

## Molecular cloning of the Ornithine decarboxylase(ODC) antizyme from flounder

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### Introduction

Polyamines are present in all living cells and are essential for cell proliferation, differentiation, and migration (Qing yuna *et al.*,2000). In rapidly growing cells, polyamine levels are up-regulated by an increase in their synthesis and cellular uptake. The feedback regulation is mediated by Ornithine decarboxylase (ODC) antizyme that represses ODC, a key enzyme for polyamine synthesis (Takatoshi *et al.*, 2000).

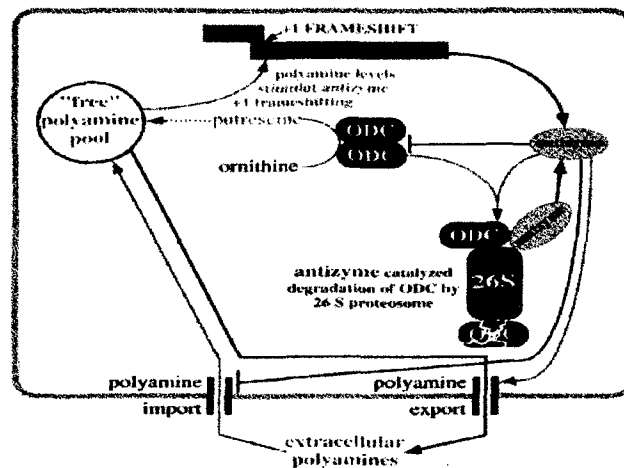


Fig. 1. The up-regulated polyamine stimulates antizyme +1 frameshifting, which ORF2 is in the +1 frame with respect to ORF1. The antizyme which is translated in the frameshifting catalyzed degradation of ODC by 26S proteasome(Ivaylo *et al.*,2000)

## Materials and Methods

### *Isolation of total RNA and poly A+ RNA*

Total RNA was isolated using TRIzol reagent(Gibco)

### *Construction of flounder pituitary cDNA library*

Flounder cDNA library was constructed by the Manufacture's instruction(Stratagene, cDNA library construction kit).

### *Screening of ODC antizyme*

The ORF of ODC antizyme was amplified by PCR on the subcloned cDNA species.

### *Purification of plasmid DNA and Sequence analysis*

The clone obtained from screenig of library was purified using Wizard Plus SV minipreps DNA purification(Promega). The nucleotide sequence of cloned cDNAs were determined by ABI PRISMTM 310 automatic sequencer(Perkin Elmer).

## Results and Discussion

We screened Ornithine decarboxylase (ODC) antizyme, which consists of 1100bp open reading frame(ORF); the sequence of 50 codons (ORF1) overlaps a longer ORF (ORF2) from a flounder cDNA library. Taking account of +1 frameshifting, AZS and AZL products were 214 and 218 residues long respectively and shared 51.8% amino acid identity. The amino-acid sequences encoded by ORF1 of both AZS and AZL are similar to that of mammalian AZ1; there is more than one AUG codon and the first AUG is followed by a highly conserved region of approx. 20 residues.

## References

- Ivanov IP, Gesteland RF, Atkins JF.(2000). Antizyme expression: a subversion of triplet decoding, which is remarkably conserved by evolution, is a sensor for an autoregulatory circuit.pp.3185-96, Oxford University Press, Nucleic Acids Res.
- Saito T, Hascilowicz T, Ohkido I, Kikuchi Y, Okamoto H, Hayashi S, Murakami Y, Matsufuji S(2000).Two zebrafish (Danio rerio) antizymes with different expression and activities.pp. 99-106, Biochem J.
- Yuan Q, Ray RM, Viar MJ, Johnson LR(2000).Polyamine regulation of ornithine decarboxylase and its antizyme in intestinal epithelial cells.pp.G130-8,Am J Physiol Gastrointest Liver Physiol.