課題番号（Project number） 4

Identification and characterization of mitochondrial translesion (TLS) machinery

［1］組織（Research group）
代表者（Principal Investigator (PI)）：
Lene Juel Rasmussen
(Center for Healthy Aging, University of Copenhagen)
対応者（Host researcher at IDAC）：
Akira Yasui
（IDAC, Tohoku University）
分担者（Co-investigator）：

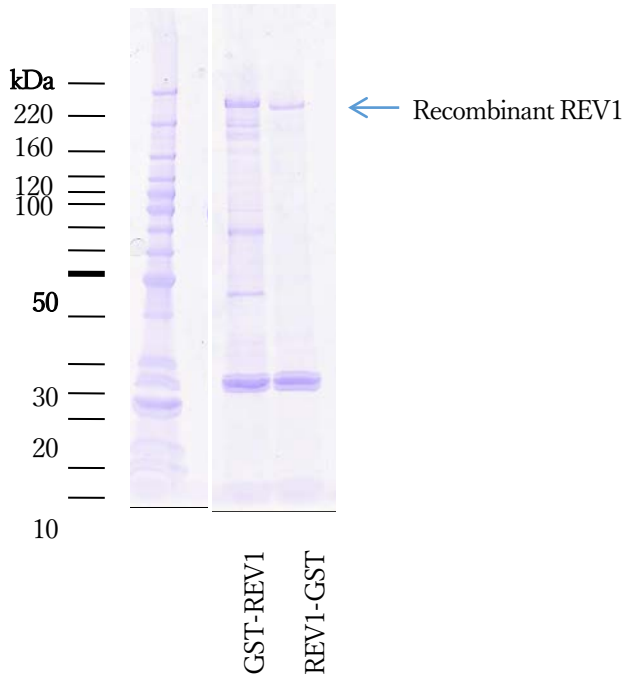
研究費(Expenditure report of research funds)：
material cost 665,000 YEN, travel cost 0 YEN

［2］研究経過（Research setup）Translesion synthesis (TLS) is activated in response to replication stress and fork arrest and carried out by the Y family of DNA polymerases that can replicate the damaged DNA templates. REV1 is believed to function as matchmaker of polymerases. REV1 interacts with several TLS polymerases and provides polymerase appropriate for the damage on the template strand to replicate. Most interestingly, mice lacking the TLS Rev1 protein show premature aging phenotypes, which resemble physiological disorders related to aging such as liver degeneration, type 2 diabetes, and obesity. It suggests a novel role of REV1 other than the matchmaker of TLS. Our data show that mitochondrial functions are impaired in both MEF cells as well as tissue from these mice (manuscript in preparation).

The purpose of the proposal was to identify and characterize proteins that are contained in mitochondrial translesion synthesis complex.

［3］成果（Research outcomes）
（3－1）研究成果（Results）
In order to identify the role of REV1 in mitochondria, we prepared mitochondrial extracts from human cells (HEK293) by standard method. GST-tagged recombinant REV1 was prepared from insect cells. While expression of recombinant REV1 was quite difficult, by changing the growth condition of SF9 insect cell and baculo virus transfection condition we obtained enough amount of GST-REV1 protein for affinity column screening of human mitochondrial extracts. (Fig. 1).

Fig. 1 Recombinant REV1 protein purified from SF9.



1. GST-REV1 2. REV1-GST

Affinity column screening of human mitochondrial extracts and careful washing followed by elution gave rise to several distinct protein bands in Fig. 2. Our results show that Rev1 interacts with a number of proteins (because of planned publication the name of the proteins are not shown). One interesting Rev1 interaction protein was found, which we think represents a novel role of REV1 in the mitochondria as well as in the nucleus. We are currently studying more intense in our laboratory.

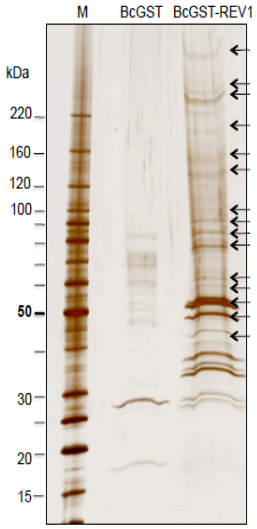


Fig. 2 Putative mitochondrial binding proteins to REV1.

（３－２）波及効果と発展性など（Future perspectives）

We expect that the novel Rev1-SHMT2 interaction will reveal interesting information about the role of Rev1 in DNA damage response and repair.

［4］成果資料（List of Papers）

We expect that the work described above will results in future common publications.