【Grant-in-Aid for Scientific Research(S)】

Biological Sciences (Medicine, dentistry, and pharmacy Ⅰ)

Title of Project : Dissection of mRNA degradation pathways and anomalies associated with targeted disruption of CCR4-NOT deadenylase complex

Tadashi Yamamoto
(The University of Tokyo, Institute of Medical Science, Professor)

Research Area : Medicine, dentistry, and pharmacy

Keyword : Molecules

【Purpose and Background of the Research】

Gene expression is regulated at the levels of transcription and post-transcription. In the posttranscriptional regulation, factors that affect mRNA stability are vitally important. A major mechanism that determines mRNA stability is deadenylation-dependent, though possible involvements of microRNAs in mRNA decay are also suggested. The CCR4-NOT complex is a major deadenylase that triggers mRNA decay and is conserved from yeast to humans (Fig. 1). In this study, we analyze

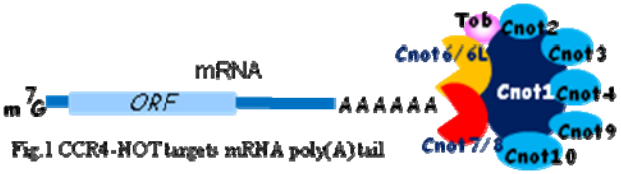

Fig.1 CCR4-NOT targets mRNA poly(A) tail

phenotypes of gene-manipulated mice in which each component of the CCR4-NOT complex is mutated one by one. By doing so, we will identify target mRNAs of CCR4-NOT deadenylases, and address how the CCR4-NOT complex together with Tob, microRNAs and RNA binding proteins specifically recognizes the target mRNAs.

【Research Methods】

Anomalies of mice in which each component of the CCR4-NOT complex is mutated by gene targeting will be examined. Through the analysis, we will look for mRNA species whose expression is under the control of CCR4-NOT deadenylases. To help identify such mRNAs, we employ microarray analysis in conjunction with various biochemical and molecular biological methods to compare the mRNA species expressed in relevant tissues of wild-type and gene-targeted mice. For example, because *CNOT7* disruption causes anomaly in spermatogenesis, we will determine targets of the CNOT7 deadenylase by comparing mRNA expression profiles in the testis between wild-type and mutant mice. The targets will be scrutinized for the presence of microRNAs binding sites and AU-rich elements in their untranslated regions. Their responsiveness to the decay of the target mRNAs is also examined. Eventually, we will determine mRNA species that are recognized by CCR4-NOT deadenylases in different tissues and in different conditions. Basing on these data as well as structural information on the CCR4-NOT components obtained from the X-ray analysis of their crystal structures, we will establish a model for the mRNA decay controlled by CCR4-NOT and possibly by various factors such as microRNAs, RNA binding proteins, poly(A) binding proteins and extracellular signals.

【Expected Research Achievements and Scientific Significance】

In this study we will dissect anomalies caused by disruption of the CCR4-NOT deadenylases and reveal the biological importance of the CCR4-NOT deadenylases. Basing on the significance of the CCR4-NOT deadenylases, we will establish a novel, yet fundamental mechanism by which mRNA decay is controlled. Because regulation of gene expression is a central issue of all biological phenomena, and because gene expression largely depends on mRNA decay, our results would give a great impact in the basic biological sciences as well as medical sciences.

【Publications Relevant to the Project】

・Yoshida Y, Tanaka S, Yamamoto T et al. Negative regulation of BMP/Smad signaling by Tob in osteoblasts. ***Cell*** **103**, 1085-1097. (2000)

・Oligo-astheno-teratozoospermia in mice lacking Cnot7, a regulator of retinoid X receptor beta, ***Nature Genet*** 36: 528-533. (2004)

・Morita M, Nakamura T, Yamamoto T. et al, Depletion of mammalian CCR4b deadenylase triggers increment of the $p27^{Kip1}$ mRNA level and impairs cell growth. ***Mol Cell Biol*** 27: 4980-4990. (2007)

【Term of Project】 FY2009-2013

【Budget Allocation】 159,200 Thousand Yen

【Homepage Address and Other Contact Information】

http://www.ims.u-tokyo.ac.jp/gannsig/
tyamamot@ims.u-tokyo.ac.jp