ABSTRACT

Ubiquitin is a protein of eukaryotic origin that is indispensable for the survival of the organism. Its name, derived from the word “ubiquitous”, implies that it is constitutively expressed in all cell types in the body, and across all eukaryotic species from yeast to humans. The cell uses it to post-translationally tag proteins, a process called ubiquitination. Ubiquitination can either modulate the activity of the target proteins, or cause their degradation. Ubiquitin is a small protein, with a length of just 76 amino acids, and is single domain. It has a globular shape, with a tertiary structure consisting of a β grasp fold. It also has two β bulges, one near the C terminal and the other near the N terminal. Ubiquitin shows an incredibly high degree of sequence conservation across species, with just a few residues differing between yeast and human ubiquitin. This makes it interesting to study structure function relationships in ubiquitin.

Two different approaches have been adopted in our laboratory to study structure function relationships in ubiquitin. One approach is generating random mutations in ubiquitin through error prone PCR, and then evaluating their effects on the protein’s structure and function. Using this approach, our laboratory generated the UbEP42 mutation, which consists of S20F, A46S, L50P, and I61T substitutions. The advantage of this approach is that it does not depend on the current understanding of the roles of different residues in the protein, and can uncover previously unknown structure function relationships. The other approach involves using site directed mutagenesis to replace selected residues, whose roles have to be studied. In our laboratory, this approach was used to generate Q2N, E64G, and S65D substitutions, to study the roles of the Q2, E64 and S65 residues of the G1 β bulge in ubiquitin.

In case of UbEP42 mutation, the four substitutions have been studied in different combinations, including single, double and triple mutants. This work involved evaluating the functional effects of the double mutants derived from the UbEP42 mutation, namely S20F-A46S, S20F-L50P, S20FI61T, A46S-L50P, A46S-I61T, and L50P-I61T. In this work, the effects of these double mutations on K-48 linked polyubiquitination, Cdc28 levels, UFD pathway, lysosomal degradation, endosomal sorting, and susceptibility to antibiotics, have been studied, and the results have been looked at in context of earlier findings in our laboratory, on the effects of these double mutations on susceptibility of cells to heat stress, and the growth curve and viability of the cells. The results indicate that L50P-I61T is the most detrimental of all the double mutations, followed by S20F-I61T and A46S-I61T.

The results of this study on the double mutations indicate that while L50P and I61T substitutions are highly detrimental, the S20F and A46S substitutions might compensate the negative effects of L50P and I61T substitutions under certain circumstances. The results also indicate that these substitutions

might competitively inhibit the functioning of wild type ubiquitin, and might thus be useful in the management of certain diseases resulting from ubiquitination.

When compared to the UbEP42 derived double mutations, the Q2N, E64G, and S65D substitutions are more benign. The previous studies in our laboratory found that these β bulge substitutions do not affect the growth curve under normal conditions, survival under heat stress, or adherence to the N end rule. However, the substitutions made cells more sensitive to cycloheximide. In this work, the effect of these substitutions on susceptibility of the cells to hygromycin B, G418 and gentamicin, as well as their effect on K48 linked polyubiquitination, lysosomal degradation of uracil permease, Cdc28 levels, UFD pathway and endosomal sorting, were tested. It was found that all three substitutions make cells more sensitive to G418, and the Q2N and E64G substitutions seem to have a limited impact on the K48 linked polyubiquitination. All the other functions tested, however, remain unaffected by the three β bulge substitutions.