

Introduction

The brain makes use of highly specialized and organized network of spiking neurons, which acts as the basic unit of the nervous system (Hahn et al., 2019). Neuronal populations are primary electrically active cells in the body with their involvement in various neurological processes. Several diseases and disorders linked with the functioning and health of neurons are often difficult to treat. For instance, Neurodegeneration can be viewed simply as slow progression of sick neurons towards agony (Przedborski et al., 2003). The death of neurons also leads to the damage of information and incapacitates cognitive, motor and behavioral functions (Seeley et al., 2009). The cellular or molecular disturbances that leads to pathogenesis of disease are generally progressive and usually initiate in mid-adult life as a result of faulty aging and deteriorates with age, manifesting motor and cognitive defects (Lessing and Bonini, 2009). However, it is also equally important to consider these degenerative mechanisms to be arising due to neuron's incapacity to abolish such toxic factors. One example is in case of protein aggregation, neuron's ability to relieve from repercussions is diminished ultimately leading to onset of apoptotic pathways (Loh et al., 2006; Nie et al., 2007; Chakraborty et al., 2015; Sze et al., 2015). Hence, uplifting such stressed neurons in their ability to defend themselves against such holistic damages would be a vital inclusion in designing a therapeutic approach.

Proteins are one of the essential biomolecules responsible for performing critical physiological functions of the cell. The unique sequence of amino acids in the polypeptide provides structural and functional diversity to these molecules. The sequence of amino acids is dictated by information coded in genome, which is accessed using DNA transcription and later manifested into a polypeptide with the help of ribosome-mediated translation. The synthesized protein then undergoes a highly conserved mechanism of folding to attain a specific three-dimensional structure. Several molecular workforces corroborate to ensure the proteins with non-native confirmations do not accumulate in the cell; thus, maintaining the quality of cellular proteome (Figure 1.1). The cellular environment is crowded with many proteinaceous species, which may amount to more than 250 mg/ml of concentration occupying 25-30% of cellular volume (Wisniewski et al., 2014). Under such congested surrounding, to ensure that protein folds in its native conformation, molecular chaperones perform significant functions by regulating the interaction between the amino acids (Rosenzweig et al., 2019). Molecular systems that centers in dealing with unfolded or misfolded proteins involve the chaperone-based protein folding or refolding and the two principal regulated protein disintegrators; the proteasome system and the autophagy. Mitochondria and endoplasmic reticulum (ER) forms critical cellular organelles involved in dealing with aberrant protein aggregates. The protein quality is continuously overseen by ER-based unfolded protein response (UPR). The misfolding events in ER instigate three UPR activators viz. IRE1, ATF6, and PERK. These proteins further mediate an array of responses by activating the transcription of protein quality control (PQC) genes (Ron and Walter, 2007).

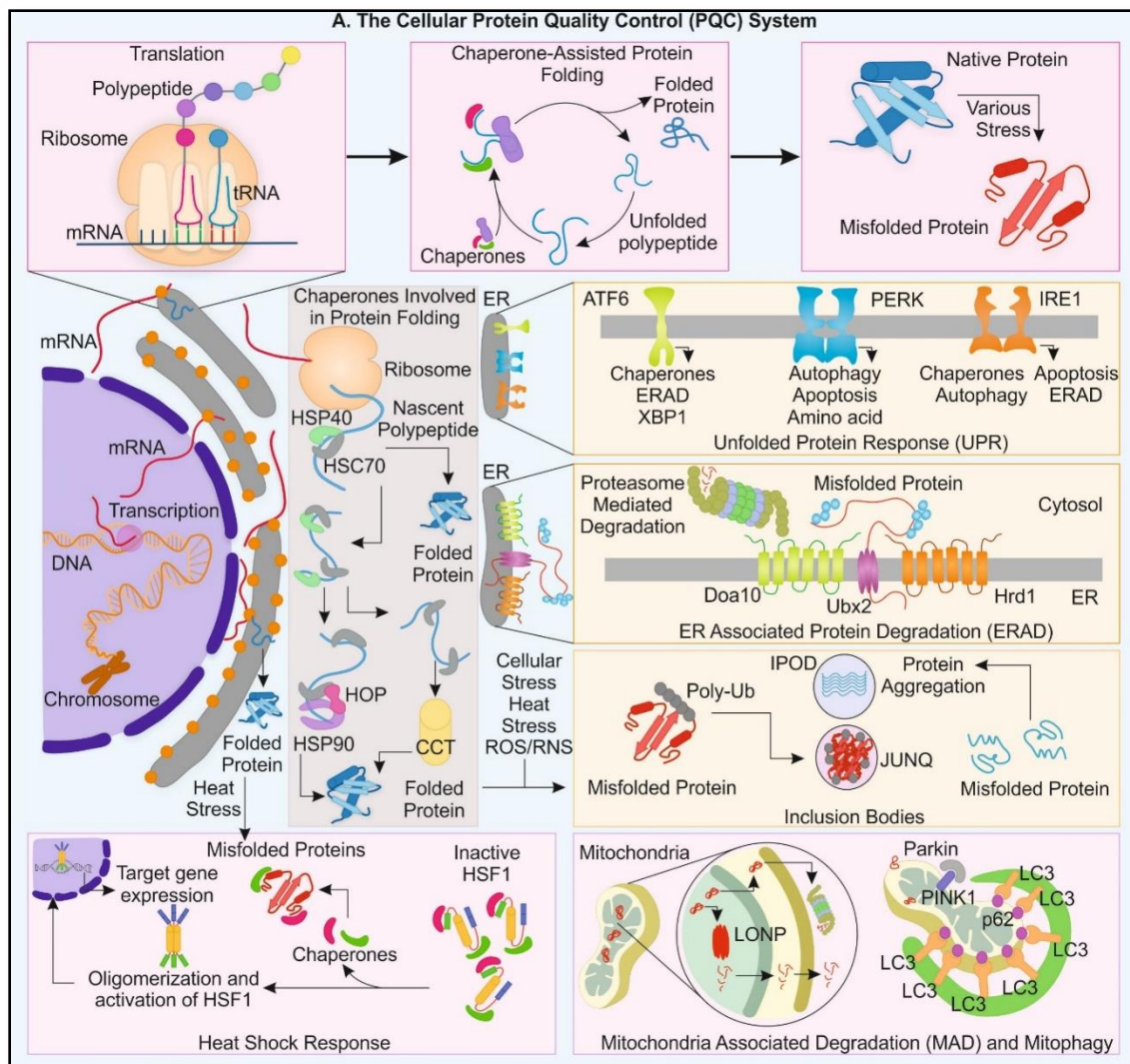


Figure 1.1 : The cellular Protein Quality Control (PQC) System: The quality of cellular protein is constantly reviewed to avoid the build-up of misfolded protein accumulates and to perform such vibrant function there is a comprehensive protein quality control system in the cell. Chaperones assist in folding of protein with proteasome and lysosome system responsible for removal of worn-out proteins. Endoplasmic reticulum and mitochondria houses prime site for dealing with misfolded protein aggregates with several protein disintegration mechanisms. (Source: Ankur R. Dubey and Amit Mishra et al, *Molecular neurobiology*, 2022)

If the folding of certain proteins is not conceivable, such proteins are cleared using ER-associated protein degradation (ERAD). The ER membrane-associated quality control E3 enzymes, e.g., HRD1, gp78, and MARCH6, ubiquitinate (tag) aberrant protein clients for proteasomal degradation (Kaneko et al., 2016; Joshi et al., 2017). The cytosolic proteins that get misfolded are often compartmentalised at several perinuclear inclusion bodies. Such protein inclusions avoid the interaction of misfolded elements with other healthy cellular proteins and are found positive for several quality control elements indicative of attempts to deal with misfolded proteins (Kaganovich et al., 2008). Apart from this, during extensive misfolding events, the protein aggregates are often transported to mitochondria, where they are dealt with the help of the MAGIC mechanism (Ruan et al., 2017). The misfolded cytosolic proteins, susceptible to aggregation, can be degraded by mitochondrial matrix proteases, like Pim1, in a phenomenon termed as Mitochondria as guardian in cytosol (MAGIC). On the other hand, mitochondrial aberrant proteins can be degraded by cytosolic proteasome, once ubiquitinated by E3 enzymes and extracted from mitochondrial outer membrane with the help of Cdc48 complex (mitochondrial-associated degradation; MAD) (Labbadia and Morimoto, 2015).

1.1 PURPOSE OF THE INVESTIGATION

Despite the presence of efficient protein disposal mechanism the occurrence of misfolded protein aggregates is a tough challenge to deal. Suppression of misfolded protein clearance generates proteotoxic inclusions, which subsequently participate in the molecular pathomechanisms of different neurodegenerative diseases (Ciechanover and Kwon, 2015; Sweeney et al., 2017). Numerous findings have shown that depletion of cellular protein quality control mechanism (PQC) affects normal degradation of aberrant proteins (Volpi et al., 2016; Zheng et al., 2016). Previous evidences signify that inhibiting proteasome instigates ER chaperones and heat shock response (Bush et al., 1997). Despite knowing that chaperones, the ubiquitin proteasome system (UPS) (Mishra et al., 2019), and lysosome-based autophagic process are physiologically linked (Joshi et al., 2020), the adequate clearance of disease-associated aggregated proteins is still a therapeutic challenge. A search of PQC modulators that can accelerate the removal of disease-associated aggregates may serve as a critical regulatory factor for the re-establishment of defective proteostasis (Plate et al., 2016).

In certain cases, aggregated polypeptides are seen to form regular structures that tend to clutch together to form small slender fibrils of large insoluble unfolded protein aggregates called amyloid fibrils. These fibrils are widely studied and categorized as one of the significant causative functions of neurodegeneration (Rambaran and Serpell, 2008). One such example causing Alzheimer's disease is the synthesis of amyloid β peptide through sequential processing of membrane-associated Amyloid precursor protein (APP) (Chen et al., 2017). These structures are large making it difficult to target by the intracellular degradation machinery like proteasome and lysosome, worsening the condition. In order to circumvent such proteotoxic problems, several plant derived natural compounds have demonstrated promising outcomes.

Furthermore, the proteolytic apparatus are found to be overburdened during degradation of misfolded-ubiquitin positive inclusions by dysfunctional proteasome machinery (Thibaut et al., 2018). The massive build-up of oligomers or protein aggregation can impair proteasome functions (Tseng et al., 2008). This could be possible because the over accumulated mutant proteins may block the proteasome-dependent degradation (Verhoef et al., 2002); therefore, search for molecules, which can induce depleted activities of the proteasome and help in re-establishing the proteostasis balance is essential for overall cellular viability, under stress conditions. The major challenge is to identify the modulators that can target such highly conserved eukaryotic proteolytic machinery, help in the removal of aberrant proteins, and re-establish cellular health.

1.2 CONCISE REPORT ON MAJOR OUTCOMES AND FUTURE PROSPECTS

The present study aims to identify and characterize a compound that can induce proteasome functions and demonstrate the anti-aggregation capacity for effective abnormal protein degradation without disturbing cellular homeostasis. We report the use of itraconazole, a well-known antifungal compound, as a proteasome activator. The concentration and time manner effect of itraconazole was able to enhance chymotrypsin-like and post-glutamyl peptidyl hydrolase-like functions of proteasome without any significant cytotoxicity. Furthermore, the enhancement of proteasome function by itraconazole was able to improve the clearance of several known neurodegenerative and modeled misfolded protein. The cumulative effect of itraconazole on cytoprotection was also checked under several known proteotoxic stressors. Moreover, our current findings also suggest that resveratrol, a stilbenoid belonging to

a natural phenol group, may alter the PQC mechanism through specific modulations in the levels of LRSAM1 E3 enzyme associated with spongiform neurodegeneration.

We have found for the first time that LRSAM1 can collectively improve the functioning of cellular proteasome. The resveratrol mediated induction of LRSAM1 enhanced the removal of aberrant polypeptides as well improve cellular proteostasis potential. An increased understanding of specific elimination of damaged or old proteins via selective activation of PQC mechanism may open new therapeutic interventions and effective treatment against protein conformational disorders such as neurodegeneration and imperfect aging. The results portrayed in this work mutually implicate new possibilities to enhance the disintegration of aberrant proteins via the treatment of itraconazole or resveratrol and most likely open novel promising therapeutic insights for neurodegeneration and imperfect aging-associated disorders. Furthermore, increasing the fundamental understanding of cellular mechanisms involved in processing misfolded protein aggregates can help define newer and better cellular therapeutic targets against such class of disorders.

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