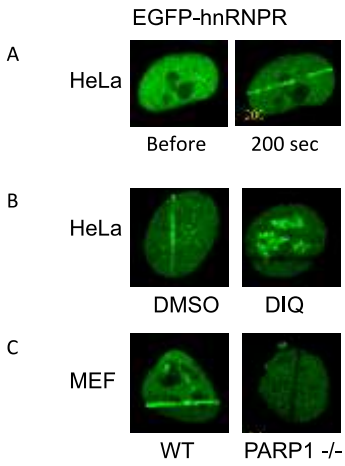
The function of hnRPUL1 and hnRNPG in DNA damage response have confirmed by our study and other groups. In here, we will explore the function of hnRNPR in DNA damage response, It will help us to understand the function of hnRNPs proteins in genome stability.

1. Explore interaction proteins of hnRNPR by affinity column chromatography performed in IDAC at Prof Akira Yasui's lab.
2. Analyze the influence of the suppression of each interacting protein on genome integrity and cellular aging

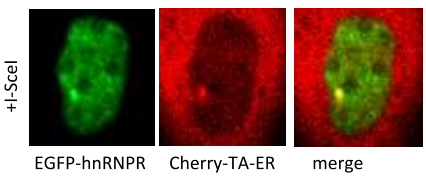
［3］**成果**（Research outcomes）
（3－1）**研究成果**（Results）
1. We have determined the response of GFP-tagged hnRNPR to DNA damage by laser-micro irradiation (Fig. 2). It accumulated to laser-irradiated site in Poly(ADP-libose) and PARP1-dependent manners. GFP-tagged hnRNPR accumulated at I-*Sce*I-induced DNA double-strand break. Therefore, hnRNPR accumulates at DNA strand breaks whereby poly(ADP-ribosyl)ation plays an important role.

Fig. 2. Damage response of hnRNPR protein.

1. Recruitment of hnRNPR to damage sites induced by laser is dependent on poly (ADP-ribosyl) ation mediated by PARP1.



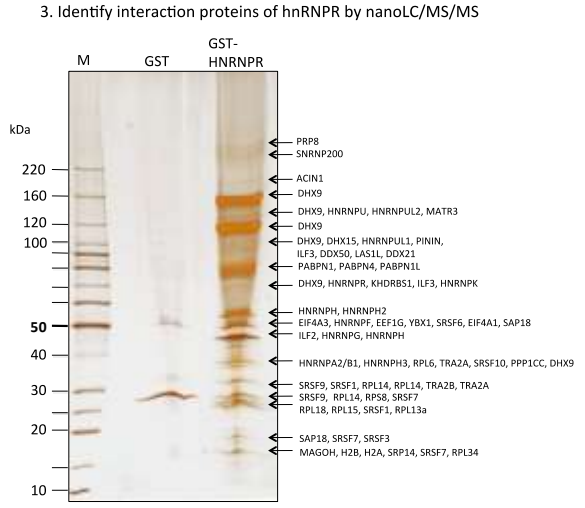
2. HnRPUL1 is recruited to DSBs sites induced by I -SceI



2. Determination of interacting proteins of hnRNPR
There are a number of hnRNP species within the nucleus, which regulate transcription, We wanted to determine how hnRNPR interacts with other

proteins and function in response to DNA damage. hnRNPR was fused behind GST and expressed in insect cell and purified. Affinity screening of hnRNPR protein gave rise to the determination of nuclear proteins possibly interacting with hnRNPR (Fig. 3). Fig. 3 indicates the presence of a large complex consisting of various RNA binding proteins. This complex may be important for regulation of transcription and translation in response to DNA damage.

Fig. 3. Identification of proteins interacting with hnRNPRy mass spectrometry.



（３－２）波及効果と発展性など（Future perspectives）

We here characterized hnRNPR by using laser-micro irradiation and human cell line with multiple I-*Sce*I sites. hnRNP accumulates at DNA strand breaks including double-strand break in a PARP-dependent manner. Since PARP regulates translation by inhibiting the interaction of hnRNP with mRNA (Fig. 1), PARP-dependent accumulation of hnRNPR at DNA damage site may be related to the repression of transcription at DNA damage site. It will explain a mechanism of DNA damage-induced transcriptional repression and DNA repair (transcription-coupled repair, TCR). Because of the relation n¥between defective TCR and aging in neural cell, influence of the hnRNP species on TCR may be quite important for future aging research.

［4］成果資料（List of Papers）

The results shown in this report is a part of paper submitted for publication.