課題番号（Project number） 12

Identification and characterization of mitochondrial nucleoids

［1］組織（Research group）
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consumable goods 300,000 Yen,

［2］研究経過（Research setup） In the last decade it has become increasingly clear that mitochondrial DNA (mitDNA) is not naked but associated with proteins in structure called nucleoids that are essential for mitDNA maintenance. Mitochondrial transcription factor A (TFAM) is an essential component of mitochondrial nucleoids and plays important roles in mitochondrial transcription and replication. The purpose of the proposal is to identify and characterize proteins in mitochondrial nucleoids by determining TFAM complex.

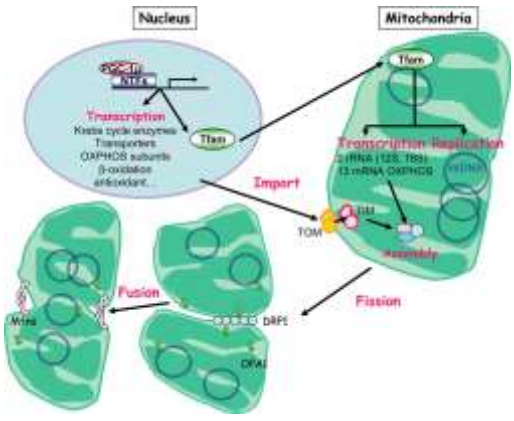

Fig. 1. The role of TFAM in mitochondria (Ventura-Clapier et al. Cardiovascular Research 2008.)

Because of the importance of mtDNA maintenance in cancer and aging we wanted to know the structure of TFAM complex in details by using domain-based affinity screening of binding proteins. All the experiments were performed in the division of dynamic proteome in IDAC, Tohoku University. We had discussion about the project and the results always with e-mail.

［3］成果（Research outcomes）

（3－1）研究成果（Results）

For the analysis of TFAM function in mitochondria our approach is to identify the proteins from human mitochondrial extracts, which interact with TFAM and its complex. We applied a domain-based affinity screening of binding proteins developed in the IDAC laboratory. There are five supposedly functional domains in TFAM (Fig. 2A). We used the amino half HMG domain indicated with N and the C-terminal half domain (C).

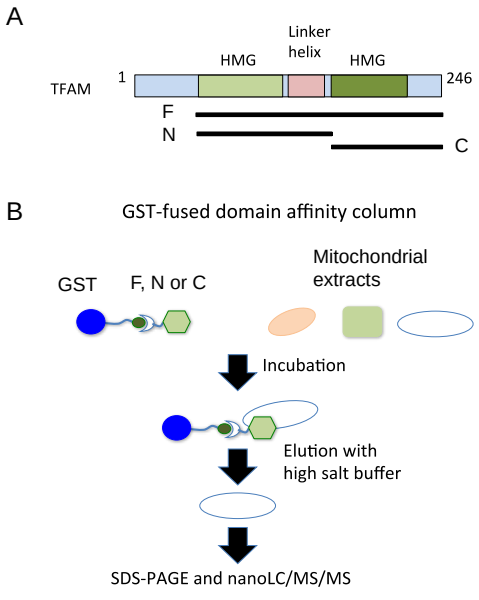

Fig. 2. Experimental procedure

These regions were attached behind GST, expressed in E. coli and purified. Human mitochondrial extracts were prepared from a human cell culture by conventional method in Dr. V. Bohr's laboratory as well as in IDAC.

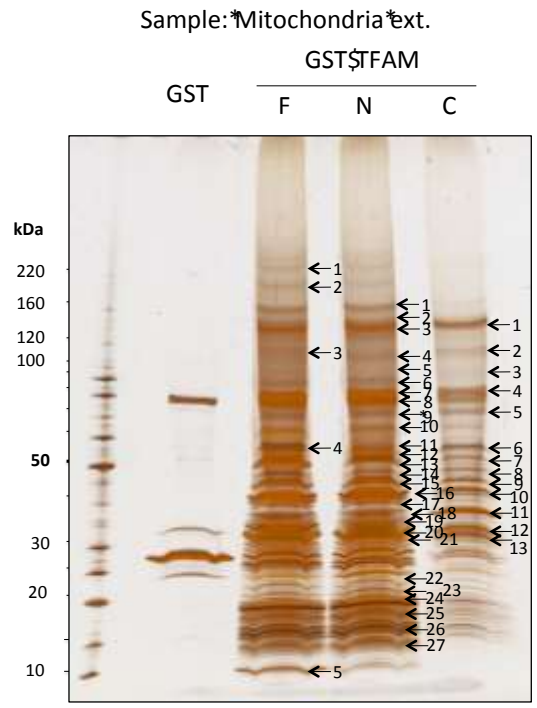

Fig. 3. Protein bands eluted after binding with each GST-fusion proteins.

Five protein samples from the affinity with full-length (F), 27 samples from N region and 13 samples from C region were determined by nano LC-MS/MS analysis (Fig. 3).

While the details of the 45 interacting proteins can not be disclosed in this report, the following classification of the TFAM-interacting proteins was obtained;

1. The putative interactive proteins of TFAM were divided into five categories; 1) mitochondria metabolism 2) RNA metabolism, 3) translation and chaperon, other functions and 5) ribosomes.
2. We identified 5 proteins, of which function in mitochondria have never been reported.
3. At least 12 proteins were identified, of which interaction with TFAM has never been reported.
4. Because of the interaction of TFAM with protein modification enzymes, TFAM may be involved in a process essential for protein maintenance system within mitochondria.

（３－２）波及効果と発展性など（Future perspectives）

This may be the first trial for the determination of TFAM protein by a domain-based affinity screening of binding proteins by using human mitochondrial cell extracts. We obtained a number of interesting candidate proteins of TFAM interaction. These data suggest more important roles of TFAM and the nucleoids played in mitochondria than it is known. Based on the results here obtained we will continue to find out how mitochondrial genome integrity is maintained and determine new roles of TFAM complex.

［4］成果資料（List of Papers）

In preparation.