2
Review of Literature

2.1 INTRODUCTION TO PROTEIN QUALITY CONTROL

Proteins are essential macromolecules, accounting for both structure and function of cells; considering the wide array of their functions, a healthy proteome is vital for maintaining cellular health (LaPelusa and Kaushik, 2022). Newly synthesized proteins require to undergo native folding for achieving their functionally active three-dimensional structure. However, under various cellular stress conditions, including mutations, proteins often misfold, which may cause their functional loss or gaining of detrimental set of functions, inducing toxic aggregate formations (Dobson, 2003). Protein aggregates are reported to be linked with many pathological conditions, like neurodegenerative diseases (NDDs), cancer, and lysosomal storage diseases (Yang-Hartwich et al., 2015;Sweeney et al., 2017;Monaco and Fraldi, 2020). To counter such pathological conditions, cells possess protein quality control (PQC) pathways - chaperones, UPS, and autophagy (Chen et al., 2011). These multiple branches of PQC work in harmony to achieve dynamic but steady proteostatic conditions (Figure 2.1). Chaperones, generally act as the earliest responders to identify misfolded proteins and encourage their refolding; upon failure to achieve native folding, degradation via UPS or autophagy can be promoted in a chaperone-dependent manner (Hartl et al., 2011a). Most of the short-lived and small aberrant proteins in cell are targeted for proteolytic degradation by UPS (Kleiger and Mayor, 2014).

On the other hand, lysosome-autophagy system generally targets large misfolded protein aggregates or damaged cellular organelles for degradation (Bento et al., 2016). These PQC pathways help maintain a healthy proteome, essential for cellular homeostasis. However, with aging, ability of PQC pathways to maintain proteostasis declines, leading to toxic malfolded protein accumulation inside cell (Taylor and Dillin, 2011). The impairment of PQC pathways is one of the critical elements in pathology of NDDs like Amyotrophic Lateral Sclerosis (ALS), Parkinson's disease (PD), Huntington's disease (HD), and Alzheimer's disease (AD) (Ruz et al., 2020). Considering its interplay with compromised PQC pathways, aging is regarded as prime risk factor for NDDs (Wyss-Coray, 2016). NDDs are reported in many older aged people, signifying the importance of declined PQC pathways as a key characteristic feature of aging (Hou et al., 2019). The question that "is aging a disease" and whether we have to address it as any other disease persists from ancient times (Franceschi et al., 2018). Investigating aging and developing anti-aging therapeutics encompass multiple domains of biological sciences.

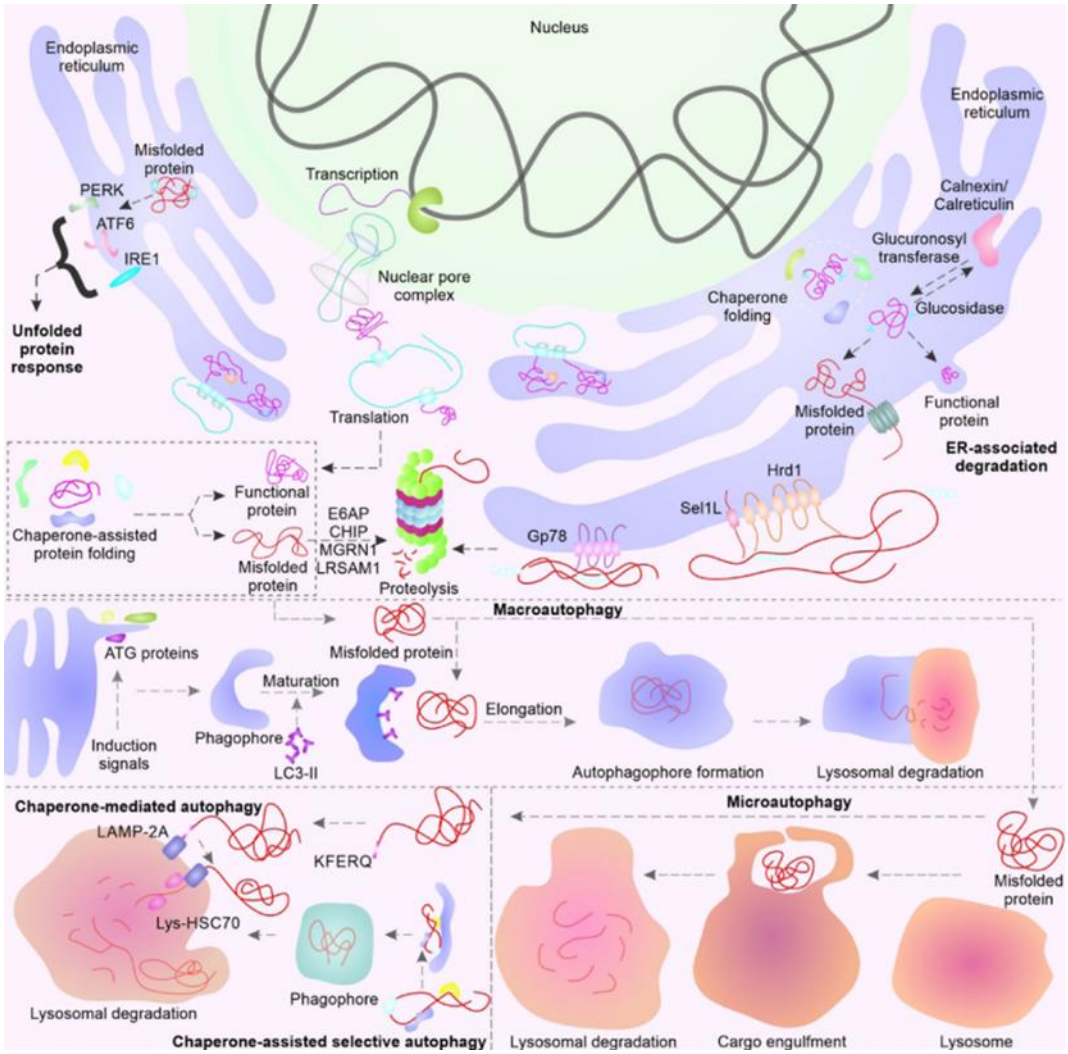


Figure 2.1: Major PQC mechanisms that help to maintain the proteostasis network in the cell: Often cancer cells are found to possess enhanced PQC mechanisms. Some of the quality control mechanisms like ERAD, UPR are represented. Besides, ER also involves the calnexin-calreticulin cycle to monitor the status of protein folding in the ER lumen. The major cytosolic protein quality is maintained by the chaperones, proteasome pathway and autophagy. (*Source: Prashant Kumar and Amit Mishra* et al. (2021), Journal of Cellular Physiology)*

2.2 THE MAJOR COMPONENTS OF PROTEIN QUALITY CONTROL SYSTEM IN CELLS

A prolonged healthy life is based on the optimal activity of an organism's organ systems, and healthy cells are at the core of this proper functioning. Cellular homeostasis is of utmost importance, and a cell deploys several cytoprotective mechanisms to maintain this balance. One such mechanism is PQC to preserve proteostasis and maintain functionality of proteins. In PQC, the chaperone system and proteolytic pathways like autophagy and UPS are primary cell devices preventing misfolding/aggregation of proteins and clearing out toxic protein aggregates and dysfunctional organelles. Unfolded protein response (UPR) is a stress response system linked with ER, which also regulates cell fate (Hetz and Papa, 2018). Aging is an unavoidable biological phenomenon observed in many organisms that negatively affect the functionality of multiple organs systems, thus reducing the life span. It constitutes a significant risk factor for impairment of PQC elements and proteostasis disruption, linked with physiological dysfunction of organelles along with other anomalies like cancer and neurodegeneration. Aging presents various medicinal challenges as it affects multiple physiological processes at once. In aging, declined PQC capacity can lead to increased incidence of several age-associated diseases, including neurodegenerative disorders. Proper maintenance and modulation of these PQC elements present an attractive therapeutic intervention

opportunity for such disorders. PQC and its components as a system affected in imperfect aging, its potential for modulation to improve healthspan and counter aging associated disorders, along with challenges linked with inherent complex nature of aging biology.

2.2.1 Molecular Chaperones: Chaperones – A Critical Component of Protein Quality Control

A loss of protein's native structure, failure of its attainment, and exposed hydrophobic amino acids make them prone to aggregate formation (Chiti and Dobson, 2006). Protein aggregation and inclusion formation in different brain regions has long been associated with neurodegeneration (Ross and Poirier, 2004). Chaperones are one of the critical elements of cellular proteostasis network and are required from the earliest stages of a protein's life – *de novo* folding upon translation, refolding upon misfolding stress, and directing them to degradation pathways upon terminal misfolding. These proteins, heat shock proteins (Hsps), are primarily categorized into chaperone families – Hsp70, Hsp60, Hsp40, Hsp90, Hsp110, and small chaperones (Vos et al., 2008). Chaperone dimers NAC and RAC associate with ribosomes and emerging nascent protein from exit tunnel of ribosomes, preventing their premature folding (Preissler and Deuerling, 2012). Proteins that are unable to gain native folding are acted upon next by cytosolic chaperones, which prevent any unwanted interactions of these proteins, aiding their folding.

Hsp70 binds to non-native proteins via their exposed hydrophobic amino acids and attempts to refold them in ATP-dependent fashion with bind-and-release cycles (Clerico et al., 2015). The J domain-consisting Hsp40 bind with Hsp70 and increase ATP hydrolysis on it, promoting closed Hsp70-ADP state with high affinity for client (Rosenzweig et al., 2019). Hsp110, a NEF co-chaperone for Hsp70, promotes its ADP-ATP exchange, resulting in release of client due to reduced affinity (Dragovic et al., 2006;Andreasson et al., 2008;Polier et al., 2008). A portion of cellular proteins to be folded are transferred to TRiC/CCT chaperonin from ribosome or from Hsp70/40 complex. It is a multiprotein complex consisting of 2 rings with 8 subunits each, which provide a cage-like environment and undergo conformational changes for folding of protein (Jin et al., 2019). Certain proteins close to their native state are transferred to homodimer of Hsp90 from Hsp70/40 via Hop co-chaperone, which can bind both Hsp70 and 90 via its TPR domains (Scheufler et al., 2000). Generally, Hsp90 action aids in activation, assembly, and facilitating ligand binding of client proteins via promoting folding by undergoing changes in conformation in an ATP-dependent fashion (Schopf et al., 2017). Furthermore, proteins which fail to achieve native folding or are aggregation-prone are directed by chaperones to degradation pathways. For instance, Hsp70 in association with CHIP E3 ligase can guide misfolded substrate proteins to degradation via UPS or autophagy (Joshi et al., 2016).

2.2.2 Chaperones Can Provide Protection Against Neurodegeneration

Decrease in proteostasis capacity is also linked with neurodegenerative conditions. Simultaneously, the generation of misfolded proteins and aggregation sustains pressure on PQC machinery, ultimately overwhelming it. Additionally, sequestration of chaperones by aggregates of misfolded proteins is a well-known feature among neurodegenerative conditions that reduce the chaperone availability for their substrates in multiple cellular pathways (Sinnige et al., 2020). AD and PD are two of the most prevalent NDDs, primarily characterised by amyloid β fibrils/neurofibrillary tangles and α-synuclein-consisting Lewy bodies in brain, respectively (Nussbaum and Ellis, 2003). Many chaperones, including Hsp70, Hsp40, and Hsp90 are reported to be sequestered by protein inclusions in AD (tau aggregates) and PD (α-synuclein inclusions), possibly affecting proteostasis to the neuronal detriment (Petrucelli et al., 2004;Uryu et al., 2006;Leverenz et al., 2007;Yu et al., 2019). Hsp70 and CHIP are involved in increased clearance of tau, whereas Hsp90 can aid in refolding (Koren et al., 2009). α-synuclein aggregates in PD can promote the proteasome-mediated degradation of HSF1, reducing the chaperone expression, possibly contributing to disease pathogenesis (Kim et al., 2016). Small Hsps αB-crystallin can hamper fibril elongation of α-synuclein and amyloid β, while Hsp22 and

Hsp27 can prevent aggregate formation of α-synuclein (Waudby et al., 2010;Bruinsma et al., 2011;Shammas et al., 2011) (Figure 2.2).

ALS is a motor neuron condition exhibiting heterogenous pathological mechanisms involving multiple aberrant proteins, such as mutant SOD1, TDP43, and C9orf72 (Hardiman et al., 2017). Hsp70, PDIA, and CCS chaperones along with other PQC components, like p62 are reported to be sequestered with ALS-linked protein inclusions (Ciryam et al., 2017). This can compromise protein folding and stress-responsive functions of chaperones along with degradation capability of PQC in motor neurons. Chaperones are also reported to provide protection to motor neurons against ALS-associated pathogenic proteins like SOD1 by inhibiting their misfolding (e.g., MIF) and aggregation (e.g., Hsp27, αB-crystallin) or improving their clearance via autophagy (e.g., Hsc70, Hsp22) (Crippa et al., 2010;Yerbury et al., 2013;Leyton-Jaimes et al., 2019). Chaperone mutants and variants, like of DJ-1 and DNAJC7 are linked with possible pathogenesis of PD and ALS, respectively (Bonifati et al., 2003;Farhan et al., 2019). Such studies indicate the potential of chaperones as therapeutic targets for various NDDs, given their capability to provide cellular protection through different mechanisms.

HD affects motor as well as cognitive functions with expanded polyglutamine repeats in huntingtin (Htt) protein forming aggregates and inclusions in neuronal cytoplasm and nucleus (Labbadia and Morimoto, 2013). Mutant Htt possibly sequesters Hsp70 chaperones, which can be a critical factor in HD pathogenesis (Lotz et al., 2010). Moreover, decreased HSF1 stability and NF-Y- and HSF1-mediated chaperone expression in HD animal models is observed (Yamanaka et al., 2008;Labbadia et al., 2011;Gomez-Pastor et al., 2017). Interestingly, HSF1 activation is associated with decreased load of aggregation and improved symptoms in HD mice model. Similarly, spinocerebellar ataxia (SCA) polyglutamine disorders are characterised by loss of motor coordination and intranuclear inclusions of expanded polyglutamine ataxins (Klockgether et al., 2019). Chaperones Hsp70, Hsp40, TRiC, and CHIP are reported to suppress aggregation and toxicity of mHtt and expanded polyglutamine ataxins; increased levels of Hsp70 even improved motor phenotype in SCA mice model (Jana et al., 2000;Cummings et al., 2001;Jana et al., 2005;Shahmoradian et al., 2013). The entrapment of chaperones in misfolded protein inclusions, transcriptional dysregulation, and even mutations can impair their functions and proteostasis with aging. Increasing the chaperone capacity of cell via different means is being investigated as potential therapeutic strategy, such as HSF1 activation, chemical chaperones, and others.

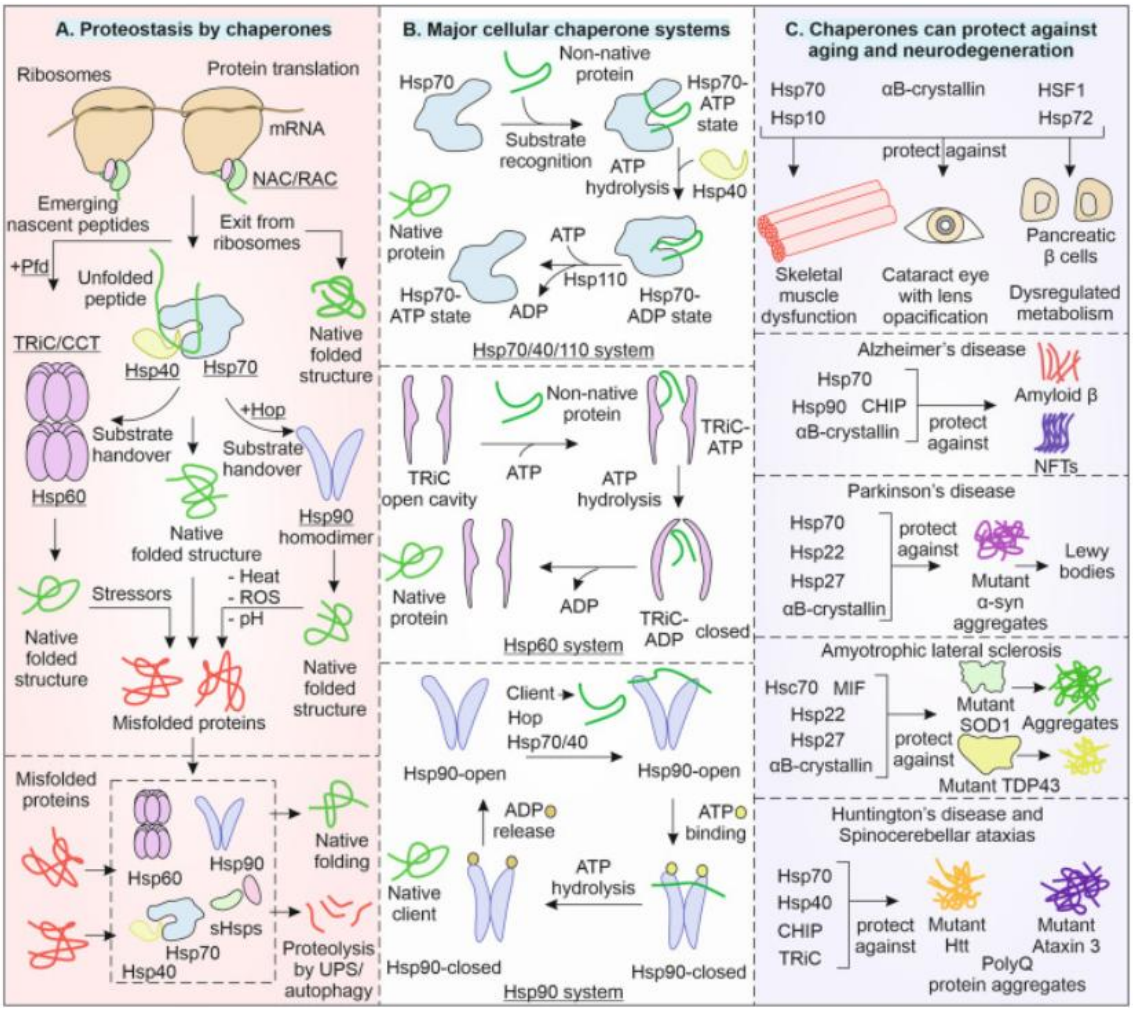


Figure 2.2: Involvement of chaperones in PQC: A) Chaperones facilitate de novo folding of multiple proteins from the time of translation. Chaperones also respond to misfolding of proteins in the presence of stressors by promoting refolding or clearance. B) Hsp70 (with Hsp40/110), TRiC chaperonin, and Hsp90 undergo conformational changes and promote protein folding in an ATP-dependent fashion. C) Different chaperones protect against aging associated diseases, including neurodegenerative conditions, by maintaining proteostasis. *(Source: Prashant Kumar and Amit Mishra* et al. (2023), Translational Medicine of Aging)*

2.2.3 Targeting Protein Clearance: Ubiquitin-Proteasome System

UPS removes proteins that have completed their lifespan or failed to attain chaperone-mediated folding (Amm et al., 2014a). UPS works in a two-step manner whereby target protein is initially labelled with a chain of highly conserved small protein ubiquitin, after which it is degraded in 26S proteasome-dependent fashion (Hershko and Ciechanover, 1998). A sequence of reactions, including E1, E2, and E3 enzymes, process polyubiquitin tag on client protein and promotes its identification and clearance by 26S proteasome (Li and Ye, 2008;Komander and Rape, 2012). The E1 enzyme adenylates ubiquitin for its activation by utilizing ATP (Schulman and Harper, 2009). Upon activation, ubiquitin is transferred from E2 enzyme (ubiquitin-conjugating), to substrate linked with E3 enzyme (ubiquitin ligase) (Ye and Rape, 2009;Metzger et al., 2012).

Based on domain architecture, E3 ligases can be RING (most common), HECT, and RBR. For RING E3s, ubiquitin is translocated from E2 to substrate in a direct manner, whereas for RBR and HECT E3s, ubiquitin is translocated from E2, to E3 first and afterwards to substrate (Morreale and Walden, 2016). The proteasome complex possesses multiple catalytic subunits with protease activity and is formed of 19S RP and 20S CP (Tanaka, 2009). 20S complex, having barrel-like structure, comprises four rings (α β β α) arranged in a stack, where some of the β subunits possess varying proteolytic activities. The 19S complex acts as a cap on the 20S and is

critical as it binds and translocates ubiquitinylated substrates to 20S complex (Mishra et al., 2018).

2.2.4 Ubiquitin-Proteasome System Impairment in Neurodegenerative Disorders

The altered activity of UPS elements is one of the most prominent features of many NDDs. In conditions like AD, PD, ALS, and HD, impaired activities of multiple E3s and proteasomes are noted. The reduced level of Ube3A/E6-AP E3 ligase in AD mice model hippocampus is associated with the loss of dendritic spines and various behavioural impairments (Olabarria et al., 2019). An ER-localized E3 enzyme, HRD1, regulates the removal of ER-resident aberrantly folded proteins through ER-associated degradation (ERAD). A decline in HRD1 levels in AD patient's cerebral cortex hampers the clearance of its substrate amyloid precursor protein (APP), increasing amyloid β levels (Kaneko et al., 2010). Previously, we have showed involvement of different E3 ligases like E6-AP, ITCH1, and MGRN1 in targeting the neurodegenerative diseases-associated proteins for their clearance (Upadhyay et al., 2017). Increasing the levels of such E3 enzymes can possibly alleviate disease pathomechanisms at phenotypic as well as molecular levels, making them attractive targets for NDDs. For instance, natural compounds resveratrol and myricetin are linked with increased LRSAM1 and E6-AP E3 enzyme levels as well as decreased misfolded protein aggregation (Joshi et al., 2019a;Dubey et al., 2022).

Moreover, mutations that alter the function of E3 enzymes have also been detected in various NDDs. For instance, mutations in *PARK2*, which codes for parkin E3 enzyme, are associated with its impaired activity and PD pathogenesis (Kitada et al., 1998). In addition to E3 enzymes, deubiquitinating enzymes (DUBs) can also be involved in NDD-linked pathomechanisms, possibly involving protein aggregation and degradation. A functional mutation in the *UCHL1*, associated with reduced UCHL1 (DUB) activity, is observed in familial form of PD (Leroy et al., 1998). The polyglutamine (polyQ) extension in ataxin-3 (DUB) results in an increased autophagic clearance of its substrate Parkin, contributing to the pathomechanism of Machado-Joseph disease (MJD) (Durcan et al., 2011). Additionally, oligomers of malfolded proteins from AD, PD, and HD are reported to inhibit proteasome activity, thereby impeding the ubiquitin-dependent and -independent proteolytic activity of proteasome (Thibaudeau et al., 2018). Investigating the modulators of the proteasome activity for improved clearance of misfolded protein can also be an important therapeutic approach. Recently, our group has shown Itraconazole to induce proteasome proteolytic activities, leading to efficient elimination of misfolded proteins associated with NDDs (Dubey et al., 2023).

2.2.5 Autophagy at Work: Clearing Cellular Junk

Autophagy is a multi-step phenomenon where cellular constituents, including proteins, and damaged organelles, are transferred to vacuole/lysosome for their clearance and salvaging (Yamamoto et al., 2023). Deficiency of essential nutrients, like amino acids and carbon can trigger autophagy (Takeshige et al., 1992). Autophagy is vital in maintaining cellular homeostasis during normal physiological conditions by clearing aberrant aggregated proteins and damaged organelles and degrading invading pathogens (Lamark and Johansen, 2012;Anding and Baehrecke, 2017;Sharma et al., 2018). Based on mechanics of delivery of cargo, the three significant autophagy types observed are macroautophagy, chaperone mediated autophagy (CMA), and microautophagy (Mizushima et al., 2008). Besides them, two other selective forms of autophagy are also reported for removal of misfolded and aberrant proteins that are chaperone-assisted selective autophagy (CASA) and endosomal microautophagy (eMI) (Arndt et al., 2010;Chauhan et al., 2019). Macroautophagy is distinguished as a catabolic mechanism where the cytoplasmic constituents in bulk are sequestered in a double membraned vesicle termed autophagosomes and subsequently fused to lysosomes for its removal and recycling (Feng et al., 2014). CASA is utilized to clear the damaged proteins and organelles, initially described for maintenance of protein homeostasis in muscle cells (Arndt et al., 2010). The CASA involves heteromeric complex formation with involvement of chaperones (Hsc70 and Hsp22), co-chaperone (BAG3), and E3 enzyme (CHIP) (Tedesco et al., 2023) (Figure 2.3).

In microautophagy, the cargo is seized via direct invagination of lysosome/vacuole membrane (Li et al., 2012). Similarly, eMI is executed by ESCRT complex where the substrate carries pentapeptide KFERQ-like sequence identified by Hsc70, later delivered to endosomes (Yim and Mizushima, 2020). CMA observed in mammalian cells is selective autophagy targeting cytosolic protein (Cuervo, 2011). In CMA, the cargo bearing a KFERQ sequence is recognized by a chaperone in cytosol (Hsc70), which, together with other co-chaperones, brings the cargo to the surface of lysosome. The Hsc70 transfers the cargo to LAMP2A (lysosomal membrane protein and CMA receptor), leading to cargo protein unfolding. Next, LAMP2A stabilisation and organization into multimeric complex requires lys-Hsp90 and substrate translocation requires lys-Hsc70 (Kaushik and Cuervo, 2012). Autophagy components work in a coherent harmony for maintaining homeostasis. Any disturbance in autophagic machinery causes an additional stress on other cellular processes to maintain proteostasis, eventually disrupting cellular functions, which get manifested later as the symptomatic failure of organs or organ systems

2.2.6 Significant Attention on Tumorigenesis Suppression Via Autophagy Pathway

The evidences show autophagy is engaged in cell homeostasis maintenance and recycling of nutrient metabolites (Sridhar et al., 2012;Boya et al., 2013;Galluzzi et al., 2014;Jiang et al., 2015). The autophagy function in cancer is reported to be multifaceted as it is engaged in both death and progression of carcinogenic cells (White, 2015). In the initial stages of cancer during oncogenesis and malignant transformation, the basal autophagy works as a cellular housekeeper. It helps in maintaining the genome stability, removes the damaged organelles, and provides the constituents for different mechanisms by degrading macromolecules (Singh et al., 2009;Singh and Cuervo, 2011). In the case of an established tumor, autophagy act as a pro-survival and protective system under stress and promotes the tumor survival in conditions like nutrition deprivation and hypoxia, growth factor scarcity, ischemia, and in the presence of chemotherapeutic agents (Kuma et al., 2004;Lum et al., 2005;Degenhardt et al., 2006;Karantza-Wadsworth et al., 2007;Sui et al., 2013). Autophagy can provide resistance against many chemotherapeutic strategies (Sui et al., 2013). Excessive autophagy can result in autophagic cell death. Autophagy also regulates several cellular processes and cell death pathways, like apoptosis. Therefore, an in-depth knowledge of the autophagy process and its regulating molecules is critically important (Kumar et al., 2025). In many therapeutic strategies, a chemotherapy combination with inhibitors of autophagy can increase the efficacy of therapy, so autophagic modulators and pathways are promising therapeutic targets.

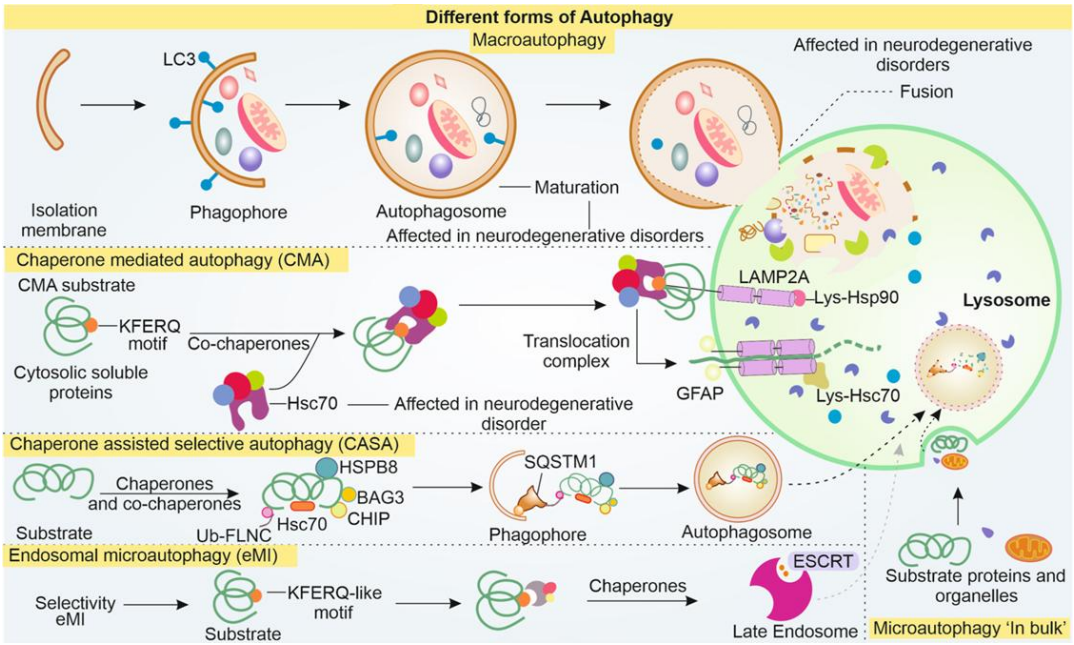


Figure 2.3: Overview of autophagy: Macroautophagy: A double membraned autophagophore is formed to sequester the cytoplasmic content and fuse to lysosome for degrading cargo. Chaperone-assisted selective autophagy (CASA): A particular type of degradation where chaperones like Hsp70 identifies and sequester cargo with the aid of the p62 of autophagosome and cargo is degraded upon lysosomal fusion. Chaperone mediated autophagy (CMA): Hsc70 with other co-chaperones recognises CMA substrate with pentapeptide signal KFERQ-like sequences and associates with LAMP2A on membranes of lysosomes. Protein transport to lumen needs lysosomal Hsc70. Microautophagy and endosomal microautophagy: In microautophagy, the lysosomal membrane invaginates to internalise organelles and proteins. Endosomal microautophagy (eMI) selectively degrades proteins and organelles.

2.3 IMPORTANCE OF MODULATION OF PROTEIN QUALITY CONTROL MECHANISM

Aging presents itself as a condition that, if improper, can increase the chances of occurrence of several diseases. The proper care and management during aging can reduce the risk factor for these diseases; for example, NDDs are often linked with aging, but its early onset detection and management can reduce its effect in later stages of life (Wyss-Coray, 2016;Perneczky, 2019). Aging biology also presents several medicinal challenges as the origin of age-associated diseases as well as their effects involve multiple systems and are heterogeneous in nature. Accordingly, many of the existing therapeutic strategies intervenes on the basis of symptoms and are not disease-modifying in nature (Frozza et al., 2018). Moreover, late-stage interventions probably bring less improvement in quality of life and amelioration of disease pathomechanisms as many of the body's physiological systems (e.g., immune system) are already in decline with aging. To solve such challenges, through research investigation into validating more reliable aging associated targets and biomarkers is needed.

Novel therapeutic approaches need to be developed for management and treatment of aging-associated diseases. For instance, the multi-systemic nature of aging can be targeted with improved efficiency via combination therapies. Still, other challenges exist for higher treatment efficacies, such as requirement to cross blood brain barrier for molecules targeting NDDs, minimising off-target effects, requirement of models for more accurate disease representation, and addressing the gap for clinical translation of drugs, possibly via drug repurposing. Exploring different natural compounds for modulation of PQC pathways targeting NDDs and other aging-related conditions is also an attractive option as they represent a large diversity of chemical classes. Furthermore, widening the age of participation for clinical trials can be helpful in developing most suitable therapies for this particular age group for better prognosis and

quality of life with lesser side effects (Shenoy and Harugeri, 2015). The future approach should involve development of more personalized medicine, better institutional infrastructure for management of diseases, lower cost of treatment, and deeper understanding of aging biology.

Furthermore, owing to its multi-responsive nature towards diverse stress stimuli, autophagy and UPS becomes an attractive therapeutic target. A unique role of mitochondria has emerged in cancer due to its critical bioenergetic nature and a potential cause of DNA damage. Investigating diverse factors that assist in releasing apoptosis regulator cytochrome *c* has illustrated the complex control of cell death. In cancer cells, mitochondrial lipids have proven to be essential in mitophagy and apoptosis. Besides, the function of mitochondrial DNA mutation is still absurd in the cancer scenario and being an essential player in PQC. The PQC components that aid in mitochondrial health include TOM, UBXD8, p97, LON, and ClpXP are all significantly upregulated in cancer conditions. In totality, the PQC elements in cancer conditions is highly expressed and can be considered as the driving force towards oncogenicity and aiding their survival.

2.4 TARGETING OF PROTEIN QUALITY CONTROL MECHANISM

The proteostasis is very crucial for proper cellular health, and in this context, role of PQC machinery is vital. Defective PQC mechanisms are feature of various NDDs; protein aggregation is the common feature of these diseases, research into PQC activators is rapidly picking pace. Contrarily, enhanced dependence of cancer cells on network of proteostasis also places inhibitors of PQC elements, like proteasomes, into focus as a potentially useful class of therapeutic interventions. Some natural compounds and NSAIDs can be explored for their modulation effect on proteasome activity.

2.4.1 Trehalose- A Natural Sugar May Potentially Affect Proteasome Activity

Trehalose (a non-reducing disaccharide) exists in many life forms, such as plants, invertebrates, yeast, and bacteria, where it acts as carbon and energy source as well as cytoprotective and stress-responsive signaling molecule (Elbein et al., 2003). It is a cellular protectant against various stress, such as osmotic, oxidative, and proteotoxic stressors. Trehalose prevents in yeast heat-denatured proteins aggregation, acting in the capacity of a chemical chaperone while keeping them in a chaperone-mediated re-foldable state (Singer and Lindquist, 1998). Many reports have suggested Trehalose modulates different branches of PQC and acts as a promising neuroprotectant. Trehalose oral administration in Lewy body disease mice model modulates protein aggregation, possibly via induced autophagy and chaperones, such as Hsp90 (Tanji et al., 2015). The neuroprotective effect of Trehalose by alleviating misfolded protein and aggregation stress is studies in multiple NDD animal models, such as transgenic rodents with AD, ALS, HD, and PD (Tanaka et al., 2004;Castillo et al., 2013;Du et al., 2013;He et al., 2016). Trehalose is also determined to activate TFEB and increase translocation to nucleus of FOXO1 transcription factors of lysosome and autophagy genes (Palmieri et al., 2017;Rusmini et al., 2019). Interestingly, Trehalose prevents protein aggregation via stabilizing Proteasome, another branch of PQC, in response to ischemia (Li et al., 2017). The effect of Trehalose on clearing neurodegeneration-associated pathogenic proteins via UPS is not investigated. Our earlier studies reported that PQC modulators could enhance misfolded protein clearance [28-30]. In our current study, we observe that using Trehalose elevates Proteasome activities, which effectively remove the aggregation-prone proteins from the cells and alleviate the cytotoxic potential of aberrant protein accumulation.

2.4.2 Ketorolac – An NSAID with Potential to Modulate Proteasomal Activity

NSAIDs are majorly consumed as prescription pharmaceuticals and, in certain instances, as a medication to reduce inflammation, discomfort, and fever (Peterson et al., 2011). Inhibition of cyclooxygenases (COXs), enzymes that regulate the conversion to prostanoids from

arachidonic acid, active lipids that control the inflammation response, is the mechanism by which NSAIDs exert their pharmacological effect (Vane, 1971;Weissmann, 1993;Ghlichloo and Gerriets, 2019). The reactive oxygen species (ROS) generated by NSAIDs may elicit damage to proteins, which can have different repercussions for the proteasome. Oxidation of proteasome subunits might consequently lead to suppression of the proteasome; however, increasing numbers of oxidised proteins may also overwhelm the proteasome, leading to malfunction (Brandolini et al., 2020). Apart from this, inhibition of proteasome is linked to cell death; one of the responsible mechanisms is NF-kB pathway inhibition (Nunes and Annunziata, 2017). NSAIDs like indomethacin can induce mitochondrial apoptosis (Amanullah et al., 2018). In myeloma cells, proteasomes inhibition is connected with inducing apoptosis via ER stress (Obeng et al., 2006).

For the management and therapy of acute pain that ranges from mild to severe, Ketorolac is an FDA-approved drug. It inhibits both COX enzymes, though a few studies have shown it to have higher selectivity for COX-1 than COX-2 (Waterbury et al., 2006;Mahmoodi et al., 2024). The tromethamine salt of Ketorolac improves its water solubility, making its administration by both oral and parenteral routes possible. Ketorolac is a derivative of pyrrolizine carboxylic acid (Amul et al., 2020) and like indomethacin, belongs to acetic acid derivatives family of NSAIDs. Evidence suggests that in triple negative patients, Ketorolac may decrease the risk of an early relapse of breast cancer (Retsky et al., 2012). Ketorolac is reported to successfully trigger cancer cells death via suppressing expression of and interacting with DDX3, an inflammatory marker, in oral cancer cell lines; it also upregulates Par-4 (tumor suppressor) moderates Rac-1/HIF-1α/DDX3/β-catenin pathway in renal cell carcinoma (Samal et al., 2015;Sonawane et al., 2023). Ketorolac also reportedly induces apoptosis in rat osteosarcoma cells (Zuckerman et al., 2019). It is not fully known how NSAIDs play a part in proteasome failure leading to apoptosis.

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