課題番号（Project number） 22

Models for normal human aging by focusing on Werner syndrome and bioenergetics in cells and mice; the presence of a WRN-interacting protein, Polymerase β, in mitochondria

［1］組織（Research group）
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研究費（Expenditure report of research funds）： consumable goods 300,000 Yen,

［2］研究経過（Research setup）

Werner syndrome (WS) is a progeroid disease caused by mutations in the gene coding for Werner protein (WRN). WS patients demonstrate many symptoms of normal human aging when they are adolescent or young adult patients. Therefore, it is generally accepted that WS is a good model for normal human aging. At the cellular level, evidence of cellular pathology is also similar in cells from WS patients and older normal individuals. Importantly, WS shares features with human mitochondrial disorders. While WRN contributes directly to nuclear genome integrity (Bohr, 2008), very little is known about the role of WRN in mitochondrial metabolism and why WS patient shows early aging remains still obscure.

Fig. 1 Werner syndrome patient

Since we showed previously the interaction between WRN and Polβ and the presence of WRN in mitochondria, we wanted to know whether polβ is present in mitochondria as well. Until now DNA polymerase γ is believed to be the only DNA polymerase in mitochondria. We have separately determined the presence of Polβ in human mitochondria by immune-staining and western blotting. Here we wanted to characterize mitochondrial proteins interacting with Polβ by affinity screening of human mitochondrial extracts. Mitochondrial fraction was prepared from human cell in NIA/NIH and transferred to IDAC for affinity screening of interacting proteins to Polβ. We discussed the project and the results with Skype and e-mail communication. Mitochondrial extracts were prepared in NIA/NIH and sent to IDAC/Tohoku University for affinity screening of the proteins in the extracts to GST-fused Polβ (domains). The results of this collaboration are now in a paper submitted for publication, and, therefore, we are not able to disclose the data in details in this report.

［3］成果（Research outcomes）

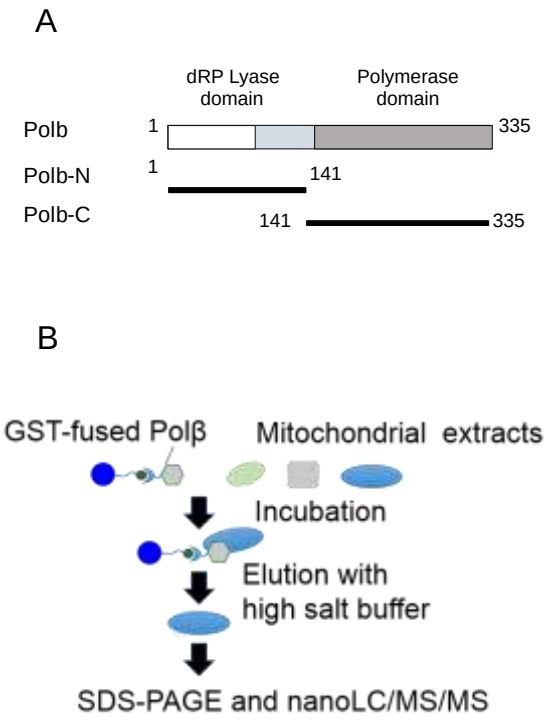
（3－1）研究成果（Results）

For the analysis of the function of Polβ in mitochondria our approach is to identify the human mitochondrial proteins, which interact with Polβ. We prepared recombinant proteins of GST-fused Polβ-N and Polβ-C domains and screened binding proteins in human mitochondrial extracts *in vitro* (Fig. 2A and 2B). The bound proteins to each domain were separated by SDS gel and 18 proteins were determined with mass spectrometry using nano LC-MS/MS (Fig. 3). Thus determined mitochondrial proteins were classified as 1. DNA maintenance. 2. Stress response 3. RNA processing. 4. Metabolism. All the data are novel

interactions between Polβ and mitochondrial proteins, which should be further characterized to understand the function of Polβ in mitochondria.

In addition to the determination by mass spectrometry through the screening, we applied western analysis of the blotted mitochondrial proteins by using antibodies against proteins important for protein transport into mitochondria, TOM70 and TIM50, for replication and transcription, TFAM as well as for helicase, TWINCLE. We found the presence of these proteins on the blot of affinity screening done with GST-Polβ-N (not shown). The interaction between Polβ and WRN in mitochondria remains to be determined.

Fig. 2. Experimental procedure



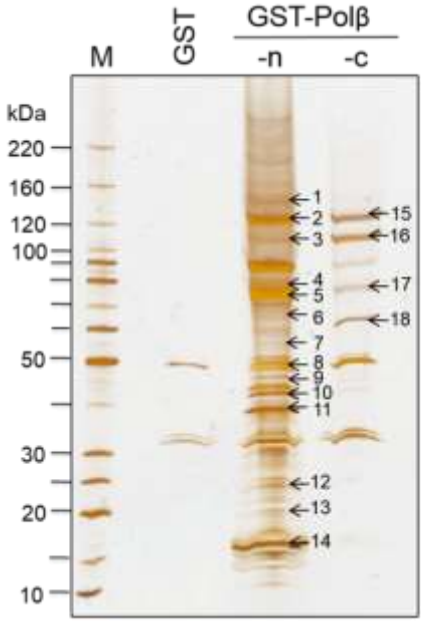


Fig. 3. Gel electrophoresis of affinity screening with GST-Polβ-N and -C

［4］成果資料（List of Papers）

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