Summary:

Ornithine decarboxylase (ODC) is key regulatory enzyme in polyamine biosynthesis pathway. In both yeast and mammals ODC is degraded by antizyme mediated mechanism by proteasome. Antizyme delivers ODC to proteasome for degradation. But only antizyme is not sufficient for ODC degradation. Some degradation signals and structured and unstructured regions lie within the ODC, which make it a favorable substrate for proteasome. These different degradation signals (degrons) were characterized in this study. Main aim of the present work was to engineering the degrons of ODC as efficient vehicle for targeted protein degradation, keeping the degrons intact and pruning ODC to minimal size. Degrons were selected for this study from yeast ODC and mouse ODC sequences.

Initially structural characterizations were carried out for selected degrons of yeast and mouse ODCs. Structural studies have established that the degrons N50 and Nα/β from yeast ODC take up secondary structure independent of rest of the protein. Moreover, this study has changed the earlier assumption regarding N50 that it is not unstructured region, it has secondary structure. It has also established that N50 region helps to maintain structural integrity of N-terminal domain of yeast ODC because after deletion of N50 region from Nα/β degrons, α/β failed to fold. In addition, after fusion of yeast α/β with CmODC, the resultant α/β-mODC peptide adopts helical conformation. These observations suggest that α/β peptide needs supportive residues at N-terminal or at C-terminal to take up native conformation. Structural studies of chimeric degrons from yeast and mouse have established that combination of two fragments from different host proteins can maintain their structural content when expressed in fusion protein.

Present study also demonstrated the potential of selected degrons of ODCs to act as efficient vehicle for targeted protein degradation. The degrons of ODCs have been tagged to target protein at N-terminal, C-terminal and both N & C terminal. Initially three degrons N50, α/β and Nα/β of yeast ODC were tagged to three target proteins namely yEGFP, β-galactosidase and Ura3. Among the three degrons, N50 and Nα/β caused rapid degradation of target proteins in the cells, while α/β failed to act as degron. The additional feature of Nα/β degron is that it has antizyme binding site. The degradation of Nα/β was regulated by the expression of antizyme, this regulatory feature makes it an ideal degron for targeted protein degradation. Further combination of two strong degrons (Nα/β withCmODC), increased the rate of degradation of target protein than only Nα/β.

In our results strong correlation was observed between functional efficacy of the peptides and their structural integrity. N50, which was believed to be unstructured, displayed propensity for helical conformation. Nα/β exhibited optimal structure, while α/β failed to adopt native like conformation. Nα/β and N50 could target chimeric proteins to degradation. However, α/β failed to act as a degron. Failure of α/β in targeting the chimeric protein for degradation establishes the importance of N50. N50 acts as an effective degron, but its degradation activity cannot be regulated. So, it cannot justify the purpose of targeted protein degradation. On the other hand the regulatory feature in Nα/β makes it an ideal degron to achieve targeted protein degradation. The degron Nα/β carries many important features: i) it cannot dimerize because C-terminal sheet domain is absent, ii) It is enzymatically inactive after over expression. So polyamine levels remain same and iii) Nα/β is an interesting degron with recognition sequence for yeast antizyme.

In the present studies we have shown interaction between N50 and α/β regions, which may influence the degradation by Nα/β. The interaction of Nα/β with antizyme probably introduces a conformational change and exposes N50 region for degradation. The N50 region then extends away from the protein and inserts itself into proteasomal portal once the protein is delivered to proteasome. It appears that the interaction of antizyme is required not only to prevent formation of a dimer by monomers, but also to destabilize the conformation of the N50 region to expose it for degradation. In the dimeric state the N50 region is most likely buried to avoid degradation. The two degrons which are acting in tandem are responsible for the enhancement of degradation observed with Nα/β when compared to N50.

The mechanism of degradation of chimeric protein assisted by degrons is presented in following Figures.