Abstract

Cells rely on a number of quality control pathways which maintain the composition of the proteome, function, concentration and quality of individual proteins both during and after synthesis. Post-translational modification of proteins is one of the strategies employed by cells towards this end. In all eukaryotic cells, post-translational modification of proteins by ubiquitination serves several important purposes, which include regulating their function, location and degradation in a targeted manner by ubiquitin-proteasome system (UPS). The trio of enzymes, ubiquitin activating enzyme or E1, ubiquitin conjugating enzyme or E2 and ubiquitin ligase or E3 catalyse post-translational modification of protein substrates with monoubiquitination, multiubiquitination or polyubiquitination. The number of E2s encoded by yeast and human genomes reflects the inherent specificity and complexity of ubiquitin system. E2s have emerged as important mediators of ubiquitin chain assembly and have been validated to govern the switch from chain initiation to elongation as well as in regulating chain processivity. Thirteen ubiquitin conjugating enzymes have been identified in *Saccharomyces cerevisiae*. Ubiquitin conjugating enzymes UBC1 and UBC4 of *S. cerevisiae* are the main focus of the present study. UBC1 is required during early stages of spore germination. UBC1 is involved in synthesizing polyubiquitin chains on proteins leading to turnover of regulatory proteins, degradation of short-lived and abnormal proteins. It is essential for growth and viability of the organism. It is an α/β protein consisting of two well-defined domains separated by a long flexible tether. The two domains are ubiquitin conjugating domain (UBC) in the N-terminus and ubiquitin associated domain (van Leeuwen et al.) in the C-terminus. UBC domain is the most conserved being present in all E2 enzymes. The N-terminus or the UBC/E2 domain is responsible for the transfer of activated ubiquitin. E2-25K, the human homolog of UBC1 is involved in degradation of crucial proteins of neurons, failure of which leads to neurodegenerative disorders (e.g. Alzheimer's disease). A chimeric protein was constructed in which the UBA domain and linker of E2-25K was fused to the UBC domain of yeast UBC4. The chimeric

protein showed no polyubiquitin chain synthesizing activity, suggesting that polyubiquitination by E2-25K is dependent on the relative conformations of the UBC and UBA domains and their specific interactions

Sequence alignment of UBC1 and E2-25K protein sequences by online available bioinformatics tool, Clustal Omega showed these two protein sequences mainly differed at linker region with 43.81% conservation at the N and C-terminus. The size of linker in yeast UBC1 is of the size of 22 residues, while the linker of human homlog E2-25K with 6 residues has decreased mobility and restricted flexibility. This observation leaves a question regarding the importance of the linker and the associated flexibility. In the present study, to understand the structural and functional importance of the linker, a chimeric protein c-UBC1 was created by swapping E2-25K linker with that of UBC1 keeping the two domains of N and C-terminals intact. The chimeric protein, c-UBC1 was assessed for its functional activities as well as characterised in vitro for its structural aspects. Functional characterisation included checking its growth, development of thermotolerance, survival under stress conditions such as heat stress and in presence of various post translational inhibitors (L-Canavanine, Hygromycin B and Cycloheximide). Results of in vivo studies carried out in Δubc1 yeast strain showed that the cells expressing c-UBC1 were viable and the protein was not lethal to *S. cerevisiae.* Moreover, cells expressing c-UBC1 survived when subjected to various stress conditions. Structural studies revealed, c-UBC1 has a compact structure with few alterations to its secondary structure. Also, the chimeric construct performed equally well as UBC1 with respect to its enzymatic activity of forming polyubiquitin chains. Hence, c-UBC1 construct with shorter linker performed equally well in yeast system as compared to wild type UBC1 with longer linker.

The second ubiquitin conjugating investigated in this study was UBC4. There are two E2s namely, UBC4 and UBC5 of *S. cerevisiae*, which have identical sequence differing only by 11 residues. They show functional complementation with respect to protein degradation, especially during stress response. Existence of two almost identical proteins suggests cell's specialized

necessity of one of them under selective conditions. To understand the reasons for the residue differences between them, mutations were introduced in the UBC4 gene to generate single residue variants by swapping with codons from UBC5. Though the variants are found to be functionally active in Δubc4Δubc5 strain of yeast, they cause reduced growth under normal conditions, altered survival under heat and antibiotic stresses, when compared with UBC4. The variants indicated decrease in protein stability theoretically. Hence, the residues of UBC5 individually do not confer any structural advantage to UBC4. Interactive proteins of UBC4 are nearly three times more than those of UBC5. UBC5, therefore, is a functionally minimized version, evolved as another means of regulation to meet cell stage specific needs.

Briefly, the work presented in this thesis is a collation of investigations carried out on the importance of structural features of two E2 enzymes UBC1 and UBC4 and highlights their functional relevance.