1
Introduction

Cellular Protein Quality Control (QC) System can be defined as a subsystem of living cells that comprises of biomolecules which help in maintaining functional protein pool through regulating both degradation and folding of proteins. This system prevents non-functional proteins to reside in cells. A central dogma in cells and organism is the biogenesis of proteins by the conversion of genetic information into active proteins. To make sure that cells do their function properly, rapid and efficient folding of each nascent polypeptide into mature functional protein is essential. Abnormal protein accumulation leads to impairment in Ubiquitin Proteasome System (UPS), and misfolded protein aggregation generates multifactorial toxic effects in cells [Bence *et al.,* 2001; Bennett *et al.,* 2005; Olzscha *et al.,* 2011]. Deregulation or inefficient folding leads to protein misfolding, aggregation, and accumulation in the various cellular compartments. Several studies and emerging evidences suggest that protein misfolding is one of the possible causal factors of various neurodegenerative and protein conformation disorders [Carrell and Lomas, 1997; Dobson, 1999; Martin, 1999]. Thus regulation of misfolded protein degradation becomes important for the cells in order to avoid cytotoxicity.

1.1 PURPOSE OF THE STUDY

There are more than 12 million patients worldwide, suffering from neurodegenerative diseases if we just account for America and Europe; World Health Organization (WHO) predicts this number to reach 30 million by 2050. India already has nearly 20-30 million neurological disorder patients [Gourie-Devi, 2008]. Apart from Alzheimer's Disease (AD), there are various other neurodegenerative diseases that include Parkinson's, Huntington, multiple sclerosis and Amyotrophic Lateral Sclerosis (ALS) diseases. All these diseases are associated with accumulation of aberrant protein aggregates. Neurodegeneration progresses in old age and leads to death of the patients as there is no cure for these devastating diseases; methods to reduce progression of the diseases are also inefficient. Hence, there in an intense need to find out firstly the basic mechanism underlying the causes of neurodegeneration so that useful diagnostics and therapeutics can be developed.

This study is aimed to investigate the role of protein quality control mechanism in cellular physiology as well as in the context of proteinopathy through finding novel E3 Ubiquitin ligase which could serve as Quality Control E3 (QCE3) Ubiquitin ligase. There exist more than 500 E3 Ubiquitin ligases, among which very few E3 have been reported to serve in protein quality control system in cells. As the mechanism of protein quality control is not yet fully revealed, more investigation of its components such as QCE3s is one of the fundamental need in this area. Perhaps, the most basic question is how misfolded proteins are recognized by these QCE3s and targeted towards various degradation pathways? QCE3s could take an advantage of chaperones' capabilities in order to recognize the misfolded proteins. This would happen through association or interaction of QCE3s with chaperones. This study has also been planned to understand the role of such QCE3s in various stress conditions as well as how these

QCE3s and their association with certain chaperones can help in overcoming aggregation of model misfolded proteins both in animal cell culture experiments and in mice model. Overall, the purpose of the study is to seed a novel approach of exploring the quality control function of E3 Ubiquitin ligases, which may help us in understanding the cytoprotective mechanisms against protein conformation disorders. Literature available for MGRN1 indicates that it has been found as a protein involved in mouse coat color mutation, and later explored for various biochemical and physiological effects like endosomal trafficking, mitochondrial dysfunction, obesity, embryonic developmental defects and spongiform neurodegeneration. As there were less explorations of neurodegeneration as only prion or related forms were studies; this prompted us to investigate the role of MGRN1 in misfolded proteins other than prions. Moreover, the earlier known role of MGRN1 in mitochondrial dysfunction and oxidative stress led to designing experiment where MGRN1 levels in such stress conditions, could be analyzed. It became important for us to investigate in details whether MGRN1 play role in the clearance of misfolded proteins or not? Where MGRN1 protein gets sequestered with aggregates, if yes, whether this sequestration is recruitment? To find out the answers of these basic questions and to understand the molecular mechanism of misfolded protein degradation, the study of MGRN1 was carried out. The whole study has been summarized in the following out comes.

1.2 BRIEF RESULTS, SCOPE AND FUTURE PROSPECTS OF THE WORK

During the course of the study, vast literature was referred in order to adopt appropriate approaches towards experimental designing. Following outcomes summarizes the current findings through this study.

Mahogunin RING Finger-1 (MGRN1) suppresses chaperone-associated misfolded protein aggregation and toxicity (Chapter 3: published as Chhangani and Mishra, 2013; *Scientific Reports 3*)

Impairment in the elimination of misfolded proteins generates cellular toxicity and leads to various late-onset neurodegenerative diseases. However, the mechanisms by which cells recognize abnormal cellular proteins for selective clearance remain unknown. Lack of the mahogunin ring finger-1 (MGRN1) E3 Ubiquitin ligase in mice causes the development of age-dependent spongiform neurodegeneration. Here, it is reported for the first time that the MGRN1 E3 Ubiquitin ligase interacts and nicely colocalizes with the cytosolic molecular chaperone Hsp70. The expression of MGRN1 increased following exposures to a variety of stressors. The inhibition of autophagy not only elevated endogenous MGRN1 levels but also caused MGRN1 to be recruited to cytosolic Ubiquitin-positive inclusion bodies. Finally, it is shown that the overexpression of MGRN1 protects against cell death mediated by oxidative and endoplasmic reticulum stress. These data suggest that MGRN1 selectively targets misfolded proteins for degradation and may exhibit viable therapeutic potential for the treatment of spongiform neurodegeneration.

Mahogunin RING Finger 1 Suppresses Misfolded Polyglutamine Aggregation and Cytotoxicity (Chapter 4: published as Chhangani, et al., 2014; *BBA-Molecular Basis of Disease; 1842:9*)

Polyglutamine diseases are a family of inherited neurodegenerative diseases caused by the expansion of CAG repeats within the coding region of target genes. Still the mechanism(s) by which polyglutamine proteins are ubiquitinated and degraded remains obscure. Here, for the first time, it is demonstrated that MGRN1 (mahogunin ring finger 1) E3 Ubiquitin protein ligase is depleted in cells that expresses expanded-polyglutamine proteins. MGRN1 protein gets coimmunoprecipitated with expanded-polyglutamine Huntingtin and Ataxin-3 proteins. Furthermore, it is shown that MGRN1 is predominantly colocalized and recruits with polyglutamine aggregates in both cellular and transgenic mouse models. Finally, the work demonstrates that the partial depletion of MGRN1 increases the rate of aggregate formation and

cell death, whereas the overexpression of MGRN1 reduces the frequency of aggregate formation and provides cytoprotection against polyglutamine-induced proteotoxicity.

These observations suggest that stimulating the activity of MGRN1 Ubiquitin ligase might be a potential target to eliminate the cytotoxic threat of misfolded proteinaceous species such as expanded polyglutamine proteins; this could serve as a contribution in developing therapeutics to expanded polyglutamine proteins related or other neurodegenerative diseases.

The results indicate an important role of a novel E3 Ubiquitin ligase in protein quality control mechanism as well as in neurodegeneration. However, the cellular system comprises of, perhaps, more complex mechanism than yet understood. There are several proteins that were found to be present in various kinds of aggregate or inclusion bodies. Not all of them are known for their role in aggregation. Figure 1.1 shows that different components of a cell could be sequestered in misfolded protein aggregates, which leads to impairment in their cellular functions. More studies and comprehensive analyses are needed for detailed understanding of the protein quality control mechanisms.

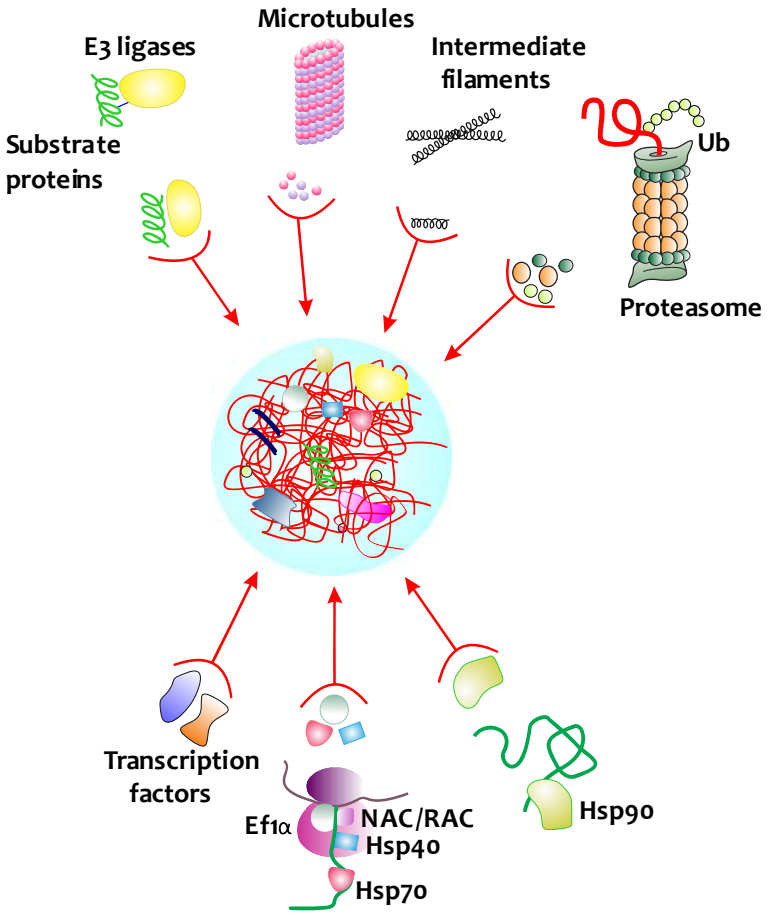

Figure 1.1 : *Sequestration of various cellular components with protein aggregates:* While aggregation progresses, it is evident that many of the cellular components get sequestered with aggregated proteins. This leads to impairment of their own function from their sites of action. Overall, such effect causes global impairment; this phenomenon makes it difficult as well as important to study if particular proteins are there with aggregates for the sake of clearance specifically or they have got sequestered unintentionally.