Synopsis of the thesis on

Structural and functional characterization of the degradation determinant signals of Ornithine Decarboxylase (ODC)

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The Maharaja Sayajirao University of Baroda

For the degree of

Doctor of Philosophy in Biochemistry

By

Rushikesh G. Joshi, M.Sc.

Under Supervision of

Prof. C. Ratna Prabha
(Macro-Molecular Structure and Biology Lab)

Department of Biochemistry
Faculty of Science
The Maharaja Sayajirao University of Baroda
Vadodara- 390 002

From:
Rushikesh G. Joshi
Dept. of Biochemistry,
Faculty of Science,
The M. S. University of Baroda,
Vadodara - 390 002.

To
The Registrar (Academic Section),
The M. S. University of Baroda,
Vadodara - 390 002.

7.10.2014

Subject: Submission of synopsis of the Ph. D. work entitled – "Structural and functional characterization of the degradation determinant signals of Ornithine Decarboxylase (ODC)"

Respected Sir,

Kindly accept the synopsis of my Ph. D work entitled – "**Structural and functional characterization of the degradation determinant signals of Ornithine Decarboxylase (ODC)**". My date of registration was 24/08/2009 and registration no. is 439.

Thanking you,

Yours sincerely,

(Rushikesh G. Joshi)

(Prof. C. Ratna Prabha)
Guide

Head
Department of Biochemistry

Dean
Faculty of Science

Introduction:

There are many purposes for protein degradation such as, removal of abnormal proteins, recycling of amino acids, protection of cell from damaged proteins and maintain cellular homeostasis. Protein degradation is required not only for elimination of defective proteins but also to control the level of proteins which are involved in cell cycle control, transcription, apoptosis and other processes (Rosenberg-Hasson et al., 1989). Protein degradation is an irreversible process. Therefore, recognition of high specificity is required to avoid destruction of wrong proteins.

In the eukaryotic cells, there are mainly two types of protein degradation present. They are: 1) lysosomal protein degradation (De Duve and Wattiaux, 1966) and another is 2) proteasome mediated degradation. In the second type of protein degradation mainly ubiquitin proteasome system (UPS) is involved. UPS is a pathway meant for specific protein degradation (Etlinger and Goldberg, 1977). In proteasomal pathway some proteins are specifically targeted for degradation by their recognition sequences, because of a degradation determining sequence also known as degron. The process is called targeted protein degradation. The degrons are responsible for protein degradation by ubiquitin dependent and independent mechanisms. We are focusing on ubiquitin independent mechanism of protein degradation.

There are numbers of protein substrates reported, which are degraded by proteasome but by ubiquitin independent mechanism. These include Ornithine decaroxylase (ODC), the first enzyme in the polyamine biosynthesis pathway (Pegg, 1988). Interestingly ODC is not degraded by ubiquitination. It is the first described and is the most studied example for ubiquitin-independent targeted protein degradation by proteasomes (Coffino, 2001; Matsufuji et al., 1995; Pegg, 2006). ODC interacts with a protein called antizyme (Az) and degraded by proteasomes, which obviates the need for ubiquitination (Matsufuji et al., 1995).

Ornithine decarboxylase is key regulatory enzyme in the biosynthesis of polyamines, (Tabor and Tabor, 1984; Wallace et al., 2003). It catalyzes the decarboxylation of ornithine to putrescine. It is catalytically active in homodimer form, which is formed by head to tail interaction of the two monomers of ODC. It is approximately 450 amino acids long and has a molecular weight of 52 kDa. Monomeric form of ODC is not

enzymatically active. ODC is one of the most rapidly degraded proteins in mammalian cells. Degradation of ODC is tightly regulated by feedback mechanism mediated through interaction of antizyme. ODC dimer catalyzes the production of polyamines, when the level of polyamines rise in the cell, antizyme is synthesized via a +1 frameshift of translation of the mRNA having two overlapping ORF1 and ORF2 (Matsufuji et al., 1995). Antizyme interact with ODC and make heterodimer (ODC-Az) and drag ODC in the direction of the 26S proteasome for degradation. The affinity of antizyme towards ODC subunits is higher than the affinity of ODC subunits for each other (Mamroud-Kidron et al., 1994). Hence, antizyme binds to ODC monomers to form inactive ODC-antizyme heterodimers that are recognized by the 26S proteasome. This mechanism is completely ubiquitin-independent and leads to degradation of ODC by proteasome.

The structure of ornithine decarboxylase has two domains namely, N terminal α/β barrel domain and C-terminal β-sheet domain (Jackson et al., 2004). Some degradation signals lie within the sequence of ODC. Antizyme recognizes a large electropositive patch on the surface of the αβ-barrel domain of ODC monomer termed as Antizyme Binding Element (AzBE) (Kern et al., 1999) and targets it for degradation. Experimental data show that residues 117-145 of mammalian ODC are recognized by antizyme. But only AzBE region is not sufficient for degradation of ODC. There are other structural elements present within ODC which are responsible for rapid degradation of ODC. In mammals, when antizyme and ODC come together, the carboxyl terminus of ODC becomes exposed, leading to conformational change in ODC (Li and Coffino, 1993). The unstructured C-terminal region pulls whole ODC towards proteasome for degradation. Actually proteasome begins degradation of ODC at the C-terminus (Zhang et al., 2004). This region is absent in *T. brucei* ODC, making the protein more stable (Persson et al., 2003). Deletion of the 37 residues at the C-terminal part of mammalian ODC stabilizes the protein even in the presence of antizyme. Attachment of only the C-terminal residues of mouse ODC to other stable proteins caused their degradation via proteasome (Zhang et al., 2003). Mutation of Cys441, which is located in this sequence, stabilized ODC even in the presence of excess antizyme (Hayashi and Murakami, 1995). This C-terminal region is disordered in human ODC crystal structure (Almrud et al., 2000; Kern et al., 1999). These observations imply that the last few amino acids of ODC are critical for proteasomal degradation of protein. The C-terminal half of antizyme interacts with ODC. And the interaction is enough to expose the C-terminus of ODC. Antizyme increases

accessibility of the C- terminus of ODC by changing conformation of ODC and the exposed C- terminus recognized by proteasomes.

When mammalian and yeast ODCs are aligned, mODC has an extended C-terminus, not present in yODC. On the other hand, yODC contains an N- terminal extension not found in the mammalian ODC. From sequence alignment it is found that the residues 164-187 of yeast ODC correspond to antizyme binding element. The mODC degradation signal present in its C-terminus (Ghoda et al., 1990; Ghoda et al., 1989), and the N- terminus of yODC are required for rapid degradation of respective ODCs (Gandre and Kahana, 2002). Although the mODC degradation signal is recognized by both mammalian and yeast proteasomes but yODC is stable in mammalian cells (Gandre and Kahana, 2002), suggesting that yeast ODC degradation signal is specific for yeast proteasomes. N-terminal domain of yeast ODC is essential for antizyme dependent degradation. In *S. cerevisiae* a deletion of 47 residues from the ODC N-terminus led to accumulation of the protein in wild-type cells to levels similar to those in *oaz1*-deleted cells (Godderz et al., 2011; Palanimurugan et al., 2004).

Targeting the over-accumulated protein in the cell using specific degrons is an emerging research area. The degradation of the vast majority of cellular proteins is targeted by the ubiquitin-proteasome pathway (Hershko and Ciechanover, 1998). But in the case of ubiquitin independent targeted protein degradation, ODC/AZ system is more effective in achieving targeted protein degradation than other types of degradation (Matsuzawa et al., 2005). Main advantage with ODC/Az system is that it does not require post-translational modification. Another advantage is we can over express the antizyme in the cells by externally transfected plasmid or by increasing polyamine concentration (Prabha et al., 2012).

Present study focuses on accomplishing targeted protein degradation by degradation signals of yeast and mouse ODC. Though the N-terminal unstructured extension of yODC is not found in mODC, it is replaced by unstructured C-terminal in mammals. Crystal structure of yODC is not solved so far. Present study is focusing on N-terminal domain of yODC and its combination with C-terminal (only 37 residues) of mouse ODC. Three segments from N-terminal region of yODC have been selected for the purpose: (i) first 50 residues (N50), (ii) α/β barrel domain and (iii) Combination of N50 and α/β

barrel domain (Nα/β). The questions addressed in this study are: (i) What is the exact role of N-terminal residues of yODC in its rapid degradation? (ii) Has the N-terminal got any role in the structure formation of α/β barrel domain? (iii) Are different segments of N-terminal part of yeast ODC independently work as degradation signals? (iv) Can they adopt stable conformation without rest of the protein? (v) Have the two proteins ODC and antizyme co-evolved?

The main objective of this study is to check the potential of degradation determining signals of yeast and mouse ODC in isolation and in combination for targeted protein degradation in *S.cerevisiae*. Simultaneously the study aims to determine the structure of above peptides without rest of the protein.

For functional characterization we have selected N-terminal part of yeast ODC and last 37 residues of mouse ODC. Those degradation signals are 1) first 50 residues of yeast ODC (N50), 2) α/β barrel domain of yeast ODC (α/β) from 39 to 346 residues, 3) N-terminal 1 to 346 residues (N50+ α/β) and 4) last 37 residues of mouse ODC (mODC) and its combination with Nα/β degradation signal of yeast ODC.

The peptides selected for structural studies are 1) Yeast antizyme binding element (AzBE), 2) last 37 residues of mouse ODC (mODC), 3) AzBE in combination with mODC (AzBE + mODC), 4) α/β barrel domain with mODC (α/β + mODC) and 5) fusion of first 50 residues of yODC and last 37 residues of mouse ODC (N50 + mODC). These peptides were selected for structural studies as the structures of peptides listed in the last paragraph were already characterized using circular dichroism (CD) and fluorescence spectroscopy in our laboratory.

Specific objectives: Major objectives of the present study are –

1. 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.

2. To determine the potential of degradation determinant signals of ornithine decarboxylase for targeted protein degradation in *S.cerevisiae.*

3. To study the co-evolution of the proteins ornithine decarboxylase and antizyme.

Objective 1: 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.

In order to test the hypothesis on successful application of degrons of ODC for targeted protein degradation, structural integrity of degron peptides of ODC was required to be determined in isolation from rest of the protein sequence. Under this objective the structures of different peptides of ODC, which are important for its degradation, were characterized.

Construction of plasmids, expression and purification of protein

The gene fragment encoding selected peptide was isolated and amplified through PCR by using gene specific primers. pET30a bacterial expression vector was used which has 6x His tag sequence for purification purpose. The gene fragments for all the selected peptides were successfully cloned using pET30a vector in *E.coli* DH5α strain. Restriction digestions and ligations were done using standard methods (Sambrook, 1989). All the constructs were confirmed by sequencing. His-tagged degrons were over expressed as fusion peptides in *E. coli* BL21 (DE3) cells. Freshly transformed cells were grown in Luria−Bertani medium (Himedia) containing 50µg/ml kanamycin at 37°C up to OD_{600} of 0.5, at which point protein expression was induced with 1 mM isopropyl β-D-1-thiogalactopyranoside (IPTG). Cells were further allowed to grow for an additional 4 h at 37°C. The cells were sonicated and lysate was centrifuged at 12000g for 15min at 4°C to remove cell debris. His-tagged degrons were purified from the supernatant via immobilized affinity chromatography by using Ni-NTA agarose resin (Qiagen). Further dialyzed the protein against 10mM sodium phosphate buffer (pH 8) to remove imidazole and other salts. The purity of sample was checked by sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS-PAGE).

a) Circular dichroism and fluorescence spectroscopy

To study the secondary structure content of the peptides far UV-CD spectra were recorded in the range of 180-260nm (Jasco J-815). Purified 30µM peptides in 10mM phosphate buffer were used for recording CD. The CD spectra of AzBE, mODC, AzBE+mODC, N50+mODC do not display any characteristic secondary structure. Since

AzBE is a small fragment in isolation from rest of yeast ODC it may not be able to gain secondary structure. N50 and mODC are unstructured even in the native protein and the lack of structure apparently is associated with functional significance. 2,2,2-Trifluoroethanol (TFE) is a solvent which is known to induce and stabilize α-helicity in many peptides. Addition of 40% TFE induced α-helical structure to different extents in AzBE, AzBE+mODC and N50+mODC peptides. In case of mODC secondary structure was not present and it was not induced in TFE. The peptide α/β displayed high content of β-sheet. The chimeric peptide α/β + mODC do not display secondary structure initially, but addition of TFE induced α/β like structure. Earlier studies from our laboratory established that Nα/β peptide has α/β like structure. Interestingly, removal of first 50 residues from N-terminal forces the α/β barrel domain into completely β-sheet like structure. Hence, N50 is apparently important for inducing and/or stabilizing α/β barrel structure in the protein. It means that only α/β domain has β-sheet like structure but in presence of N and C terminal extension it is able to gain α/β like structure. From these results it is established that N50 is important for the N-terminal domain yeast ODC to take stable conformation.

b) Fluorescence spectroscopic studies to characterize the structure of the peptides:

Fluorescence studies were carried out to characterize tertiary structural features of the peptides using intrinsic and extrinsic fluorophores. Fluorescence spectra were recorded using Hitachi Fluorescence Spectrophotometer FL-7000.

Intrinsic fluorescence spectra were recorded to know the tertiary conformation of selected degradation signals of yODC and mODC and their combination. Peptides were excited at 280nm and emission spectra were recorded in the range of 300-400nm wavelength. Increasing concentrations of guanidine hydrochloride (0 to 6M) were used to study the denaturation of the peptides. From guanidine hydrochloride denaturation studies of α/β+mODC shows increase in fluorescence intenstity with increased concentration of guanidine hydrochloride, indicating loss of tertiary structure. AzBE+mODC and N50+mODC show erratic changes in fluorescence intensities with changing guanidine hydrochloride concentrations, suggesting a possibility for lack of

tertiary structure leading to aggregation at lower concentrations of guanidine hydrochloride.

Extrinsic fluorophore 1-anilino 8-naphthalene sulphonic acid (ANS) was used in fluorescence resonance energy transfer (FRET) studies. The peptides AzBE, mODC and AzBE + mODC did not show fluorescence resonance energy transfer, indicating failure of binding of ANS to them, due to the possible absence of hydrophobic patches in the sequences. Chimeric peptides α/β+mODC and N50+mODC showed transfer of energy from intrinsic (aromatic amino acids) to extrinsic (ANS) fuorophore in FRET study. It means that these peptides have hydrophobic patches exposed to the outer environment, an indication of lack of tertiary structure.

Objective 2: To determine the potential of degradation determinant signals of ornithine decarboxylase for targeted protein degradation in *S.cerevisiae.*

In this objective, reporter protein has been tagged with degradation signals of ODC to check their potential to act as signals for targeted protein degradation. The reporter protein has been tagged with different degradation signals of yeast ODC to study the stability of reporter protein *in vivo*. This part of the work concentrates on the ability of degradation signals of ODC for targeted protein degradation. Yeast Enhanced Green Fluorescent Protein (yEGFP) was selected as a reporter protein in the model organism budding yeast *S.cerevisiae* BY4741 strain.

Construction of chimeric plasmids and its expression

For this study pUG35 yeast expression vector was used which expresses yEGFP as a reporter protein under ADH promoter. The sequences of selected degradation signals of yODC were amplified by PCR. These degradation signals were fused upstream (N-terminal in protein) to yEGFP by using *Bam*HI and *Eco*RI restriction sites. All the constructs were confirmed by sequencing.

After that the chimeric plasmids were transformed into *S. cerevisiae* BY4741 strain. The cells were grown in fresh synthetic dextrose medium alone and with proteasome inhibitor MG132 (50µM). The log phase and stationary phase cells were harvested. Number of cellswere normalized in all experiments. The rate of degradation of yEGFP was

monitored by using different techniques like fluorescence spectroscopy, flow cytometry, fluorescence microscopy and western blot.

To record fluorescence spectra, the cells containing yEGFP and chimeric proteins of yEGFP were excited at 488nm and emission spectra were scanned in the range of 505 to 550 nm. Fluorescence spectra of cells expressed unmodified yEGFP showed that with the stage of cell growth, there was a change in the expression of yEGFP. But the fusion of the degradation signals of yODC, N50 and Nα/β to the N- terminus of yEGFP led to the rapid turnover of fusion protein. The N50-yEGFP fusion protein and unmodified yEGFP were constitutively expressed in yeast transformants under the ADH1 promoter. The steady-state level of N50-yEGFP was greatly reduced compared to unmodified yEGFP in the yeast transformants. yEGFP fluorescence was similarly reduced in cells expressing the Nα/β-yEGFP fusion. But fusion of α/β with yEGFP to make α/β-yEGFP fusion protein has not seen any intensity difference with respect to cell growth. However, expression of yEGFP is decreased with respect to unmodified yEGFP. To confirm that the reduction in steady-state levels of the fusion proteins was because of increased degradation by the 26 S proteasome, the levels of the yEGFP fusion protein were monitored in presence of proteasome inhibitor MG132 (50μM). After treatment of MG132, fluorescence intensity of yEGFP is increased in all fusion proteins. These results established that degradation signals N50 and Nα/β of yeast ODC can cause degradation of reporter protein (yEGFP) via proteasome. This observation was confirmed by fluorescence microscopy.

For further confirmation of the above results, the samples were analyzed with flow cytometry. FITC channel was used to measure yEGFP expression and 30,000 events were recorded. The observations made with flow cytometry are in agreement with the results of fluorescence spectroscopy.

The results established that degradation signals of yeast ODC are able to degrade reporter protein. It was observed that N50 and Nα/β peptides are able to degrade reporter protein more efficiently than α/β peptide. From these observations two conclusion can be drawn, (i) first 50 amino acids of yeast ODC (N50) are helping the peptide to take up native like conformation of protein by which antizyme can interact with ODC and (ii) like C-terminal of mouse ODC, N50 is important for degradation of yeast ODC via proteasome.

The same studies are going on with last 37 residues of mouse ODC and its combination with Nα/β degradation signal of yeast ODC.

Objective 3: To study the co-evolution of the proteins ornithine decarboxylase and antizyme.

Under this objective the degree to which the two proteins ODC and antizyme have coevolved is being investigated *in silico*. Ornithine decarboxylase (ODC) is a key enzyme in the biosynthesis of polyamines. Antizyme (Az) regulates the levels of polyamines in cells by targeting ODC for degradation. The interaction between ODC and antizyme is important for the regulation of the levels of polyamines in the cells. Hence, the question is these two proteins ODC and antizyme coevolved to what extent to maintain their interaction?

To check the hypothesis of coevolution, interacting residues of ODC and antizyme have been chosen for the study. If residue Xxx of yeast ODC interacts with residue Bbb of yeast antizyme and if Xxx undergoes a mutation to Xxx* in an organism, then it is associated with a corresponding change in residue Bbb in the antizyme of this organism so that the interaction between Xxx and Bbb is maintained. However, it can be the same interaction or some other interaction that contributes favourably to the interaction energy.

Sequences of ODC and antizyme of different organisms were collected. Multiple sequence alignment were done. Now, mutation found in interacting residues will be checked for any specific pattern and correlated to the process of coevolution.

Conclusion:

Some of the degradation signals (degrons) of yeast and mouse ODC are able to gain secondary as well as tertiary conformation independently, while the degrons yeast and mouse ODC, which are mostly devoid of any secondary structure in the native proteins remained unstructured. From the results of functional studies it can be concluded that degradation signals of yeast ODC act as a tag for targeted protein degradation. Structural studies carried out earlier by us established that Nα/β domain has α/β-helix like structure. But with the deletion of first 50 amino acids (N50) it became β-sheet like structure. Correlating the structural results with the results of functional studies, it can be concluded

that the first 50 amino acids of yeast ODC are important for its degradation. This unstructured N-terminal region is helping in maintaining structure of yeast ODC by which it can be recognized by antizyme. In yeast, truncation of its N-terminus of ODC leads to stabilization of whole protein. When antizyme interact with ODC, these N-terminal residues are exposed and they act as degradation signal of yeast ODC, similar to the C-terminal of mammalian ODC. Present work also reports that N50 act as a tag for targeted protein degradation both efficiently and independently.

References:

Almrud, J. J., Oliveira, M. A., Kern, A. D., Grishin, N. V., Phillips, M. A., and Hackert, M. L. (2000). Crystal structure of human ornithine decarboxylase at 2.1 A resolution: structural insights to antizyme binding. J Mol Biol *295*, 7-16.

Coffino, P. (2001). Regulation of cellular polyamines by antizyme. Nat Rev Mol Cell Biol *2*, 188-194.

De Duve, C., and Wattiaux, R. (1966). Functions of lysosomes. Annu Rev Physiol *28*, 435-492.

Etlinger, J. D., and Goldberg, A. L. (1977). A soluble ATP-dependent proteolytic system responsible for the degradation of abnormal proteins in reticulocytes. Proc Natl Acad Sci U S A *74*, 54-58.

Gandre, S., and Kahana, C. (2002). Degradation of ornithine decarboxylase in Saccharomyces cerevisiae is ubiquitin independent. Biochem Biophys Res Commun *293*, 139-144.

Ghoda, L., Phillips, M. A., Bass, K. E., Wang, C. C., and Coffino, P. (1990). Trypanosome ornithine decarboxylase is stable because it lacks sequences found in the carboxyl terminus of the mouse enzyme which target the latter for intracellular degradation. J Biol Chem *265*, 11823-11826.

Ghoda, L., van Daalen Wetters, T., Macrae, M., Ascherman, D., and Coffino, P. (1989). Prevention of rapid intracellular degradation of ODC by a carboxyl-terminal truncation. Science *243*, 1493-1495.

Godderz, D., Schafer, E., Palanimurugan, R., and Dohmen, R. J. (2011). The N-terminal unstructured domain of yeast ODC functions as a transplantable and replaceable ubiquitin-independent degron. J Mol Biol *407*, 354-367.

Hayashi, S., and Murakami, Y. (1995). Rapid and regulated degradation of ornithine decarboxylase. Biochem J *306 (Pt 1)*, 1-10.

Hershko, A., and Ciechanover, A. (1998). The ubiquitin system. Annu Rev Biochem *67*, 425-479.

Jackson, L. K., Baldwin, J., Akella, R., Goldsmith, E. J., and Phillips, M. A. (2004). Multiple active site conformations revealed by distant site mutation in ornithine decarboxylase. Biochemistry *43*, 12990-12999.

Kern, A. D., Oliveira, M. A., Coffino, P., and Hackert, M. L. (1999). Structure of mammalian ornithine decarboxylase at 1.6 A resolution: stereochemical implications of PLP-dependent amino acid decarboxylases. Structure *7*, 567-581.

Li, X., and Coffino, P. (1993). Degradation of ornithine decarboxylase: exposure of the C-terminal target by a polyamine-inducible inhibitory protein. Mol Cell Biol *13*, 2377-2383.

Mamroud-Kidron, E., Omer-Itsicovich, M., Bercovich, Z., Tobias, K. E., Rom, E., and Kahana, C. (1994). A unified pathway for the degradation of ornithine decarboxylase in reticulocyte lysate requires interaction with the polyamine-induced protein, ornithine decarboxylase antizyme. Eur J Biochem *226*, 547-554.

Matsufuji, S., Matsufuji, T., Miyazaki, Y., Murakami, Y., Atkins, J. F., Gesteland, R. F., and Hayashi, S. (1995). Autoregulatory frameshifting in decoding mammalian ornithine decarboxylase antizyme. Cell *80*, 51-60.

Matsuzawa, S., Cuddy, M., Fukushima, T., and Reed, J. C. (2005). Method for targeting protein destruction by using a ubiquitin-independent, proteasome-mediated degradation pathway. Proc Natl Acad Sci U S A *102*, 14982-14987.

Palanimurugan, R., Scheel, H., Hofmann, K., and Dohmen, R. J. (2004). Polyamines regulate their synthesis by inducing expression and blocking degradation of ODC antizyme. Embo J *23*, 4857-4867.

Pegg, A. E. (1988). Polyamine metabolism and its importance in neoplastic growth and a target for chemotherapy. Cancer Res *48*, 759-774.

Pegg, A. E. (2006). Regulation of ornithine decarboxylase. J Biol Chem *281*, 14529-14532.

Persson, L., Jeppsson, A., and Nasizadeh, S. (2003). Turnover of trypanosomal ornithine decarboxylases. Biochem Soc Trans *31*, 411-414.

Prabha, C. R., Mukherjee, S., Raman, R., and Kulkarni, S. (2012). The ends and means of artificially induced targeted protein degradation. Appl Microbiol Biotechnol *96*, 1111-1123.

Rosenberg-Hasson, Y., Bercovich, Z., Ciechanover, A., and Kahana, C. (1989). Degradation of ornithine decarboxylase in mammalian cells is ATP dependent but ubiquitin independent. Eur J Biochem *185*, 469-474.

Sambrook, J., Fritsch, E.F., Maniatis, T., (1989). Molecular cloning: a laboratory manual, 2 edn (Cold Spring Harbor, NY, Cold Spring Harbor Laboratory Press).

Tabor, C. W., and Tabor, H. (1984). Polyamines. Annu Rev Biochem *53*, 749-790.

Wallace, H. M., Fraser, A. V., and Hughes, A. (2003). A perspective of polyamine metabolism. Biochem J *376*, 1-14.

Zhang, M., MacDonald, A. I., Hoyt, M. A., and Coffino, P. (2004). Proteasomes begin ornithine decarboxylase digestion at the C terminus. J Biol Chem *279*, 20959-20965.

Zhang, M., Pickart, C. M., and Coffino, P. (2003). Determinants of proteasome recognition of ornithine decarboxylase, a ubiquitin-independent substrate. Embo J *22*, 1488-1496.

Manuscript under preparation:

"Structural and functional characterization of the degradation determinant signals of Ornithine Decarboxylase" by Rushikesh G. Joshi, Swapnali Kulkarni and C. Ratna Prabha* (Manuscript under preparation).

Oral/Poster presented & conference/ workshop attended in Ph.D. duration:

1) Poster presentation on "Structural studies on degradation determinant signals of ODC" at Indo-US symposium, IIT, Bombay, 2011.
2) Oral presentation on "Structural characterization of degron peptides from ODC" in XXVI-Gujarat Science congress-2012, MSU, Baroda.
3) Oral presentation on "Effect of C-terminal degron tail on the stability of ubiquitin" in Regional Science congress-2012, MSU, Baroda.
4) Attended a workshop on "Analysis of cell death in mammalian cells" at Dept. of Biochemistry, MSU, Baroda, 2010.
5) National conference "CME in Immunology" organized by Department of Biochemistry, MSU Baroda, 2009.

Date: October 7, 2014 **Signature of the candidate**

Rushikesh G. Joshi

Prof. C. Ratna Prabha

Guide

Head **Dean**

Department of Biochemistry **Faculty of science**