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General Summary

Our research have focused on biological significance of regulating cellular polyamines, in particular through a polyamine-regulating protein "antizyme". Polyamines are ubiquitous biogenic amines that are essential for cell proliferation and related to various phenomena such as differentiation, development, cancer, and autophagy. Three major polyamines, putrescine, spermidine and spermine, are present in mammalian cells. When cellular polyamines increase, AZ is induced through translational frameshift. AZ binds to ornithine decarboxylase (ODC), a key enzyme for polyamine biosynthesis, and inhibits the enzymatic activity and accelerates degradation of the enzyme protein. Thus AZ provides the feedback regulation for the cellular polyamine levels. Mammalian cells express three members of the AZ family (AZ1-3) and each AZ is likely to have specific function.

Research Activities

Interaction between MYCN and AZ2 in neuroblastoma cells
We have previously found that AZ2 interacts with c-Myc and accelerates its degradation in ubiquitin-independent manner. We are interested if AZ2 also interacts with MYCN and regulates its degradation since AZ2 expression is known to correlate with the survival of neuroblastoma patients. We observed using immunofluorescent microscopy that HA-tagged AZ2 (HA-AZ2) colocalizes with endogenous MYCN in the nucleoplasm of neuroblastoma cells, and that a proteasome inhibitor, MG132, shifts the localization to the nucleolus. Knockdown of AZ2 using siRNA stabilized and elevated the level of MYCN in neuroblastoma cell lines. These results suggest that AZ2 regulates MYCN in the neuroblastoma cells as c-Myc.

Analysis of interaction between AZ and ATP citrate lyase
Screening for AZ-binding proteins identified ATP citrate lyase (ACLY), a cytosolic enzyme producing acetyl-CoA that is utilized to synthesize lipid and to acetylate cellular components. We confirmed that both AZ1 and AZ2 bind to ACLY and colocalize with ACLY in the cytoplasm. Unexpectedly, neither AZ1 nor AZ2 accelerated ACLY degradation, like ODC degradation mediated by AZs. Additionally, HA-tagged AZs purified from mammalian cells activated purified ACLY in a dose-dependent manner *in vitro*. Knockdown of AZ1 and/or AZ2 in human cancer cells significantly decreased the ACLY activity as well as cellular levels of acetyl-CoA and cholesterol (Tajima *et al*., 2016).

The effects of high-polyamine diet on metabolites
Polyamines are decreased with aging. To address the effects of ingestion of high-poly-

amine diet, we compared the metabolites between the wild-type mice fed a control diet, the wild-type mice fed a high-putrescine diet, and the Oaz1 (AZ1 gene) heterozygous mice whoes polyamine synthesis especially putrescine synthesis is increased. 132 compounds were detected in the sera of these mice by gas chromatography-mass spectrometry. Two-way ANOVA analysis showed that 25 compounds in comparison between young and old mice and 18 compounds in comparison between control diet, high-putrescine diet and the heterozygous deletion (knockout) of Oaz1 were identified with significant differences for each comparative analysis. Among them, we noted that the level of metabolites pattern of high-putrescine diet mice was similar, to some extent, to that of young mice. We are studying these physiological consequences.

Analysis of AZ +1 ribosomal frameshift mechanism with human in vitro *translation system*

It is known that the termination codon at frameshifting position in AZ mRNA is necessary to induce the +1 ribosomal frameshift. To analyze molecular mechanism of polyamine-induced +1 translational frameshift, we replaced the termination codon (UGA) at shifting position of AZ1 mRNA with sense codons, UUC, UAU or GGA. These constructs were translated in HeLa cell extract *in vitro* translation system. Unexpectedly, +1 framshift was induced by polyamines in all constructs. Mass spectrometry analysis revealed that shifting position of these constructs were the same as the original one. Furthermore, we prepared other constructs replacing the termination codon at shifting position with a leucine codons, CUG, CUA or UUA (codon usage is 4, 0.6 or 0.4%, respectively). Plus 1 framshift was also induced in all these constructs by polyamine and the frameshift efficiency was almost the same among these constructs. These results indicate that ribosome stalling by a codon at frameshifting position is not necessary for AZ +1 frameshift.

Publications

Jaafar ZA*[1], *Oguro A*, *Nakamura Y*[2], *Kieft JS*[1] (*[1]Univ of Colorado, [2]Univ of Tokyo). Translation initiation by the hepatitis C virus IRES requires eIF1A and ribosomal complex remodeling. *eLife.* 2016; **5:** e21198.

Oguro A*, *Yanagida A*[1], *Fujieda Y*[1], *Amano R*[1], *Otsu M*[1], *Sakamoto T*[1], *Kawai G*[1], *Matsufuji S* (*[1]Chiba Tech). Two stems with different characteristics and an internal loop in an RNA aptamer contribute to spermine-binding. *J Biochem.* 2017; **161:** 197-206.