T&BLE OF CONTENTS

Sr. No.	TITLE	PAGE No.
1	Introduction	1
1.1	Protein degradation	1
1.2	Targeted protein degradation	4
1.2.1	Ubiquitin and Ubiquitin proteasome system used for targeted protein degradation	5
1.2.2	Ubiquitin independent targeted protein degradation	7
1.2.3	Ubiquitin dependent and independent protein degradation	8
1.2.4	Advantages of targeted protein degradation	9
1.3	Ornithine decarboxylase (ODC)	10
1.3.1	Structure	11
1.3.2	Regulation of ODC	12
1.3.3	Comparison of mammalian and yeast ornithine decarboxylase	14
1.4	Antizyme, a competitor of ubiquitin	16
1.4.1	Structure of antizyme	17
1.4.2	Function and regulation of antizyme	18
1.5	Why ODC has special mechanisms for its regulation?	20
1.6	ODC: Antizyme interface	20
1.7	ODC as a tool for targeted protein degradation	22
1.8	Hypothesis of the present study	23
1.9	Major objectives of the present study	25
2	Structural characterization of degradation determinant signals of yeast and mouse ornithine decarboxylase, in isolation from rest of the protein sequences and in combination with other degrons.	27
2.1	Introduction	27
2.2	Materials and methods	28
2.2.1	Strains and plasmids	28
2.2.2	Construction of chimeric plasmids	28
2.2.3	Expression and purification of proteins	31
2.2.4	Structural characterizations	32
2.2.5	Homology modeling of degrons peptides	33
2.2.6	N50 structure characterization	34
2.3	Results	34
2.3.1	Cloning of degrons of yeast and mouse ornithine decarboxylase (alone and in combination) in bacterial expression vector	34
2.3.2	Expression and purification of degron peptides of ODCs	35
2.3.3	Structural characterizations of N50, α/β and N α/β peptides	38
2.3.4	Structural characterizations of AzBE, mODC, AzBE-mODC, N50-mODC and α/β-mODC	40
2.3.5	Homology modeling for degrons of ODCs	47

Theoretical sequence analysis of first 50 residues of yeast ODC (N50)	50
Discussion	51
Determination of the potential of degradation determinant signals (degrons) of ornithine decarboxylase for targeted protein degradation in S.cerevisiae	55
Introduction	55
Materials and methods	56
Strains and plasmids	56
Cultural conditions	57
Construction of chimeric plasmids	58
Fluorescencespectroscopy	60
Fluorescencemicroscopy	60
Flowcytometry	60
Western blotanalysis	61
Results	63
Tagging of yODC degrons to N-terminal of target proteins	63
Tagging of yODC and mODC degrons to C-terminal and N & C- terminals of target protein (yEGFP)	75
Discussion	79
Studies on the coevolution of ornithine decarboxylase and antizyme.	82
Introduction	82
Hypothesis of the study	82
Methodology	83
Collect the sequences of ODC and antizyme for several organisms	83
Performed multiple sequence alignment	83
Pairwise distance analysis	83
Phylogenetic tree	83
Correlation co-efficient	83
Strategy to find co-evolution between ODC and antizyme proteins	85
Results and discussion	86
Canadatian batana fallibrati ODC and artisma and income	96
Correlation between full length ODC and antizyme protein sequences	86
Summary:	93
Summary:	93
Summary: References	93
Summary: References Published Paper	93
Summary: References Published Paper List of Publications	93
Summary: References Published Paper List of Publications Awards/Oral/Poster Presentation	93
	Determination of the potential of degradation determinant signals (degrons) of ornithine decarboxylase for targeted protein degradation in S.cerevisiae Introduction Materials and methods Strains and plasmids Cultural conditions Construction of chimeric plasmids Fluorescencespectroscopy Fluorescencemicroscopy Fluorescencemicroscopy Flowcytometry Western blotanalysis Results Tagging of yODC degrons to N-terminal of target proteins Tagging of yODC and mODC degrons to C-terminal and N & C- terminals of target protein (yEGFP) Discussion Studies on the coevolution of ornithine decarboxylase and antizyme. Introduction Hypothesis of the study Methodology Collect the sequences of ODC and antizyme for several organisms Performed multiple sequence alignment Pairwise distance analysis Phylogenetic tree Correlation co-efficient Strategy to find co-evolution between ODC and antizyme proteins Results and discussion