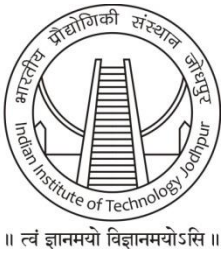
Rejuvenation Mechanisms of LRSAM1 E3 Ubiquitin Ligase Against Misfolded Proteins Aggregation Linked Neurodegenerative Diseases

A Thesis submitted by

Ribhav Mishra

in partial fulfillment of the requirements for the award of the degree of

Doctor of Philosophy



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Summary and Conclusion

E3 ubiquitin ligases maintain cellular proteostasis in different stress situations by removing misfolded proteins with the concerted action of UPS and autophagy (Figure 5.1).The present work contains two chapters, where the first chapter explains the role of LRSAM1 E3 ubiquitin ligase in alleviating cells from the toxicity of neurodegenerative associated misfolded and aberrant proteins formed under different stresses. The second chapter reveals LRSAM1 mediated modulation of PQC protein, i.e. E6-AP. The work contributes in identifying a novel substrate of LRSAM1, with a possible linkage of LRSAM1 to the neurodevelopment and to the pathology of neurodevelopmental disorder i.e. Angelman syndrome, where E6-AP becomes dysfunctional.

5.1 SUMMARY

In the first section of the doctoral work, LRSAM1 level is observed to be induced in different stress conditions, and it follows the expression profile of well-known PQC protein Hsp70. Similar to Hsp70, LRSAM1 can also remove misfolded protein aggregates from cells and provide cytoprotection. LRSAM1 was found to recruit different PQC proteins like ubiquitin and LC3 to remove such misfolded proteins from the cell. The QC function of LRSAM1 E3 ubiquitin ligase was demonstrated with its ability to mark misfolded protein inclusions of a model putative luciferase for degradation. An overexpression of LRSAM1 was critical to provide cytoprotection against the toxicity of different stress producing drugs and its knockdown results in aggravation of cell toxicity under various proteotoxic insults. Depletion of LRSAM1 was also found to affect mitochondrial and cellular health under a variety of stresses.

The second chapter of the present research helps in validating the functions of LRSAM1 in PQC and also in neurodevelopment. LRSAM1 overexpression was found to induce E6-AP protein degradation using the machinery of proteasome and a si-RNA mediated depletion of LRSAM1 lead to E6-AP accumulation in the cell. Changes in E6-AP also cause variations in the its cell cycle substrates such as p53, p27 and p21 and leads to alteration of cellular proliferation. This work shows the presence of a cellular mechanism to regulate essential PQC protein like E6-AP to its basal level in different stress conditions by another E3 ubiquitin ligase LRSAM1, and may also provide a role of LRSAM1 in neurodevelopmental disorder such as Angelman syndrome, where E6-AP functions are lost.

5.2 CONCLUSION

LRSAM1 E3 ubiquitin ligase loss in function is characterized as the main cause of Charcot-Marie-Tooth disease Type 2P (CMT2P) pathology. The first work performed in this doctoral thesis reports critical QC nature of LRSAM1 E3 ubiquitin ligase against different neurodegenerative misfolded protein causing conditions. The second part of the thesis identifies the role of LRSAM1 in the maintenance of PQC protein levels and findings of this study may also have applications in to understand the molecular mechanisms of pathology like Angelman syndrome. Identifying QC roles of LRSAM1 will be really beneficial in understanding the molecular mechanisms used by LRSAM1 in targeting the misfolded protein for removal from the cell. Hence in future this knowledge may be used in devising suitable modulators against LRSAM1 that can benefit in regulating the activity of LRSAM1 in protein

conformational disorder like in Parkinson or also in axonal neuropathology of CMT2P, where LRSAM1 activity is lost [Aerts *et al.*, 2016, Guernsey *et al.*, 2010]. These modulators can also help in neuroprotection against the protein conformational disorder of Huntington's, as LRSAM1 has been previously observed to protect against the aggregates of Huntingtin's protein [Tang *et al.*, 2011].

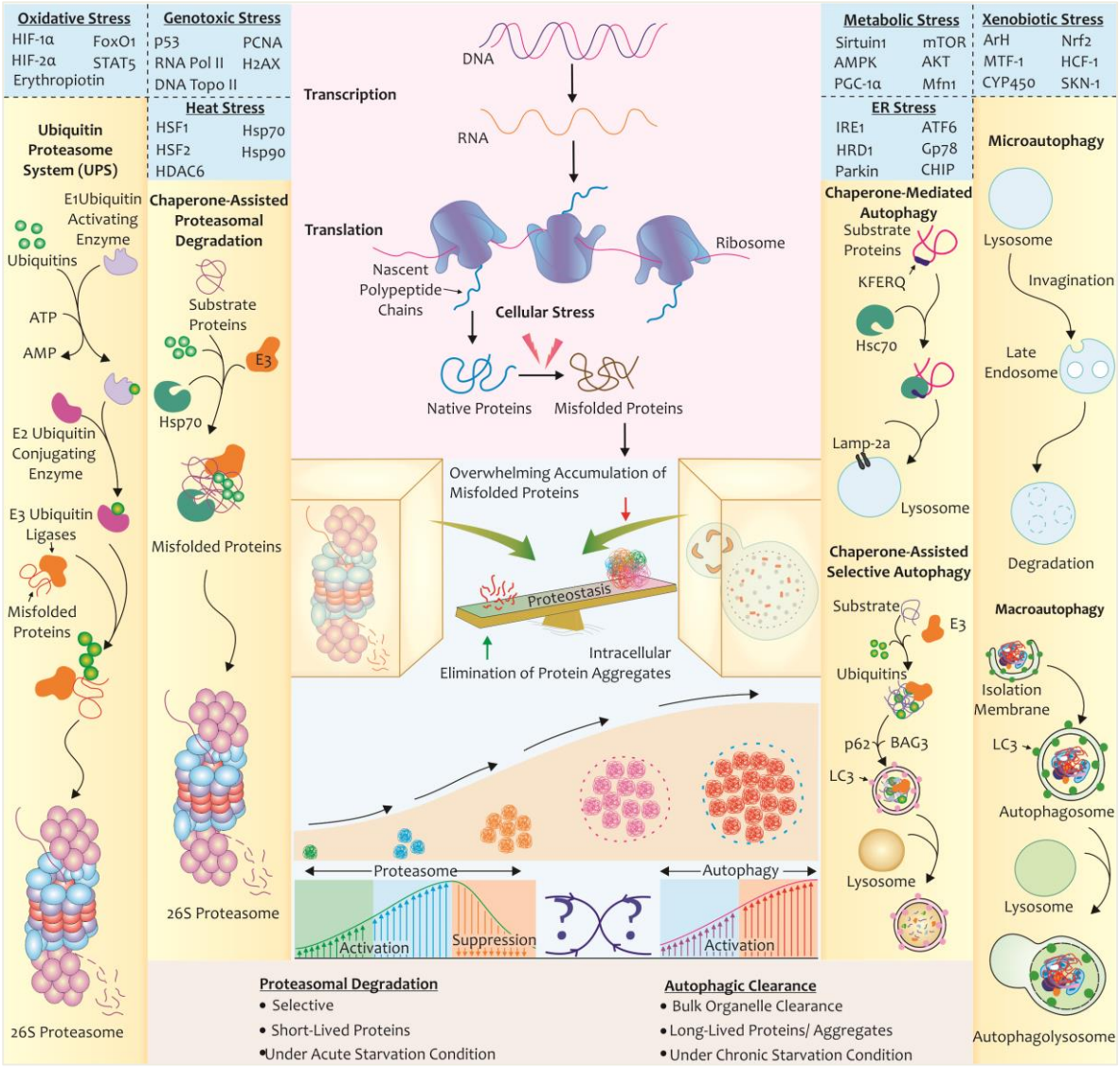

Figure 5.1 : *Orchestration of stress-response pathways by proteasome in a concerted action with autophagy*: Several proteasomal substrates have been reported to be the major regulators of different stress regulatory pathwaysrunning inside the cell under different conditions (upper blue squares). A diagrammatic representation of the multipledegradative pathways reported so far, which includes: UPS (ubiquitin proteasome system), CAP (chaperone-assisted proteasomal degradation), CASA (chaperone-assisted selective autophagy), CMA (chaperone-mediated autophagy), microautophagy, and macroautophagy (yellow boxes). The central portion illustrates the possible interaction mechanism present between two major protein degradation pathways of the cell. A nascent polypeptide chain may or may not attain its native conformation, resulting in aggregate formation under a variety of intra- or extracellular stresses. The aggregated proteins tend to be degraded by proteasome in order to maintain the cellular proteostasis. But under severe toxic conditions, the proteasomal machinery does not suffice the requirements and may even get stalled due to accumulation of massive aggregate proteins. Previous report indicates that under this critical condition, the bulk degradation of these large protein inclusions is executed via autophagic degradation pathways. **Source: Published As Ribhav Mishra and Amit Mishra et.al., 2018 in Medicinal Research Review. DOI: 10.1002/med.21502.**