2
Review of Literature

2.1 INTRODUCTION TO CELLULAR PROTEOSTASIS

Proteostasis is essential for normal function of proteins and vital for cellular health and survival. Proteostasis encompasses all stages in the "life" of a protein, that is, from translation to functional performance and, ultimately, the degradation. Proteins, generally, need native conformations for function and in the presence of multiple types of stress, their misfolding and aggregation can occur. The diversity among the types of proteins in cells, molecular crowded atmosphere (proteins and other biomolecules), wide range of biomolecular interactions, and continuous presence or risk of a variety of stressors (external or internal) necessitate the requirement of a system dedicated to proteostasis. Molecular chaperone proteins are primary players for folding new polypeptides during their translation at ribosomes, prevention of unwanted protein-protein interactions and misfolding/aggregation, maintaining solubility of proteins, solubilising aggregated proteins, and guiding the terminal misfolded or aggregated proteins to proteolysis [Hartl et al., 2011]. UPS and autophagy are predominant methods of proteolysis in cells. Many short-lived and small proteins that have completed their cellular functional roles or are aberrantly folded are linked to small protein ubiquitin so as to be degraded by a multi-subunit proteolysis machinery called 26S proteasome [Kleiger and Mayor, 2014]. Large protein aggregates, long lived proteins, and organelles undergo degradation by lysosome-dependent autophagy system [Bento et al., 2016]. These mechanisms contribute to the PQC in cells. Many cellular stressors, like heat stress, oxidative stress, and specific mutations can make the proteins more vulnerable to misfolding and aggregation. Certain proteins like Fused in sarcoma (encoded by *FUS*), TAR DNA-binding protein 43 (TDP-43; encoded by *TARDBP*), superoxide dismutase 1 (encoded by *SOD1*), α-synuclein (encoded by *SNCA*), cystic fibrosis transmembrane conductance regulator (encoded by *CFTR*), tau (encoded by *MAPT*), and huntingtin (encoded by *HTT*) can become susceptible to aberrant folding and aggregation in the presence of specific mutations. These misfolded and aggregated proteins are linked with different diseases that can involve their loss-of-function and gain-of-function [Valastyan and Lindquist, 2014]. Additionally, misfolding of a few proteins can also lead to misfolding and sequestration of other metastable proteins (with different critical functions) globally in the cells [Gidalevitz et al., 2006; Olzscha et al., 2011].

2.2 PROTEOSTASIS IN CELLS DURING PROTEIN TRANSLATION

The cellular proteostasis network constitutes of regulators at levels of transcriptional, translational, and post-translational to maintain the protein conformation, concentration, and location, which is essential for healthy proteome, cells, and organism [Balch et al., 2008]. At the time of translation of many mRNA molecules, specific chaperones are known to associate with ribosomes to guide folding of nascent polypeptides. Two components of PQC have been proposed to work at this stage – co-translational QC and ribosome-associated QC (RQC) [Brandman and Hegde, 2016] (Figure 2.1). The co-translational quality control mechanisms are possibly involved in sensing the nascent polypeptides folding status, influencing their fate. There are specific ribosome-associated chaperones dedicated for folding nascent peptide chains. The nascent polypeptide-associated complex (NAC) is a eukaryotic heterodimeric chaperone consisting of αNAC and βNAC, both of which have NAC domains required for dimerization [Wiedmann et al., 1994].

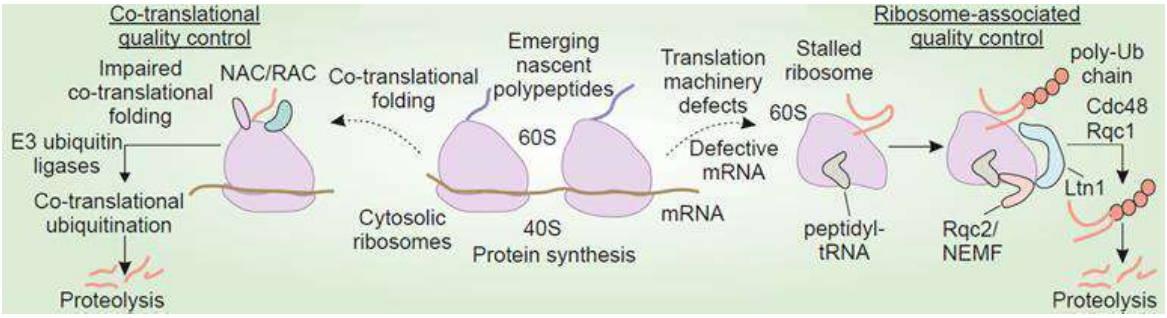

Figure 2.1: Proteostasis at the level of translation: It involves ubiquitination-dependent proteolysis of nascent polypeptides. Upon disturbance in the co-translational folding of nascent polypeptides, ubiquitination by E3 enzymes can lead to degradation, which can be referred to as co-translational QC. In the case of defects with translation machinery or mRNA, ribosome-associated QC via RQC machinery can lead to ubiquitination and proteolysis. (*Source: Sumit Kinger and Amit Mishra* et al. (2024), Advances in Clinical Chemistry*)

Ribosome-associated complex or RAC chaperone complex acts with Ssb (yeast Hsp70; can bind to ribosomes) [Preissler and Deuerling, 2012]. Ssb recruitment to ribosomes for co-translational folding of nascent polypeptides is dependent on certain factors, including the presence of intrinsic disorder, β-sheet rich regions, and hydrophobic sequences in the clients; in order to prevent aggregation in such scenario, Hsp70 co-translational function is critically important [Willmund et al., 2013]. NAC and RAC/Hsp70 play role in folding new polypeptides co-translationally and are critical for proteostasis. Recently, NAC is reported to interact with cytosolic protein aggregates, thus, shifting away from ribosomes during proteotoxic stress, thereby slowing translation [Kirstein-Miles et al., 2013]. Upon resolution of this stress, NAC can resume its role in translation, though chronic proteotoxicity can hamper this. The PQC mechanisms in cytosol can also target nascent chains for proteasomal degradation through ubiquitination upon impairment with co-translational folding [Duttler et al., 2013]. The E3 ubiquitin ligases Hel2, Doa10, Hrd1, and Hul5 are possibly associated with ubiquitination of nascent polypeptide chains unable to fold properly co-translationally whereas Ltn1 ubiquitinates translated products of damaged mRNAs. Depletion of NAC is associated with increased ubiquitination of nascent polypeptides, indicating its role in providing opportunity to emerging nascent polypeptides for folding. Co-translational quality control mechanisms can therefore, target nascent peptides during translation and affect their fate.

The second major mechanism RQC can be initiated in various scenarios, such as non-stop mRNAs (absence of termination codon) or no-go mRNAs, which can have ribosome stalling sequences, leading to impaired mRNA codon recognition at ribosomes [Joazeiro, 2017]. Stalling of ribosomes can occur through premature mRNA polyadenylation, which upon translation can form polypeptides with poly(lysine) chains at their C-terminal, possibly via impaired electrostatic interactions. RQC machinery recognised in yeast include Ltn1 (ribosome-interacting E3 ubiquitin ligase), Rqc2/NEMF (nucleotide-binding protein), Rqc1, and Cdc48/VCP (an AAA+ ATPase) [Bengtson and Joazeiro, 2010; Shao et al., 2015]. NEMF can recognise stalled ribosome via the peptidyl t-RNA and interact with 60S subunit, prevent the rejoining of 40S subunit, and recruit listerin E3 enzyme for ubiquitination of stalled nascent chains. Rqc2 in yeast has also been reported to elongate the stalled chains at 60S subunit via addition of alanine and threonine residues at their C terminals (CAT tails) [Shen et al., 2015; Kostova et al., 2017]. CAT tails can ensure recognition of poly(lysine) chain by Ltn1 and upon ubiquitylation, Cdc48 and Rqc1 aid in extraction of nascent chain, leading to proteasomal degradation [Defenouillere et al., 2013]. Impairment in function of listerin E3 ubiquitin ligase is linked with neurodegeneration in mice [Chu et al., 2009]. Interestingly, Thrun *et al.* reported degradation of nascent polypeptides in mammals independently of listerin E3 enzyme. This alternative pathway (possibly when listerin

is unavailable) involves alanine tailing at C-terminal of ribosome-bound nascent polypeptides by NEMF that are recognised upon release by Pirh2 and CRL-KLHDC10 E3 enzymes for ubiquitination and degradation [Thrun et al., 2021].

2.3 THE COMPONENTS OF CELLULAR PROTEIN QUALITY CONTROL MACHINERY

The three major branches of PQC - chaperones, UPS, and autophagy-lysosome system not only regulate diverse cellular mechanisms but their failure leads to protein misfolding and disturbed cellular proteostasis due to the generation of protease resistant aggresomes of misfolded proteins [Nedelsky et al., 2008; Chen et al., 2011].

2.3.1 Molecular Chaperones

Historically, chaperones (heat shock proteins/Hsps) were first demonstrated to be induced in presence of heat shock to *Drosophila*, indicated by chromosomal puffs as the site of transcription of Hsp genes [Ritossa, 1962; De Maio et al., 2012]. Later on, this induction was observed even in presence of other stress types, including hypoxia and chemical toxins [Ritossa, 1964]. These chaperones are majorly classified as Hsp70, Hsp90, Hsp60, Hsp40, Hsp110, and small chaperone families [Vos et al., 2008; Kampinga et al., 2009]. These proteins have a wide distribution throughout the cell, with cytosolic chaperones being the most abundant. Many cytosolic proteins unable to realize native state upon translation are acted upon by downstream chaperone systems. Hsp70 chaperone family proteins (HSPA) consist of a nucleotide-binding domain (NBD) towards N-terminal, linker region, substrate-binding domain (SBD) towards C-terminal, and an unstructured C-terminal region with EEVD motif [Zuiderweg et al., 2017]. To perform its functions, Hsp70 needs to undergo cycles of conformational changes to bind and release its client protein [Rosenzweig et al., 2019]. This binding and release of substrate proteins give them the opportunity to attain native conformation. Hsp40 family (also known as DNAJ family) chaperones are required for increasing the hydrolysis of ATP by Hsp70 as well as binding of substrate to Hsp70. Hsp40 proteins require J domain to perform these functions and are classified into DNAJA, DNAJB, and DNAJC according to similarity with bacterial Hsp40, DNAJ [Cheetham and Caplan, 1998; Qiu et al., 2006]. Hsp110 are large chaperones and members of Hsp70 superfamily that are also the most abundant NEFs for Hsp70. They interact with Hsp70 to aid in releasing ADP and binding new ATP, leading to decreased affinity of Hsp70 for substrate and their release [Dragovic et al., 2006; Andreasson et al., 2008; Polier et al., 2008]. Additionally, Hsp110 and Hsp40 are also part of disaggregation machinery in complex with Hsp70 to solubilise protein aggregates via entropic pulling [Nillegoda et al., 2015].

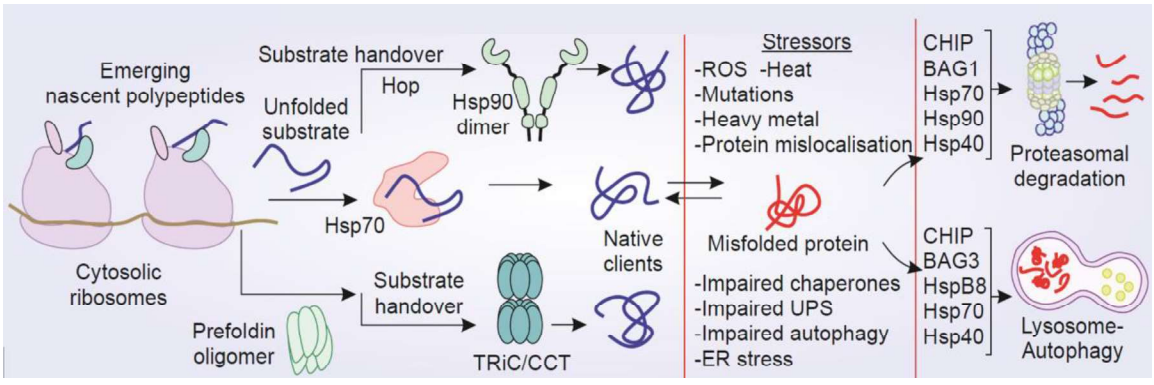

Figure 2.2: Chaperone-mediated proteostasis: Chaperones in cytosol act on nascent polypeptides at both co-translational and post-translational levels. Substrate handover can be done from Hsp70 to Hsp90 and TRiC/CCT, depending on the client protein. Various stressors can lead to misfolding of natively folded proteins, which can again be acted upon by chaperones. Chaperones can aid in their refolding or in the case of terminally misfolded proteins, chaperones can direct them to proteolytic degradation by UPS or autophagy. (*Source: Sumit Kinger and Amit Mishra* et al. (2024), Advances in Clinical Chemistry*)

The proteins not able in attaining native conformation upon release from Hsp70 system are moved to either Hsp90 system or TRiC/CCT chaperonins in cytosol (Figure 2.2). The substrate handover between cytosolic chaperones is aided via co-chaperones, such as Hip and Hop [Frydman and Hohfeld, 1997]. Hop is a co-chaperone that has multiple TPR domains through which it can bind EEVD motifs on C-terminals of both Hsp70 and Hsp90 chaperones [Scheufler et al., 2000]. Apart from their involvement in protein folding, the TPR domain containing proteins can also aid in degradation of client proteins. For example, CHIP, a TPR domains containing protein with both co-chaperone and E3 enzymatic functions, can also interact with Hsp70/Hsc70 and Hsp90 chaperones and ubiquitinate their bound clients, guiding them to degradation by proteasome [Connell et al., 2001; Stankiewicz et al., 2010]. In cytosol, many of the cellular proteins (around 10% of proteome) are folded into their native conformation by chaperonins TRiC, such as actin [Vinh and Drubin, 1994]. The nascent polypeptides can be transferred to TRiC by prefoldin oligomer for folding [Frydman et al., 1994; Martin-Benito et al., 2002]. Moreover, client proteins still unfolded, can be transferred to TRiC by Hsc70, indicating co-operation between different chaperone systems for efficient proteostasis [Cuellar et al., 2008]. Similarly, substrates can also be handed over to Hsp90 via Hop co-chaperone from Hsp70. Hsp90 proteins are chaperones present most abundantly in cytosol, have a diverse range of clients and can exist in different oligomeric forms [Chiosis et al., 2023]. Hsp90 chaperones consist of a NBD towards N-terminal, a middle domain (for binding other proteins, including co-chaperones as well as substrate), and a dimerization domain towards C-terminal [Biebl and Buchner, 2019]. Heat shock factor 1 (HSF1) is major transcription factor for expression of chaperones upon stress by translocating to nucleus from cytosol and binding promoters of chaperone genes [Vihervaara and Sistonen, 2014; Gomez-Pastor et al., 2018].

The action of molecular chaperones on different misfolded client proteins can lead them to various fates (Figure 2.3). Chaperones are actively involved in helping nascent or stress-generated non-native protein conformations to undergo a progressive folding process into native forms of protein. Key chaperones, such as HSP70, BAG3, and HSPB8 (also known as HSP22), regularly patrol the cytoplasm to detect any protein molecules with non-native conformations [Ganassi et al., 2016]. Upon detection of any of misfolded toxic species of proteins, these chaperones may initiate an intrinsic refolding process or, if required, may lead to their clearance through either the proteasome or autophagy, as seen in case of HSP70, HSPB8, BAG3, DNAJB2, BAG1, and CHIP [Connell et al., 2001; Carra et al., 2008; Crippa et al., 2010; Crippa et al., 2016; Cristofani et al., 2018]. Alternatively, for Amyotrophic Lateral Sclerosis (ALS)-linked mutant proteins, such as FUS and TDP-43, chaperone proteins HSP70, HSPB1 (also known as HSP27), HSPB8, DNAJB1, and DNAJA1 are actively involved in regulating the formation of liquid liquid phase separation-mediated membrane-less granules [Lu et al., 2022]. In addition, chaperones such as DNAJC5 and HSC70 may also mediate the extracellular secretion of ALS misfolded proteins via an endocytic pathway to clear them from the cytoplasm [Fontaine et al., 2016; Lee et al., 2016; Lee et al., 2018; Xu et al., 2018]. Small chaperones such as HSPB1, DNAJA, and DNAJB, along with HSP70 and HSP110, may critically initiate disaggregation, attempting to dissolve misfolded accumulates [Nillegoda et al., 2015].

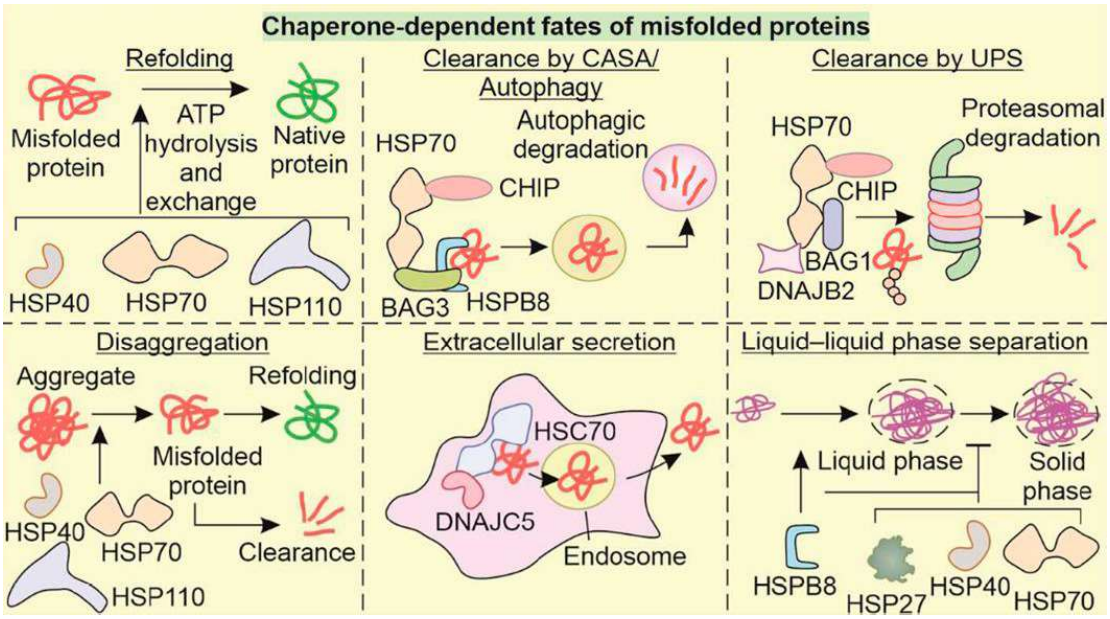

Figure 2.3: Chaperone-dependent fates of misfolded proteins: Molecular chaperones can act on their misfolded protein clients and make various fates possible. Apart from refolding, chaperones can aid in directing misfolded proteins to degradation by autophagy or UPS. Chaperones can also interact with protein aggregates and aid in their disaggregation/solubilisation. Extracellular secretion of misfolded proteins has also been reported by chaperones. Chaperones also regulate liquid liquid phase separation of certain proteins. (*Source: Sumit Kinger and Amit Mishra* et al. (2023), Cells*)

The cellular chaperone network is involved in handling proteins with abnormal conformation and aggregation, making different fates possible. The protein degradation is also essential to proteostasis network. Chaperones can direct misfolded proteins or their aggregates for degradation via UPS and autophagy through their interaction with various proteins involved in PQC. In the upcoming sections, the mechanism of working of UPS and autophagy and their involvement in neurodegeneration are described.

2.3.2 Ubiquitin-Proteasome System

The UPS is one of key cellular PQC mechanisms facilitating removal of misfolded aberrant proteins [Tanaka, 2009]. As earlier mentioned, molecular chaperones act as initial defense against aberrantly folded proteins by first trying to refold these proteins, and upon failure, target them towards cellular degradation pathways, i.e., UPS and autophagy [Hipp et al., 2019]. To clear these proteins from crowded cellular environment, tagging them with small protein ubiquitin is required, a process facilitated by three well-known enzymes collectively termed ubiquitin-modifying enzymes [Amm et al., 2014]. Three vital enzymes, E1 (ubiquitin activation), E2 (ubiquitin conjugation), and E3 (ubiquitin ligase), are required to target abnormal proteins or critical specific substrates by linking ubiquitin proteins to them for their proteasomal degradation (Figure 2.4). The barrel shaped 26S proteasome proteolytic machinery retains >50 subunits and carries various active sites responsible for protein degradation. The proteasomes (~2MDa) are composed of 19S regulatory particle (RP) and 20S core particle (CP) and are essential for PQC as well as other cellular housekeeping functions [Mishra et al., 2018]. The 20S subcomplex consists of 2 inner β subunit heptameric rings and 2 outer α subunit heptameric rings arranged in $\alpha_7\beta_7\beta_7\alpha_7$ fashion [Sahu and Glickman, 2021]. The proteolytic catalytic activity of 20S proteasome is known to reside in β1 (post-glutamyl peptide hydrolase-like), β2 (trypsin-like), and β5 (chymotrypsin-like) subunits, while the neighboring β subunits help in substrate binding in the 20S complex. The N termini of α subunit rings form a gate to prevent substrate entry into 20S core [Groll et al., 2000]. The 19S RP multiprotein complex consists of 13 non-ATPases and 6 ATPases for recognition of ubiquitinated target protein, its deubiquitination and unfolding, and opening 20S

gate for the translocation of target to 20S catalytic chamber for degradation [Sahu and Glickman, 2021].

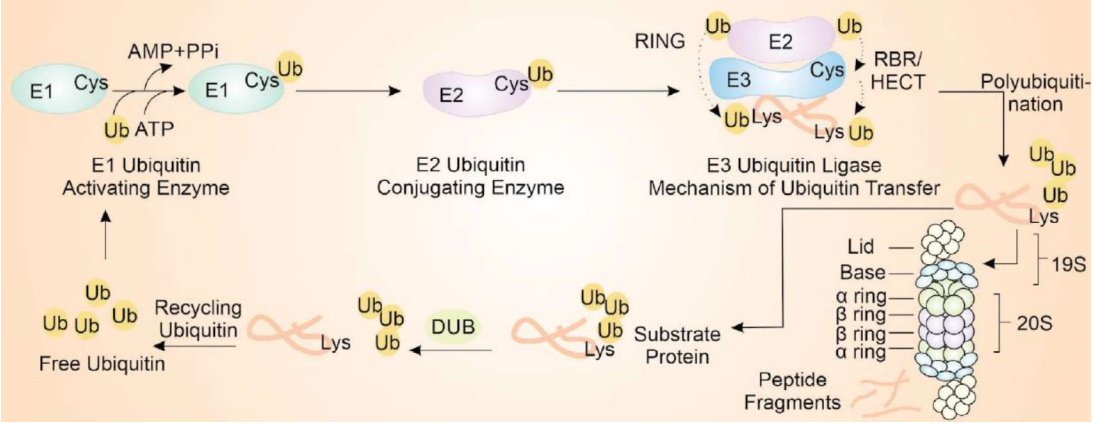

Figure 2.4: Introduction to ubiquitin-proteasome system: This system involves ubiquitination of a target protein in a series of reactions catalysed by E1, E2, and various types of E3 enzymes for activation of ubiquitin and its conjugation to substrate. Ubiquitinated protein is recognised, unfolded, and deubiquitinated by 19S RP and translocated to 20S CP for degradation. The free ubiquitin upon deubiquitinylation can be recycled and reused. *(Source: A.R. Dubey, Sumit Kinger# and Amit Mishra* et al. (2021), Cellular Physiology and Biochemistry)*

Nearly 600 E3 ligases are identified till now and are majorly categorized based on their catalytic mechanisms into RING, HECT, and RBR [Clague et al., 2015; Buetow and Huang, 2016]. The ubiquitin transfer in different classes of E3 enzymes also differs; for instance, in RING domain E3 enzymes, ubiquitin is transported from E2 enzyme directly to client protein bound with E3. In contrast, for HECT and RBR domain E3 enzymes, ubiquitin transfer occurs first from E2 to E3 and then to target protein [Morreale and Walden, 2016]. Then, the ubiquitin chain is extended upon multiple such ubiquitination cycles. Based on ubiquitin chain type (M1, K6, K11, K27, K29, K33, K48, K63) formed on client protein, its cellular fate is decided [Komander and Rape, 2012] (Figure 2.5). For example, the K48 chain linked target proteins are generally subjected to proteasomal degradation and K63 chains are involved in NF-κB signaling and autophagy. Apart from UPS, the lysosome-dependent autophagy mechanisms are the second major proteolytic system for clearing unfolded protein aggregates in human cells.

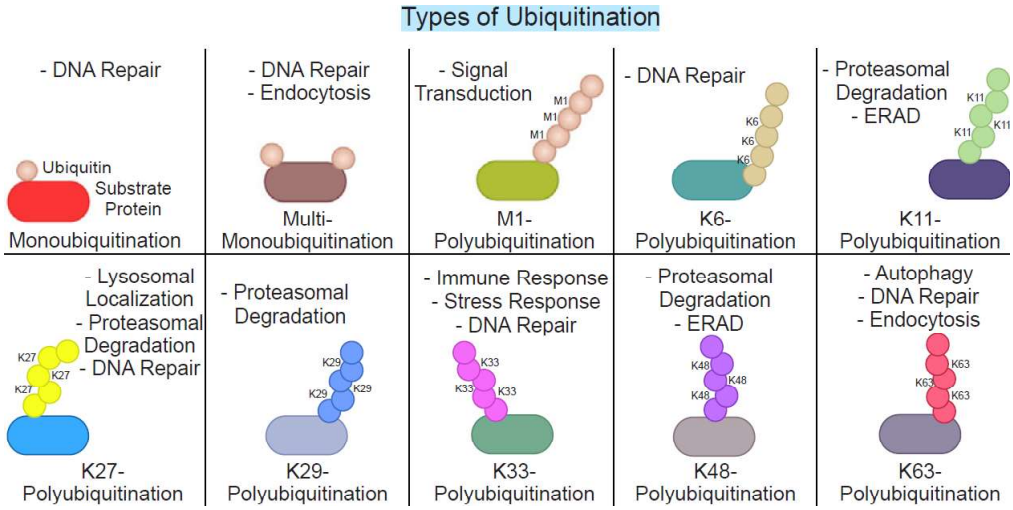

Figure 2.5: Ubiquitin code: Ubiquitination of proteins can be mono-ubiquitination as well as poly-ubiquitination. The poly-ubiquitin chains can be of different types depending on type of linkage between two ubiquitin molecules and have different cellular roles. (*Source: Sumit Kinger and Amit Mishra* et al. (2024), Advances in Clinical Chemistry*)

2.3.3 Autophagy

The word "autophagy" in Greek means "eating of oneself," and was initially used by Christian de Duve. It is a catabolic phenomenon allowing cells to break down components (proteins, organelles etc.) through lysosomes and serves a housekeeping function in ensuring cellular homeostasis [Cuervo, 2004; Klionsky, 2005]. It contributes to the regulated cycle of continuing cellular component recycling, synthesis, and degradation necessary for proper cells functioning [Chun and Kim, 2018]. It is a conserved mechanism that allows eukaryotic cells to uphold their homeostasis by clearing the senescent or defective protein and organelles via lysosomal machinery. This process occurs under normal settings as well as in responses to stressful situations such as starvation. There exist multiple types of autophagy, which may be categorised: chaperone-mediated autophagy (CMA), microautophagy, and macroautophagy [Johansen and Lamark, 2011; Feng et al., 2014; Mejlvang et al., 2018] (Figure 2.6). It is also possible to categorise macroautophagy as either selective or non-selective; in selective autophagy, specifically damaged organelles, aggregated proteins, and other cargo are selected for autophagic degradation, possibly on the basis of specific cargo receptors [Zaffagnini and Martens, 2016].

The process of macroautophagy degrades cytoplasmic components of a cell in bulk quantities. After the cytosolic materials, including protein aggregates and impaired organelles, are contained into a vesicle with double membrane; this vesicle is fused with lysosome, exposing all of material to the hydrolytic environment of the lysosome, where mainly acid hydrolases degrade the material into their fundamental elements. The creation of phagophore initiation sites is dependent on ULK1 complex action on VPS34 complex, which phosphorylates phosphatidylinositol lipids, marking the first step in macroautophagy [Russell et al., 2013]. There are many ATG proteins that are required in autophagosome synthesis [Nakatogawa et al., 2009].

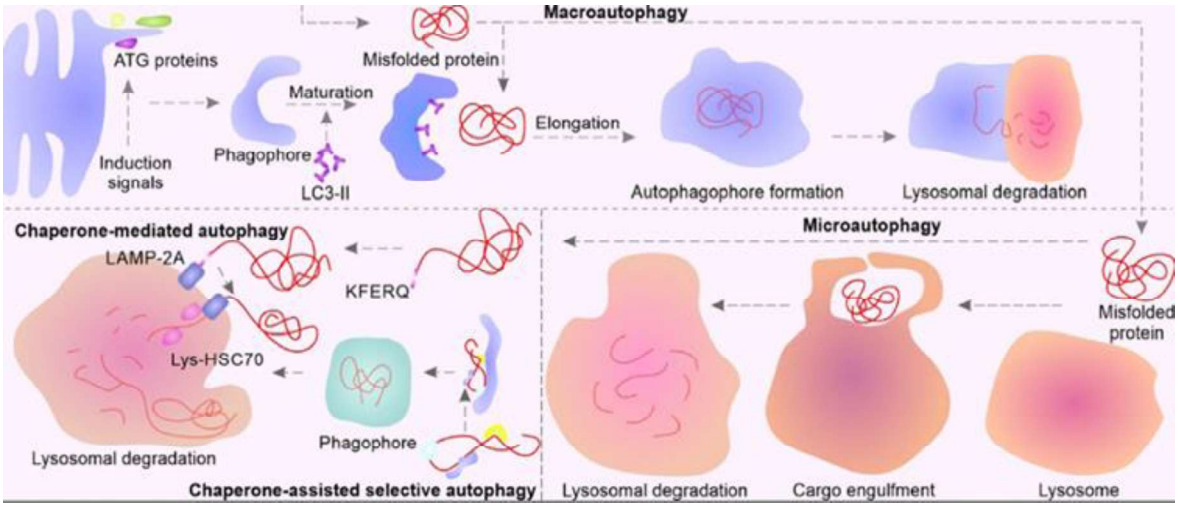

Figure 2.6: Introduction to cellular autophagy: Macroautophagy includes double-membraned autophagosome formation and conjugation with LC3-II. This autophagosome can enclose cellular particles in bulk and fuse with lysosomes, after which hydrolytic degradation of autophagosome contents occurs. Chaperone-mediated autophagy involves direct uptake of certain proteins that have KFERQ motif by lysosomes for degradation. Microautophagy also involves direct uptake of cytosolic content by lysosomes via engulfment. *(Source: P. Kumar, Sumit Kinger and Amit Mishra* et al. (2021), Journal of Cellular Physiology)*

CMA is another mechanism for selective autophagy that assists in degrading soluble cytosolic proteins. CMA was the first kind of autophagy to be proven as degrading certain proteins and being selective in nature [Dice, 1990; Cuervo, 2011; Kaushik and Cuervo, 2012]. In CMA, a KFERQ-like sequence is identified by cytosolic Hsc70 accompanied with Hop, Hsp40, Hsp90, BAG1, and Hip is then associated with a protein of lysosomal membrane LAMP-2A,

which then oligomerize [Chiang et al., 1989; Agarraberes and Dice, 2001]. During this process, a lysosomal chaperone known as Lys-Hsc70 is critical for assisting in translocating protein beyond lysosome membrane, necessary for completion of the degradation process [Kaushik and Cuervo, 2012]. Microautophagy is a non-selective bulk process in which lysosomes may directly ingest the items found in the cytosol via the invagination of the membrane [De Duve and Wattiaux, 1966].

2.4 IMPAIRED PROTEIN QUALITY CONTROL MACHINERY IS LINKED WITH DISEASE CONDITIONS

The significance of cellular proteostasis network can be gauged from numerous observations on its imbalance association with cytotoxic protein misfolding and aggregation, which is associated with various disease conditions, including neurodegenerative diseases and metabolic diseases, and cystic fibrosis among others [Balchin et al., 2016].

2.4.1 Depleted and Impaired Protein Quality Control is Linked with Neurodegenerative Diseases

The major clinical hallmark of neurodegeneration is formation of abnormal proteins inclusions in various areas of diseased brains [Ross and Poirier, 2004; Wilson et al., 2023]. The neuronal degeneration conditions Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), Prion diseases, and Amyotrophic Lateral Sclerosis (ALS) are reported with the occurrence of aggresome-like proteinaceous species in the neuronal cells [Soto, 2003]. AD (commonest neuronal degenerative condition) involves deposition of amyloid plaques (extracellular; comprising amyloid β) and neurofibrillary tangles (intracellular; comprising tau protein) and typically degeneration initiation occurs at frontal/temporal lobes and hippocampus [Masters et al., 2015]. PD is primarily recognised by dopaminergic neuronal loss in SNc as well as protein aggregate inclusions, with α-synuclein being its major component [Poewe et al., 2017].

ALS is a neuronal degenerating condition with majorly motor neuronal loss [Hardiman et al., 2017]. Aggregation of certain mutant proteins TDP-43, SOD1, FUS, and dipeptide repeats (DPRs) translated from hexanucleotide repeat expanded *C9orf72* has been linked with cytotoxicity in ALS [Johnson et al., 2009; Fushimi et al., 2011]. HD is a polyglutamine (polyQ) expansion disorder involving CAG trinucleotide expansion in *HTT* exon 1 leading to polyQ expanded HTT oligomerisation and aggregation and degeneration of striatum region, though increasingly, HD pathology is being characterised to affect whole brain [Bates et al., 2015]. The length of polyQ expanded repeat affects the onset age as well as progression of disease [Lee et al., 2012; Rosenblatt et al., 2012]. The misfolded proteins can arise in cells due to multiple sources. Certain larger proteins can exhibit slow folding kinetics (e.g., CFTR), which can make their folding less efficient, increasing the probability of misfolding [Ward and Kopito, 1994; Lukacs and Verkman, 2012]. Similarly, certain mutations can increase the probability of their impaired protein folding and/or aggregation/fibrillation propensity, such as SOD1, α-synuclein, or nucleotide repeat expanded proteins, like huntingtin (trinucleotide repeat expansion generating polyQ expansion) and C9orf72 (hexanucleotide repeat expansion generating DPRs) [Bruijn et al., 1998; Li et al., 2001; Stathopulos et al., 2006; Mori et al., 2013]. For instance, A53T and E46K mutations in α-synuclein can increase its capacity for dimer formation, which can influence further oligomerisation [Krasnoslobodtsev et al., 2013; Flagmeier et al., 2016]. Stress agents, like oxidative stress, heat stress, and heavy metals, can also denature the proteins or lead to their misfolding [Tamas et al., 2014; Jacobson et al., 2017; Abramov et al., 2020].

Decreased proteostasis during ageing, possibly via reduced PQC components (including chaperones), and their capacity can also increase susceptibility for neurodegeneration [Lopez-Otin et al., 2013; Brehme et al., 2014]. Failure of the proteolytic process is a phenomenon that may be observed in a variety of neurodegenerative diseases [Morimoto et al., 2011; Roth and Balch,

2011; Hohn et al., 2020]. The accumulating aberrant proteins resulting from defective degradative processes affect proteostasis, and aggregation of these abnormal proteins is primarily responsible for death of neuronal cells [Wong and Cuervo, 2010]. The overaccumulation of misfolded and aberrant proteins is serious threat to the function of proteasome catalytic subunits as well as substrate entry [Deriziotis et al., 2011; Thibaudeau et al., 2018]. Previous findings summarize that aggregation of abnormal proteins inhibiting proteasome functions can be a possible cause of proteotoxicity in cells [Bence et al., 2001; Labbadia and Morimoto, 2015]. Decreased function of proteasome as well as other elements of PQC is associated with neurodegenerative conditions. The failure of PQC mechanisms, including UPS, is widely recognized in AD pathology [Oddo, 2008]. One study showed that impaired proteasomal function was linked with pathologic aggregation of tau and Aβ [Tseng et al., 2008]. Moreover, tau filaments also impaired proteasome activity, resulting in neuronal damage in AD [Keck et al., 2003].

Recently, ALS-linked C9orf72 DPR aggregates are also reported to impair proteasome activity by stalling them in substrate processing conformation [Guo et al., 2018]. Another ALS-linked protein TDP-43 C terminal fragments (TDP-25) are recently reported to form neuronal inclusions rich in stalled proteasomes unable to exhibit proteolysis [Riemenschneider et al., 2022]. HD-associated mutant huntingtin (HTT) can lead to dysregulation of various transcription factors in cells (e.g., HSF1 and NF-Y; involved in chaperone expression), mitochondrial impairment (e.g., transport, dynamics, and energy production), and PQC dysfunction (folding and degradation) [Labbadia and Morimoto, 2013]. The altered proteasomal activity in presence of PD-linked mutant α-synuclein is also reported. For instance, expression of α-synuclein (A53T) suppresses the 26S proteasome, leading to UPS dysfunction and ultimately resulting in dopaminergic neuronal death [McKinnon et al., 2020]. Normally, α-synuclein can be targeted to proteasomal as well as CMA degradation due to the presence of a recognising motif [Liu et al., 2003; Cuervo et al., 2004; Vogiatzi et al., 2008; Luk et al., 2012]. Interestingly, CMA activity is reduced in PD brain. Hsc70 chaperone and LAMP-2A were also decreased in dopaminergic neurons [Alvarez-Erviti et al., 2010], making them potential targets for treatment. The PD-linked α-synuclein mutants A30P and A53T have strong affinity for LAMP-2A adaptor for CMA. Because these are not successfully transported to the lysosomal lumen, they function as uptake blockers thus limiting their own clearance as well as that of others, which combine to obstruct CMA [Cuervo et al., 2004; Malkus and Ischiropoulos, 2012]. ALS inclusion bodies might sequester components such as E3 enzymes and chaperones like Hsp70 and Hsp40, thus decreasing the effectiveness of PQC systems [Shiber and Ravid, 2014].

2.4.2 The Increased Dependence on Protein Quality Control Machinery in Abnormally Proliferating Cancer Cells

Cancer diseases have a very high incidence globally, imposing a high burden, varying in incidence rates between different age groups as well as gender [Sung et al., 2021]. Genome instability appears to be at the core of cancer pathomechanisms and is associated with diverse heterogeneity of cancer, marking a vital challenge to develop effective therapeutics. Sustained growth signaling, increased resistance to cell death, abnormal proliferation, invasion and metastasis, angiogenesis, and metabolic adaptation to hypoxic conditions are primary features of cancer cells [Hanahan and Weinberg, 2011]. To perform these functions, cancer cells depend on protein homeostasis and the necessary PQC machinery; modulating them is an attractive therapeutic strategy [Dubey et al., 2021; Kumar et al., 2022]. Cancer cells heavily rely on proteostasis for their survival. Among many proteins degraded by proteasomes are also those required for regulation of cell cycle, apoptotic cell death, metastasis, metabolism, and cell growth, which are essential for abnormal cellular proliferation of cancer cells [Adams, 2003]. Additionally, many cancer cell types have higher proteasomal expression as well as activity in comparison to normal cells [Masdehors et al., 2000; Arlt et al., 2009]. In contrast to neurodegeneration where PQC function of cells is disturbed and depleted, cancer cells have higher levels of and higher dependence on various PQC components.

The proteotoxic crisis hypothesis has been indicated for cancer cells, which proposes that cancer cells exhibit a higher dependency on PQC machinery compared to normal cells. This is due to a mismatch between load of proteasome substrates resulting from increased protein synthesis, including mutant proteins in cancer cells and the capacity of proteasomes [Deshaies, 2014]. Inhibiting proteasomes is linked with triggering apoptosis through increased cytotoxic events such as ER stress in myeloma cells [Obeng et al., 2006]. The cancer cells response to inhibition of proteasome for apoptosis induction is influenced by balance between the load of proteasome and its capacity [Bianchi et al., 2009]. Inhibition of degradation of multiple proteasome substrates can be beneficial in targeting cancer cells to death. For instance, p53, a tumor suppressor and pro-apoptotic transcription factor, is a key proteasomal substrate and can be stabilized by proteasome inhibition [Lopes et al., 1997; do Patrocinio et al., 2022]. Additionally, nuclear factor kappa B (NF-κB) transcription factor that supports survival of cancer cells, has its negative regulator IκBα as a proteasomal substrate. Inhibition of proteasome can consequently reduce activity of NF-κB [Palombella et al., 1994; Guzman et al., 2002].

2.5 PROTEIN QUALITY CONTROL MACHINERY AS THERAPEUTIC TARGET

The significance of the proteostasis mechanisms in cells, including the PQC machinery cannot be overstated. The depleted PQC functions, including of proteasome, in multiple neuronal degenerative disorders and protein aggregation being a hallmark of these diseases make the search for PQC activators desirable. Similarly, higher dependence of cancer cells on proteostasis network makes the inhibitors of PQC components, including proteasome, as attractive therapeutic agent candidates.

2.5.1 Lanosterol – An Endogenous Natural Biomolecule with Potential for Aberrant Protein Aggregate Clearance

Lanosterol is an endogenous natural sterol intermediate compound in a pathway to synthesis of cholesterol. It is formed in a cyclisation reaction catalysed by the enzyme lanosterol synthase (oxidosqualene cyclase; OSC) acting on substrate monoepoxysqualene [Huff and Telford, 2005]. It is naturally found in the eye lens and protects different lens crystallin proteins from aggregation, demonstrated both *in vivo* and *in vitro* [Zhao et al., 2015]. Given its amphipathic characteristic, lanosterol possesses the capability to bind specific hydrophobic regions of lens crystallins to prevent their aggregation [Kang et al., 2018]. Furthermore, lanosterol induces disaggregation of protein aggregates and promotes their chaperone-mediated refolding [Hu et al., 2020]. Recently, lanosterol is reported to cause disruption of amyloid β peptide aggregates, mitigating their cytotoxic effects [Zhou et al., 2019]. In another study, sterol profiling in toxin-induced PD mice model indicated decreased levels of lanosterol in brain regions, and exogenously added lanosterol provided neuroprotection in an autophagy-dependent manner [Lim et al., 2012].

Previously, we have reported lanosterol to alleviate proteotoxicity via induction of CHIP (exhibits both co-chaperoning and E3 enzyme functions) expression and autophagy [Upadhyay et al., 2018]. Interestingly, lanosterol also promotes ubiquitination and UPS-mediated proteolysis of HMG-CoA reductase, an enzyme crucial to synthesis of cholesterol [Song et al., 2005]. However, effect of lanosterol on proteasome activity for maintaining proteostasis is not known. These PQC modulation properties of lanosterol, in addition to, it being a natural compound makes it an attractive candidate as therapeutic agent for neurodegenerative diseases.

2.5.2 Valproate – A HDAC Inhibitor Drug with Potential for Protein Quality Control Modulation and Anti-cancer Characteristics

Valproic acid (VPA), a short, branched chain fatty acid and FDA-approved for seizures treatment is reported to have protective effects against metabolic and cardiovascular diseases and exhibits anti-cancer as well as anti-microbial activities [Singh et al., 2021]. Moreover, VPA is also

well-known for its inhibitory action on histone deacetylases (HDACs) activity, primarily class I HDACs [Gottlicher, 2004]. Further, it is suggested that VPA inhibits HDAC by influencing its catalytic access to substrates and exhibit reduction in tumor growth and metastasis and increases differentiation in cancer cells [Gottlicher et al., 2001]. Interestingly, VPA treatment is noted to increase proteasome-mediated degradation of HDAC2, possibly via inducing levels of E2 enzyme Ubc8 [Kramer et al., 2003]. VPA also induces apoptosis in cancer cell lines of hepatoma, endometrium, breast, ovarian, prostate, cervix, and leukemia along with increased expression of pro-apoptotic proteins (e.g., caspases, Bax, cytochrome *c*) [Kawagoe et al., 2002; Phillips et al., 2003; Takai et al., 2004a; Takai et al., 2004b; Thelen et al., 2004; Han et al., 2013; Ma et al., 2017]. Moreover, VPA is reported to arrest cell cycle and promote apoptosis in a p53-dependent manner [Chen et al., 2009; Paradis and Hales, 2015; Tran et al., 2017].

Interestingly, exposure of VPA is also shown to induce cytotoxicity by promoting ER as well as oxidative stress in lens epithelial cells [Palsamy et al., 2014]. Recently, valproate has been linked with reduced activity of deacetylase enzymes HDAC6 and HDAC8, protecting against cardiac hypertrophy in rats [Kee et al., 2013]. Previously, the inhibition of HDAC6, an enzyme required for recruitment of misfolded proteins to aggresomes via dynein motor protein, in combination with proteasome inhibitors is reported to generate enhanced accumulation of ubiquitinated proteins and cytotoxicity [Johnston et al., 2002; Kawaguchi et al., 2003; Hideshima et al., 2005]. As mentioned previously, VPA low dose is indicated for increase in N-terminal acetylation of proteasome subunits and proteolytic activity in mice myocardium [Wang et al., 2013a]. The anti-cancer potential as well as its possible association with cellular proteostasis makes valproate an attractive candidate for further studies for targeting PQC machinery in cancer cells.

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