EXAMPLE BLAST

Sequence:

SYMGFEVVRP DHPALPPLDN

Name of protein:

Ornithine Decarboxylase Antizyme 3 Isoform 1

Complete sequence:

1 MPCKRCRPSVYSLSYIKRGKTRNYLYPIWSPYAYYLYCYKYRITLREKMLPRCYKSITYKEEEDLTLQPRSCLQCS
ESLVGLQEGKSTEQGNHDQLKELYSAGNLTVLATDPLLHQDPVQLDFHFRLTSQTSAHWHGLLCDRRLFLDIPYQA
LDQGNRESLTATLEYVEEKTNVDSVFVNFQNDRNDRGALLRAFSYMGFEVVRPDHPALPPLDNVIFMVYPLERDVG
HLPSEPP 235

a. **First BLAST run**

Sequence #19

19) SYMGFEVVRP DHPALPPLDN

Ornithine Decarboxylase Antizyme 3 Isoform 1 [Homo sapiens]- NP_057262.2

Complete sequence:

2 MPCKRCRPSVYSLSYIKRGKTRNYLYPIWSPYAYYLYCYKYRITLREKMLPRCYKSITYKEEEDLTLQPRSCLQCSESLVGL
QEGKSTEQGNHDQLKELYSAGNLTVLATDPLLHQDPVQLDFHFRLTSQTSAHWHGLLCDRRLFLDIPYQALDQGNRESLTAT
LEYVEEKTNVDSVFVNFQNDRNDRGALLRAFSYMGFEVVRPDHPALPPLDNVIFMVYPLERDVGHLPSEPP 235

b. **Second BLAST run**

Subset of related sequences

NP_057262.2-Ornithine Decarboxylase Antizyme 3 Isoform 1 [Homo sapiens] (Human)

NP_001136257.1-Ornithine Decarboxylase Antizyme 2 [Taeniopygia guttata] (Finch)

CAK11023.1-Ornithine Decarboxylase Antizyme 1 [Danio rerio] (Fish)

1ZO0_A-Chain A, Nmr Structure Of Antizyme Isoform 1 From Rat (Structure)

AAL85621.2 -Ornithine Decarboxylase Antizyme [Aedes aegypti] (Mosquito)

AAH59754.1-Ornithine Decarboxylase Antizyme 1 [Xenopus (Silurana) tropicalis] (Frog)

Sequence Alignment

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1     MPCKRCRPSVYSLSYIKRGKTRNYLYPIWSPYAYYLYCYKYRITLREKMLPRCYKSITYKEEEDLTLQPRSCLQCSE---  77
1     ----------------------------------------MINTQDSSILPLSNCPQLQCCRHIVPGPLWCSDAPH---  36
1     ------------------------------------------------------------------------DVPL---  4
1ZO0  ------------------------------------------------------------------------------
1     M-MKFVASEISSIM-----EQQMWSEPISSSNR------ATKRTISSSSSSSSSSAGFDSYCVSLAVGPLWWSDVPQSRT  68
      ------------------------------------------------------------------------------

78    ------SLVGLQEGKSTEQGNHDQLKEL-YSAGNLTV---------LATDPLLHQDPVQLDFHFRLTSQTSAHWHGLLC- 140
37    ------PLSKIPGGRGGG-RDPSLSA-LIYKDEKITV---------SQDVPVHEGKPHIVHFQYKVTEVKTSSWDAVLS-  98
5     ------PPLKIPGGRGNDQRDHSLSAKLFYSDAQLLV---------LEEAPQSNSRVRFLLFERRCSVSKHLVWRGALK-  68
1     ---------------------------ILYSDERLNV---------TEE-PTSNDKTRVLSIQCTLTEAKQVTWRAVWN-  42
69    DHDRASPLKEYNRKTSIDSTTTASSEFTDYDDTESTVEFMNQHEAAVIQEVLNQPTPTQISLKLFVTPQKYSVWETVFNP 148
      ------------------------------------------------------------------------------

141   -DRRLFLDIPYQALDQGNRESLTATLEYVEEKTNVDSVFVNFQNDRNDRGALLRAFSYMGFEVVRPDHPALPP------L 213
99    -NQSLFVEIPDGLLADGSKEGLSALLEFAEEKMKVNYVFICFRKSREDRAPLLKTFSFLGFEIVRPGHPAVPS------R 171
69    -GTNLYIEIPTGVLPEGSKDSFSLLLEFAEEKLQVDHVFICFHKSRDDRASLLRTFSFMGFEIVRPGHPLVPT------R 141
43    -GGGLYIELPAGPLPEGSKDSFAALLEFAEEQLRADHVFICFPKNREDRAALLRTFSFLGFEIVRPGHPLVPK------R 115
149   LDNILYVNLPSTMTHEASKHSFISLLEFAEEKLECDAVVLCIRKDRLDRPNLVRTFSFVGFQPVSPKSPLAPPHIEEQQK 228
1     ------------------------------------------------MLLRTFRFLGFEIVIPGHPLVPK------R  24

214   DNVIFMVYPLERDVGHLPSEPP  235
172   PDVMFMVYPLDQSSSSDKE---  190
142   PDAFFMAYRIERDSDGDE----  159
116   PDACFMVYTLE-----------  126
229   NDYLFMIYNIEE----------  240
25    PDACFMAYTFERDSSDED----  42
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Figure 1: Sequence alignment of related species in the order listed above. Conserved amino acids thought to preserve structure are in yellow. Amino acids thought to be important for function are in blue.

c. Conserved Amino Acids

The structure of ornithine decarboxylase antizyme isoform 1 consists of 2 α helices and 8 β sheets. The amino acids colored in yellow above are highly conserved among related species and mostly hydrophobic. The most important of these are GLY194, PHE195, GLU196, VAL198, and PRO200.* These residues form strands that connect the β sheets. Another identically conserved amino acid is TRP130. This is a hydrophobic residue located in the core of the protein and is thought to be "critical for protein folding and stability."(1)

The amino acids colored in blue above are charged, and most likely play a role in the function of ornithine decarboxylase antizyme 3. Specifically, these are GLU161, GLU164, and GLU165*. These hydrophilic residues are on the surface of the protein and form a negatively charged "patch" on one face of the structure.(1) LYS153, ASP182, ARG183, and ARG188* are also conserved amino acids found on the surface of the antizyme that could possibly be important for intermolecular interactions and stabilize the substrate.

*The amino acid numbers stated above correlate to the sequence of 1ZO0 and are illustrated in Figure 3.

d. Known Structure of Protein

1ZO0_A-Chain A, Nmr Structure Of Antizyme Isoform 1 From Rat

PDB code: 1ZO0

e. PyMOL figures

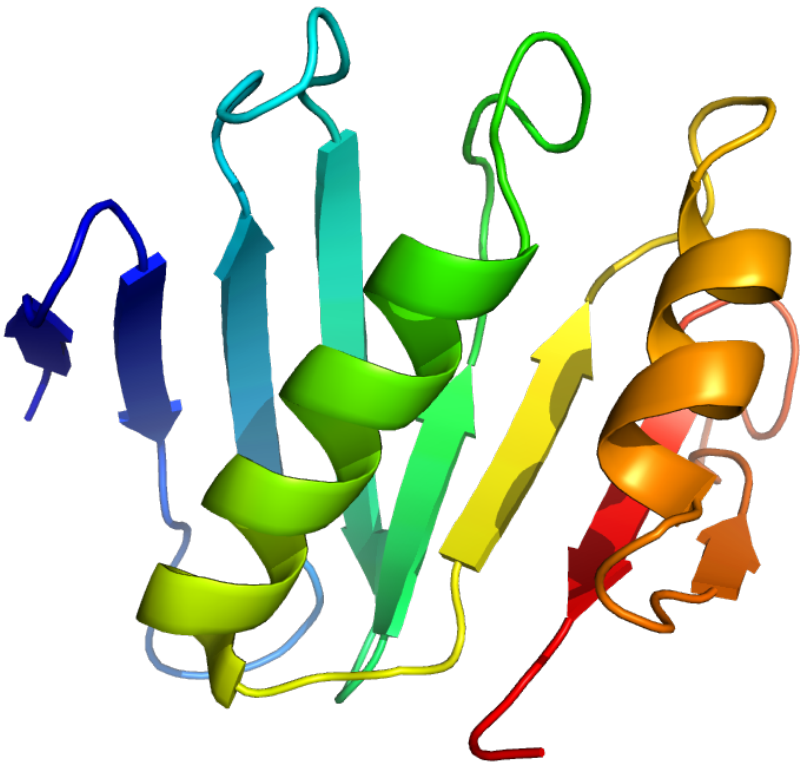

Figure 2: Structure of Ornithine Decarboxylase Antizyme Isoform 1 from a rat.

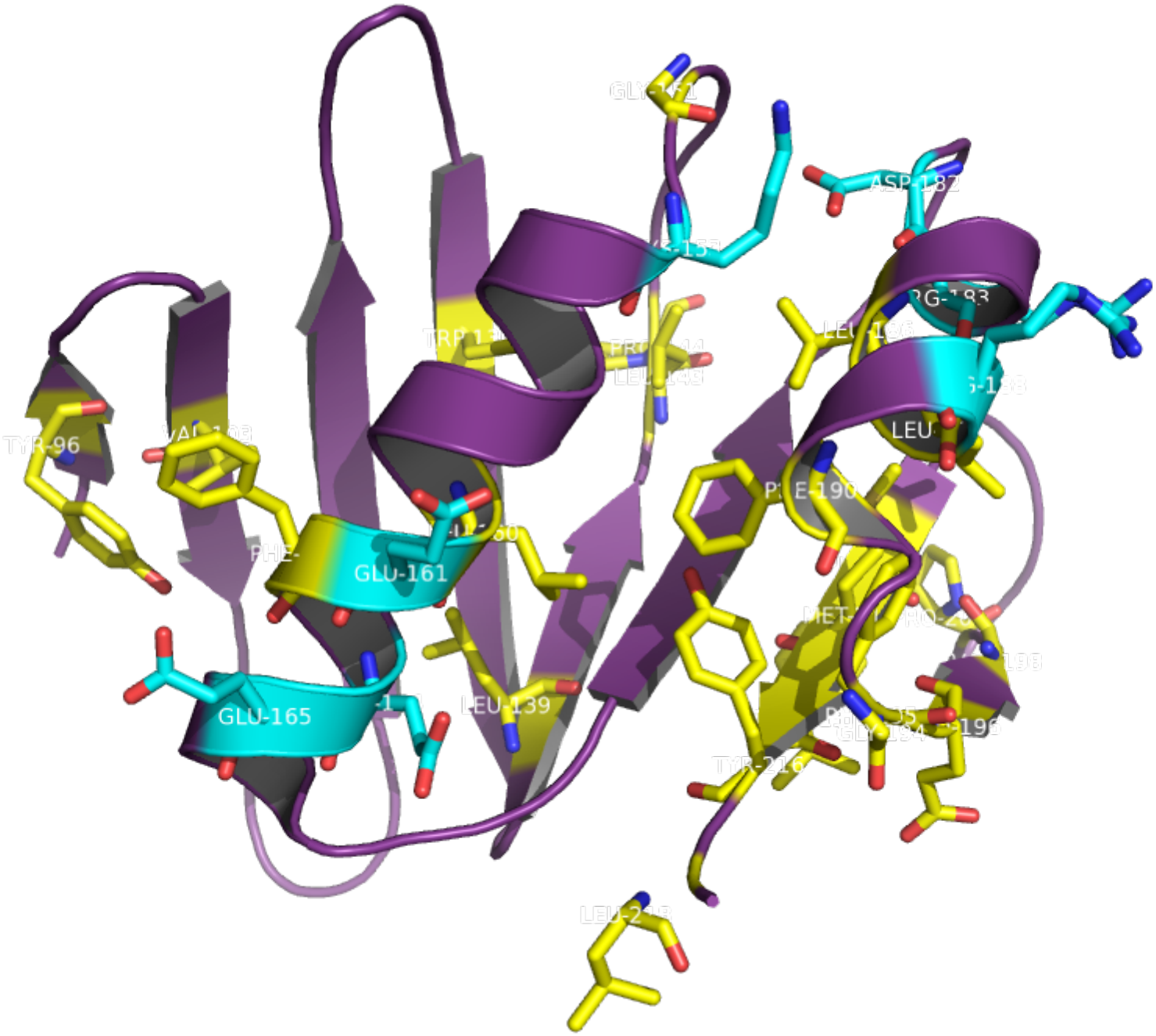

Figure 3: Structure of Ornithine decarboxylase antizyme 3 isoform 1 where the amino acids that preserve structure have been highlighted in yellow and the amino acids that are important for function are highlighted in blue.

f. Function of Ornithine Decarboxylase Antizyme 3

Ornithine decarboxylase antizymes work to regulate polyamine levels in cells.(1) Polyamines, such as spermine and spermidine, are essential for cell growth and transformation. They bind to both chromosomal DNA and RNA and aid in DNA compaction, programmed cell death, gene expression, and embryonic growth. Biosynthesis of polyamines begins with the enzyme ornithine decarboxylase (ODC), which catalyzes the removal of a carboxyl group from ornithine to form putrescine.(4) When polyamine levels become elevated, the translation of antizyme mRNA is increased. The antizyme binds to the carboxylic end of ornithine decarboxylase to inhibit its function and target it for degradation. The 26s proteosome goes on to destroy ODC. This system is an example of a negative feedback loop. A deficiency in ornithine decarboxylase antizyme would lead to a buildup of polyamines.

There are at least three forms of antizyme protein within humans. While all antizymes regulate levels of polyamines and inhibit the function of ornithine decarboxylase, studies have shown that antizyme 3 does not stimulate the degradation of ODC.(2,5) Isoform 3 is expressed solely in testicular germ cells, allowing it to potentially regulate polyamine levels during spermatogenesis.(5) Antizyme 3 is less effective than 1 and 2 in the inhibition of ornithine decarboxylase. It is usually only successful when it is in excess compared to the level of ODC in the cell.

Research has shown that ODC antizyme could be used to treat cancer.(6) ODC is required for cell growth and differentiation. It therefore makes sense that antizymes potentially have anti-tumor activity. Because the effect of ODC antizyme on tumor development is unknown, further research is necessary to determine its exact function in that application.

References

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2. Ivanov, I. P., and Rohrwasser, A. (2000) *PNAS.* 97 (9) 4808-4813.
3. Palanimurugan, R., and Scheel, H. (2004) *The EMBO Journal*. 23, 4857 – 4867.
4. Almrud, J., and Oliveira, M., and Hackert, M. (2000) *Journal of Molecular Biology*. 295, 7-16.
5. Snapir, Z., and Keren-Paz, A. (2009) *Biochem J PubMed*. 419(1):99-103.
6. Iwata, S., and Sato, Y. (1999) *Oncogene*. 18, 165 – 172.