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General Summary

Antizyme (AZ) is a negative regulator of polyamines. It is conserved in a wide range of eukaryotes from yeasts to humans, and 3 AZ subtypes (AZ1, AZ2, AZ3) have developed in mammals. We have been studying the mechanisms of polyamine-dependent translational frameshifting required for the expression of AZ and the physiological function of AZ.

Research Activities

Mechanism of antizyme translational frameshifting

The messenger (m)RNAs of AZ1 and AZ2 have a pseudoknot structure as a signal for translational frameshifting. We used the ultraviolet-crosslinking method to survey RNA-binding proteins that bind to the pseudoknot structure and affect frameshift efficiency. Some of the candidate proteins of 20 to 50 kDa identified in the previous year turned out to be nonspecific. However, a new candidate protein of 34 kDa that specifically binds to a mutant version of the pseudoknot was identified. We are testing the hypothesis that the 34-kDa protein interacts with the partially melted pseudoknot structure by elongating ribosomes. We have also purified the 34-kDa protein using affinity to the mutant pseudoknot for identification with mass spectrometry.

The mammalian AZ frameshift signal sequence directs backward hopping of ribosomes or −2, −5, and −8 frameshifting in *Escherichia coli*. To facilitate the analysis of these phenomena, we constructed a vector with upstream glutathione-S-transferase and downstream Protein A-His tag reporter genes. The frameshift sequence was inserted between the two reporters. After purification with the tags, the product was cleaved with a specific protease at the border with the upstream reporter and subjected to mass spectrometry. The backward hopping was confirmed to occur in this system. When the transfer (t)RNA-binding codon for −8 frameshifting was mutated, the corresponding product disappeared, suggesting that the tRNA-binding site is a signal for the backward hopping.

We continued screening for novel recoding (reprogrammed genetic decoding) genes among the target genes of nonsense-mediated mRNA decay (NMD) in the fission yeast *Schizosaccharomyces pombe*. DNA microarray and computer analyses revealed that mRNAs of 98 genes increased in an NMD-knockout strain and that 33 of the genes contained potential recoding sites.

Study with AZ1 knockout mice

We demonstrated that homozygous AZ1 knockout mice die from impairments of embryonic hematopoiesis. Our earlier experiments using α-difluoromethylornithine (DFMO), a specific inhibitor of ornithine decarboxylase, suggested that early

hematopoietic cells already have high levels of putrescine caused by the absence of AZ1 before they migrate to the liver from the aorta-gonad-mesonephros (AGM) region. In another experiment, oral administration of 2% DFMO in drinking water to maternal mice during the AGM period (embryonic day 9.5–11.5), but not during the period of hepatic hematopoiesis (embryonic day 11.5–13.5), rescued the phenotype of AZ1 knockout mice. In addition, colony-forming assays using cells derived from the AGM region and bone marrow showed decreases in the burst-forming unit of erythroid cells and cells of earlier stages. These results indicate that early hematopoietic cells that have not yet migrated to the liver are particularly sensitive to high concentrations of putrescine.

Analysis of AZ2-interacting proteins

We previously found that cerebellar-degeneration-related protein 2 (CDR2) binds to AZ2, but not to AZ1 or AZ3, by means of the two-hybrid system, and determined the AZ2-binding region on the CDR2 molecule. Further study using AZ1/AZ2 chimera proteins indicated that the CDR2-binding region of AZ2 is located between amino acid residues 135 and 181. We then substituted 7 conserved residues in this region with alanine and found that 2 residues, arginine 140 and valine 174, are indispensable for binding to CDR2. Interestingly, AZ1 acquired interaction with CDR2 when the corresponding 2 residues were mutated to the AZ2 type, suggesting that the 2 residues are determinants of specific binding with CDR2.

We performed yeast two-hybrid screening of a mouse kidney complimentary DNA library to identify AZ2-interacting proteins. From 1.35×10^5 independent clones, about 300 positive clones were obtained, and 75 clones with stronger signals were sequenced. These contained known AZ2-binding proteins, such as ornithine decarboxylase, antizyme inhibitor, and CDR2. We selected several candidates and are confirming the specificity of binding.

Publications

Kosuge M, Takizawa H, Maehashi H, Matsuura T, Matsufuji S. A comprehensive gene expression analysis of human hepatocellular carcinoma cell lines as components of a bioartificial liver using a radial flow bioreactor. *Liver Int* 2007; **27:** 101–8.