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Review of Literature

Cells harbor large number of different kinds of molecules that accomplish all the necessary functions for its survival. Out of these molecules, proteins are involved in majority of cellular processes ranging from gene regulation to cell growth and differentiation [Qin *et al.*, 2015]. The importance of properly functioning proteins can be understood from the evidence which suggest that organisms having longer lives consist of much stable or damage resistant proteome [Kaushik and Cuervo, 2015; Treaster *et al.*, 2013]. Therefore, cells continuously require properly functioning repertoire of proteins and to meet these requirements, cells synthesize and harbor proteins with utmost care [Frydman, 2001; Ibba *et al.*, 1999]. Regulation of protein synthesis which includes its proper folding, concentration and localization by cell can be termed as cellular proteostasis [Jackson and Hewitt, 2016]. Nascent polypeptide chains come out of the ribosome tunnel, and are taken care by a group of cellular proteins, called molecular chaperones [Ellis, 1987; Kim *et al.*, 2013; Wickner, 1999]. Chaperones assist nascent polypeptides to attain their necessary three-dimensional shape, without which they cannot function properly; instead they may turn into an unwanted aggregatory form, which start accumulating inside the cells and may sometimes cause lethal damage [Hartl *et al.*, 2011; Hartl and Hayer-Hartl, 2002]. Neurodegenerative diseases (NDDs) are the well known examples of such damage, caused by aggregation of proteins [Dobson, 1999; Gregersen *et al.*, 2006; Ross and Poirier, 2004]. Common factors that may enhance the risk of protein aggregation include genomic alterations, intra- as well as extracellular stresses, along with insufficient chaperoning capacities [Ramirez *et al.*, 2010; Selkoe, 2004]. However, to avoid and counter such obnoxious and toxic accumulation of proteins, cells have developed multiple ways, which majorly include repair systems at the genomic level, and refolding systems at the protein level [Lindahl and Wood, 1999; Tyedmers *et al.*, 2010]. If such attempts fail, cells may take final decision to degrade misfolded proteins and their inclusions inside the cells [Goldberg, 2003; Hochstrasser, 1996]

Maintenance of cellular proteostasis is the integral need for cellular health and viability. Several cellular pathways linked with aging and age associated pathologies such as redox state of proteins, translation rate, protein folding and heat shock responses are affected in response to proteostasis alteration [Rongo, 2015; Taylor and Dillin, 2011]. Further, this perturbed protein environment or proteostasis may lead to toxicity due to abnormal cell signaling, aberrant protein interactions and cellular membrane disruption [Gidalevitz *et al.*, 2010]. Proteostasis in cells is not only restricted to cytosol, but is also present in organelles such as in endoplasmic reticulum (ER) and mitochondria. Quality control mechanism in ER ensures proper folding and release of proteins [Ellgaard and Helenius, 2003]. In ER, the mechanism of proteostasis is maintained by unfolded protein response (UPR), which senses and restores the disturbed proteostasis by activating pathways that involve translation inhibition, enhanced folding and degradation [Inagi *et al.*, 2014]. Intriguingly, perturbations in the ER proteostasis is observed to be linked with neurodegeneration, cancer, and other disorders such as kidney disease [Hetz and Mollereau, 2014; Inagi *et al.*, 2014; Liu and Ye, 2011]. Similarly, proteostasis is crucial for mitochondria so that it can accomplish various cellular tasks such as ATP production through respiratory chain networks, β-oxidation of fatty acids and maintenance of Ca^{2+} ion concentration etc. [Baker *et al.*, 2011].

In mitochondria, a defective proteostasis generates UPR^{mt} response similar to ER, which utilizes mitochondrial chaperones and proteases that clear proteotoxic load of mitochondria [Jovaisaite and Auwerx, 2015]. A defective proteostasis in mitochondria can produce detrimental consequences in cells such as irregularities in ATP production and increased reactive oxygen species generation that may in turn contribute in different neurological, cancer and hereditary disorders [Haynes and Ron, 2010]. Proteostasis also plays a central role in aging, and loss of proteostasis is considered as a common characteristic of aging [Labbadia and Morimoto, 2015]. The cellular proteome of an aging cell is constantly challenged with different stress conditions that results in misfolded proteins production. These misfolded proteins with their hydrophobic surfaces exposed are highly prone to aggregation and formation of toxic inclusions in the cell [Taylor and Dillin, 2011]. In older cells, the probability of toxic inclusion formation is more, since an older cell have limited expression of various protein quality control machineries to relieve cells from the toxic load of aggregated proteins [Gidalevitz *et al.*, 2010]. Together, finding strategies that can provide us with novel tools or targets to precisely regulate the mechanism of proteostasis can prove to be beneficial from therapeutic point of view.

2.1 CELLULAR PROTEOME NETWORK AND ITS LINKAGE WITH DISEASES

Techniques such as microarrays and yeast two-hybrid screens have made it possible to efficiently acquire large amount of data that contains valuable information of cellular environment. However, to gain clear understandings of these mechanisms, datasets must be converted into meaningful sets of information. Systematic arrangement of the data acquired, into well defined interaction network, made up by universal laws may provide us with new insights in understanding of cellular organization, as well as disease occurrence [Barabási and Oltvai, 2004]. A normal cell can be considered to be composed of a complex web of interaction networks having three basic interacting components i.e. genes, proteins and metabolites [Han, 2008]. Being an important component and having diverse array of functions, understanding proteins have always remained a subject of interest to many researchers. Properly folded functional proteins interact with each other and other metabolic components in a well defined manner, to perform respective assigned physiological functions [Saghatelian and Cravatt, 2005]. Considerable studies have been performed in the past to understand the cellular machinery through generation and analysis of protein-protein interaction networks [Li *et al.*, 2016; Rual *et al.*, 2005]. The mechanism of proteostasis can itself be considered as an integral part of cellular interaction network, involved in maintenance of a healthy proteome. However, under certain stress conditions or mutations, the state of proteostasis can get perturbed, resulting in a defective proteome network, ultimately generating pathological state [Barabási *et al.*, 2011]. The severity of disease condition can be assumed to be dependent on the number of interactions or the mechanisms, the proteins have been involved in, which have been disturbed [Barabási *et al.*, 2011; Missiuro *et al.*, 2009]. As evident from a study, highly connected protein or hub protein if gets deleted results in more lethal outcomes as compared to less connected protein [He and Zhang, 2006].

Accumulating studies have demonstrated impaired proteostasis as one of the key features of aging [Ben-Zvi *et al.*, 2009; López-Otín *et al.*, 2013]. Different mechanisms involved in maintaining proteostasis like chaperones machinery and proteolytic systems have been analyzed to gain an understanding of their role in aging. Reduction in chaperone synthesis, decrease in HSF DNA binding capacity, damaged chaperones and overloaded chaperones are various factors that contribute to age associated impairment in chaperone machinery [Sőti and Csermely, 2007]. Similarly, alterations in components of ubiquitin proteasome system viz. ubiquitin, E2 conjugating enzyme, E3 ubiquitin ligases and proteasome have been observed with aging [Tsakiri and Trougakos, 2015]. Proteasome is the most extensively studied component of UPS with respect to aging. Decreased proteasome activity, reduced proteasome subunit expression, proteasome disassembly and proteasome inactivation due to clogging by protein aggregates are common features of aging [Ferrington *et al.*, 2005; Saez and Vilchez,

2014]. Moreover, a mutation in E1 activating enzyme of UPS resulted in the development of aging-like phenotypes in *Drosophila* [Liu and Pfleger, 2013]. However, further studies targeted towards understanding role of E1, E2, E3, and ubiquitin in aging are needed to unravel hidden aging associated pathways affected due to a disturbance in UPS. Like UPS, disruption in autophagy has also been reported with aging [He *et al.*, 2013]. Proteins such as autophagy related gene (ATG), sirtuin 1 and beclin which are involved in autophagy induction, have been observed to be down regulated with aging [Lipinski *et al.*, 2010; Rubinsztein *et al.*, 2011]. The numerous connections that have been discussed above between different components of proteostasis mechanisms and aging provides enough support to target these mechanisms for finding therapeutic solutions of various age-related problems.

Disturbance in proteostasis caused by various stress conditions such as genomic instability, hypoxia, mutations, nutrient deprivation, tumor suppressor protein aggregation and redox imbalance are emerging as critical factors that have implications in cancer development and progression [Ano Bom *et al.*, 2012; Urra *et al.*, 2016]. To overcome proteotoxic load caused by the proteostasis imbalance, cancer cells enhance its proteostasis maintenance capacity by up-regulating chaperones and proteolytic machinery [Deshaies, 2014; Zorzi and Bonvini, 2011]. In leukemic cells increased expressions of proteasomes have been observed [Kumatori *et al.*, 1990]. Another component of UPS, the E3 ubiquitin ligases like Mdm2 and SCF complex have also been found to be amplified in different types of cancers [Chen *et al.*, 1998; Zhang and Wang, 2000]. Similarly, both in tumor and malignant cancers chaperone machinery may help cancer cells to overcome proteostasis imbalance induced apoptotic signaling [Urra *et al.*, 2016; Whitesell and Lindquist, 2005]. Recently, it was found that CHIP E3 ubiquitin ligase targets DNA damage-induced apoptosis suppressor (DDIAS) for proteasomal degradations, having therapeutic implications in cancer treatment [Won *et al.*, 2017]. Interestingly, the elevated levels of chaperones Hsp90, Hsp70 and Hsp27 have also been shown as a contributing factor in causing resistance to various cancer therapies [Gabai *et al.*, 1995; Heinrich *et al.*, 2016; Whitesell *et al.*, 2014]. In addition to UPS and the chaperone machinery, the role of autophagy has also been extensively studied in cancer and metastasis [Mathew *et al.*, 2007; Mowers *et al.*, 2016; White, 2015]. However, the involvement of autophagy in tumorigenesis is complex. Autophagy has been found to be tumor suppressing at initial stages of tumor formation while in established tumors it promotes cell survival, thus act as a tumor promoting mechanism [Choi, 2012]. This dual behavior of autophagy can be taken as an example of how at different stages of cancer the environment of cancer cell changes. Therefore, research focusing on understanding the role of proteostasis in various stages of cancer development is required so that efficiency of currently available anticancer strategies could be improved.

An indication of disruption in proteostasis is the presence of protein inclusions containing misfolded protein aggregates; a clinical feature widely observed in several NDDs (neurodegenerative diseases) [Yerbury *et al.*, 2016]. It is obvious that being major processes involved in clearance of misfolded or aberrant proteins, the chaperone machinery, and proteolytic mechanisms have a key role to play in neurodegenerative diseases progression. Interestingly, besides removing damaged proteins in neurons, proteostasis mechanisms such as UPS also takes part in neuronal development, presynaptic functions, and postsynaptic plasticity aiding in proper functioning of the nervous system [Yi and Ehlers, 2007]. Thus any alterations in proteostasis mechanisms may also affect these processes leading to aberrant nervous system operations. It has been observed that mice lacking a maternal copy of E3 ubiquitin ligase UBE3A/E6-AP show Angelman syndrome-like characteristics i.e. motor dysfunction, seizures and learning deficit [Jiang *et al.*, 1998]. Thus the outcome of dysfunction in proteostasis is not just limited to neuronal toxicity, but it also disturbs the overall functionality of the nervous system. Therefore, studies designed to understand wider consequences of proteostasis imbalance are required. Such studies may prove to be clinically useful as it would help in understanding the mechanisms underlying disease progression in addition to disease

occurrence. Various mechanisms that are employed by cells to maintain protein homeostasis are shown in Figure 2.1.

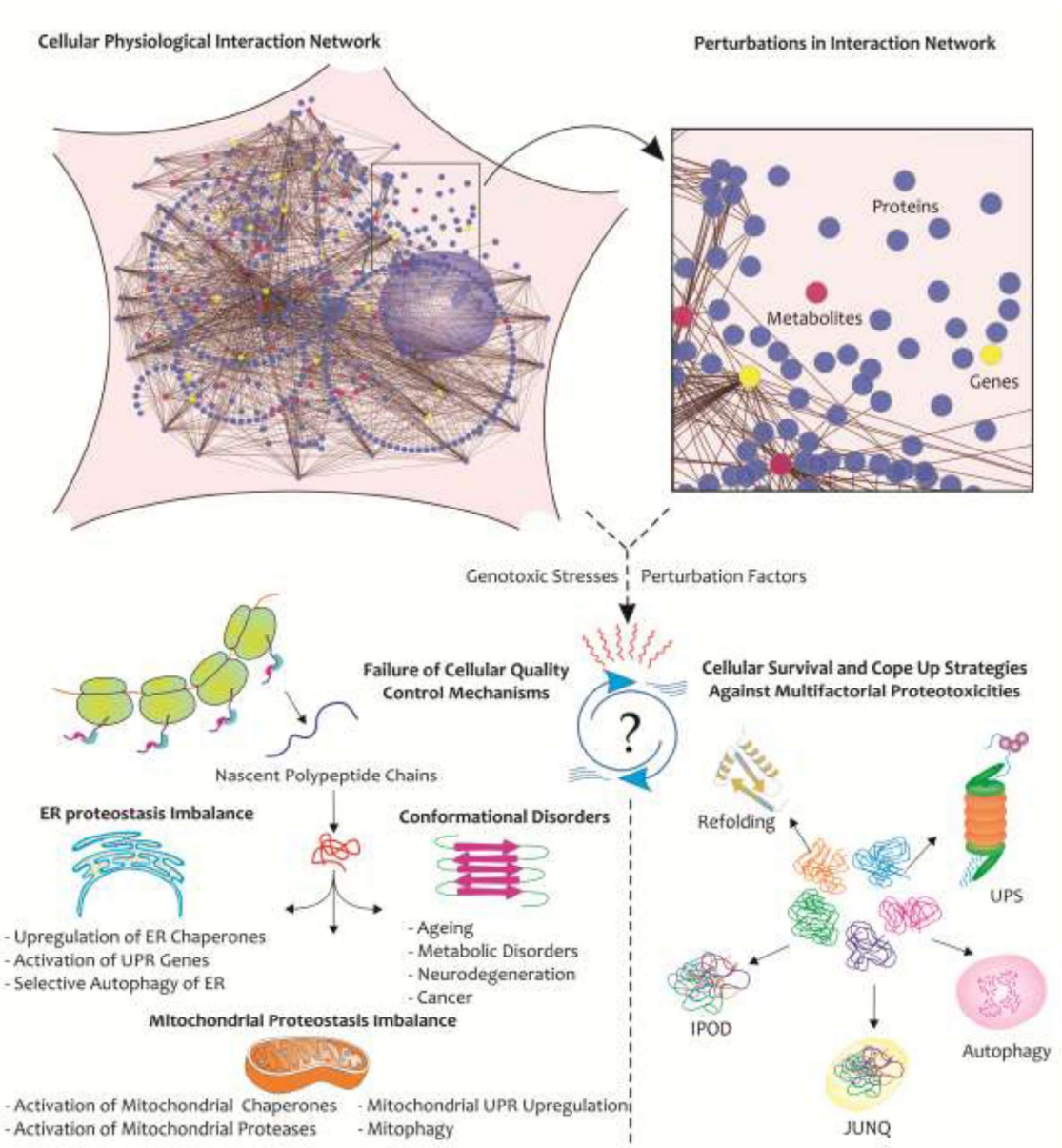

Figure 2.1 : *Downstream effects of proteome disturbance :* An exemplary representation of an interactome composed of genes, proteins and metabolites. Protein quality control mechanism (PQC) is represented as an integrated sub-network of this interactome involved in providing a healthy proteome, leading to a homeostatsis state. On exposure to perturbation factors, cells try to achieve state of proteostasis by employing various protein quality control mechanisms which include refolding, sequestration and degradation. Failure in PQC mechanisms may result in accumulation of unwanted and aberrant praoteins which may lead to proteostasis imbalance in vital cellular organelles such as endoplasmic reticulum and mitochondria.

2.2 AN OVERVIEW OF CELLULAR PROTEOSTASIS STRATEGIES

As mentioned in the previous section, to keep cellular proteome working properly and protect from diseases, cells employ various quality control mechanisms at different levels during course of protein synthesis. A schematic diagram of those mechanisms has been represented in Figure 2.2. Following subsections provides are brief overview of those mechanisms.

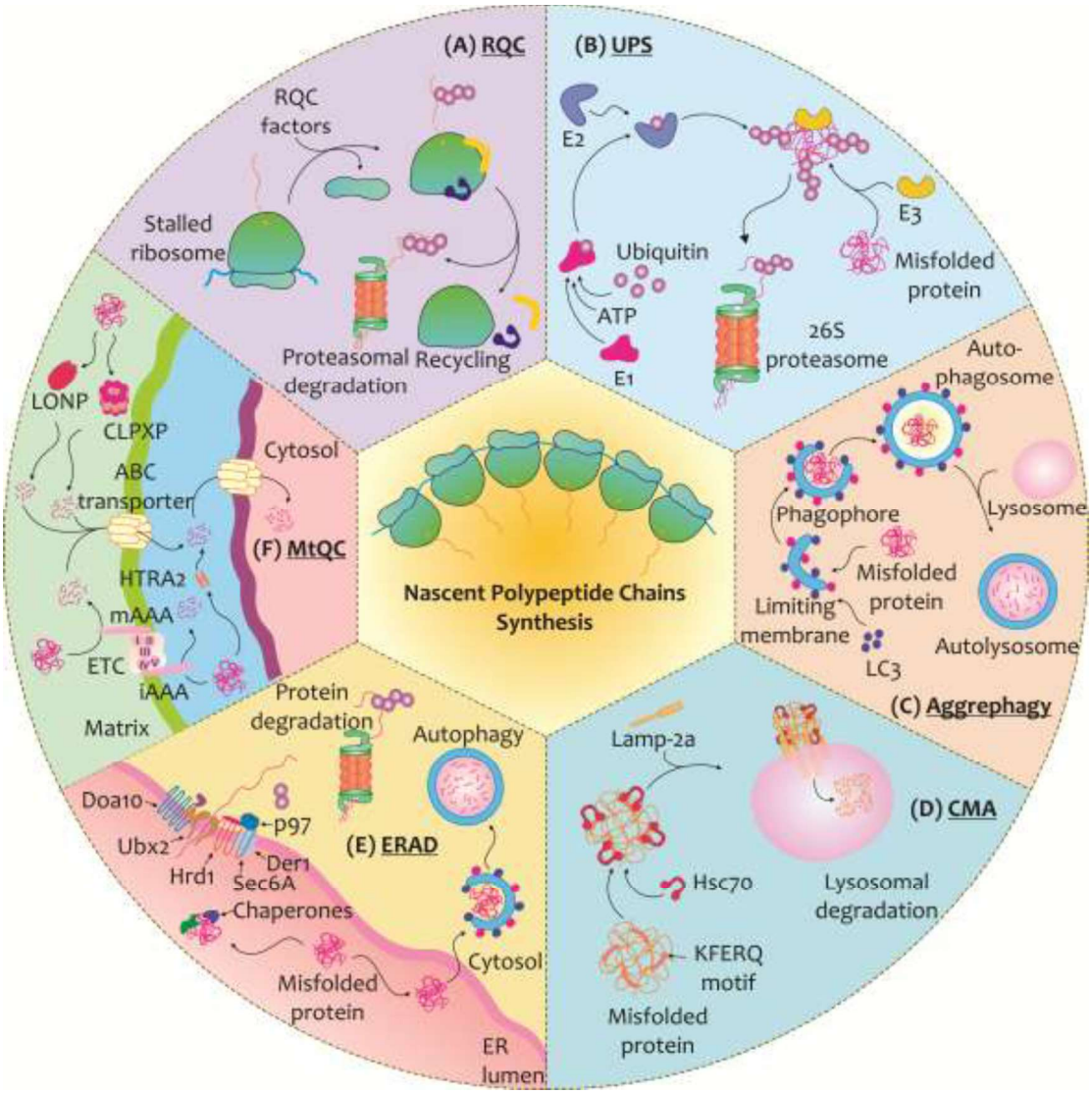

Figure 2.2 : *Schematic representation of various strategies to protect nascent polypeptide chains against misfolding:* (A) Ribosome-associated complex (RAC) and nascent polypeptide-associated complex (NAC) monitors efficient dispersal of newly synthesized polypeptide chains. (B) Ubiquitin proteasome system performs intracellular protein degradation, using a very specific approach via assistance of E3 ubiquitin ligases. (C) Unwanted bulks of cellular protein aggregosomes are cleared by process of aggrephagy. (D) Chaperone-mediated autophagy recognizes specific motif containing substrates and degrades them through lysosomal pathway. (E) There exists a separate set of ER chaperones and ER associated QC E3 ubiquitin ligases, which identify and ubiquitinate respectively, ER resident incorrectly folded proteins to translocate them to the cytosol, where proteasomal degradation of such ERAD substrates take place. (F) Mitochondria are another cellular subsystem, which separately possess its own quality control system, i.e. mitochondrial quality control (MtQC). Diverse mitochondrial proteases in mitochondrial matrix, IMM, and OMM contribute majorly in MtQC.

2.2.1 Co-translational Protein Quality Control Mechanism

It is important for the cells to prevent formation and accumulation of abnormal proteins at the initial stage of protein synthesis, the co-translational QC mechanisms act at the ribosomal sites, where proteins are synthesized and guide newly synthesized polypeptide chains into properly folded functional proteins[Hartl and Hayer-Hartl, 2002] . The co-translational QC mechanism is equipped with two complexes; nascent polypeptide-associated complex (NAC) and ribosome-associated complex (RAC) which are involved in protecting nascent

polypeptide chains from unwanted interactions and stabilize them [Amm *et al.*, 2014; Hartl and Hayer-Hartl, 2002]. Occasionally, transcriptional errors may lead to formation of defective mRNAs, like non-stop mRNA, that may result in ribosomal stalling. Cells overcome such situations with help of surveillance mechanism that includes Dom34/Pelota-Hbs1 and ATP binding cassette subfamily E member 1 (ABCE1) which senses stalled ribosome and disassemble ribosome into its subunits causing mRNA to dissociate, leading to its degradation [Inada, 2017; Pisarev *et al.*, 2010; Shoemaker and Green, 2011]. Further, E3 ubiquitin ligase like Listerin ubiquitinates the defective nascent polypeptide chain that may lead to proteasomal degradation [Shao *et al.*, 2013]. Other E3 ubiquitin ligases like Histone E3 ligase (Hel2) and Not4 have also been suggested to be involved in co-translational quality control [Duttler *et al.*, 2013; Panasenko, 2014]. Thus, quality control mechanism at the ribosome level plays a crucial role in preventing aberrant translation leading to aggregation and proteotoxicity. However, more studies are still needed to uncover underlying hidden mechanisms to gain further understanding of this crucial process.

The quality control mechanisms present at transcriptional and translational levels protect cells regularly from the formation of misfolded proteins. However, mutations, stress conditions and failures at transcription and translational levels may result in formation of misfolded proteins and toxic protein aggregates [Amm *et al.*, 2014]. To counter such situations, a cell is equipped with another set of QC system termed as post-translational protein quality control. Here, misfolded proteins are recognized by protein folding machineries called as chaperones that try to refold them into functional forms or target them to protein degrading pathways such as ubiquitin proteasome system and autophagy [Ciechanover and Kwon, 2015; Wickner, 1999]. These mechanisms will be further discussed in other section. Together, to establish a properly working and tightly regulated proteome network, comprising huge number of proteins, which keep on varying as per requirement, the cell has to be equipped with an efficient quality control mechanism. The elasticity these quality control mechanisms exhibit by getting precisely activated in response to a particular requirement in constantly changing dynamics of cellular environment, makes it a quite interesting and attractive system to gain further insights.

2.2.2 Ubiquitin Proteasome System Mediated Protein Quality Control

Cellular proteins are synthesized by ribosomes, whereas their maturation takes place at the endoplasmic reticulum (ER) membranes, to later get transported over their respective cellular locations [Braakman and Bulleid, 2011]. Proteins have certain half-lives, which are the major determinants for their cellular presence, activities and functions [Eden *et al.*, 2011; Plotkin, 2011]. To ensure the best possible regulation over their functions, cells have chosen well-regulated specific degradation machinery, which tags proteins, to be degraded, very specifically, through a small ubiquitin molecule, and directs them to a large barrel-shaped multi-protein proteolysis unit called proteasome [Hershko and Ciechanover, 1982; Hough *et al.*, 1987]. The cascade of reactions is catalyzed by three different kinds of enzymes: E1 ubiquitin activating, E2 ubiquitin conjugating and E3 ubiquitin ligase enzymes. E1 activates small ubiquitin molecules in an ATP-dependent manner, which is later conjugated to E2 enzymes. Thereafter, E3 ubiquitin ligases attach these ubiquitin molecules to the substrates proteins [Hershko and Ciechanover, 1992; Hershko and Ciechanover, 1982]. There are other enzyme classes, known as, E4 and deubiquitylating enzymes (DUBs), which also play important roles in maintaining the homeostasis of the cell. E4 enzymes lengthen the ubiquitin chain, whereas DUBs release free ubiquitin molecules once the substrate protein enters the proteasome [Koegl *et al.*, 1999; Reyes-Turcu *et al.*, 2009]. The specificity of the system is generated by a large number of E3 ubiquitin ligases, which target their specific substrates, for proteasomal degradation [Finley, 2009].

In the past, another mechanism of proteasomal degradation of non-native cellular proteins has been proposed, i.e., chaperone assisted proteasomal degradation (CAP) [Meacham *et al.*, 2001; Patterson and Höhfeld, 2008]. In CAP, ubiquitinated proteinaceous inclusions are identified by chaperones Hsc70 and are delivered to proteasome for their degradation [Arndt *et al.*, 2007; Kettern *et al.*, 2010]. Co-chaperones Hsp40 and CHIP play important roles in the functional regulation of chaperone-assisted degradation [Joshi *et al.*, 2016; Shiber and Ravid, 2014]. The proteins, which are degraded by CAP, are involved in multiple crucial cellular pathways, ranging from signaling to apoptosis; therefore, its own regulation by co-chaperones is also equally important, in order to accomplish its cellular tasks [Kettern *et al.*, 2010]. The limitation of proteasome system lies in its incapability to degrade bulky mass of protein aggregates, or insoluble inclusions present inside neuronal cells, as reported in a number of neurodegenerative diseases [Bence, 2001; Bennett *et al.*, 2005; Venkatraman *et al.*, 2004].

2.2.3 Autophagy Mediated Protein Quality Control

To overcome the limitations of the proteasome, cells encompass a dynamic recycling system, termed as 'autophagy', which not only removes bulky masses from the cytoplasm, including old or damaged cell organelles and insoluble protein inclusions, but also produces energy, and provide new building blocks for further renovation inside the cells [Mizushima and Komatsu, 2011; Ravikumar *et al.*, 2010]. Although the importance of lysosomal lysis of intracellular substances is known for around hundred years, yet the essence of this organelle for proteostasis has been established recently. Cytoplasmic protein inclusion bodies are delivered to lysosomes by similar ubiquitin like modifications, as happens in UPS degradation, and is digested thoroughly by a set of lysosomal enzymes [Cuervo, 2008; Klionsky, 2007]. As cellular proteostasis could be defined in simpler terms as a fine balance between biosynthesis and turnover, it is now believed that insufficiency of this system with increasing age could be a major cause of many diseases including cancer and neurodegeneration [He and Klionsky, 2009].

Although, the autophagy system is a bulk degradation pathway, still its own regulation at transcriptional and translational levels is very important for many physiological pathways running inside the cell. Involvement of a number of genes in its regulation over the induction and the magnitude of autophagy is indicative of its regulatory importance. Several attempts to modulate the system, in order to maintain its balanced state, are under investigation [Feng *et al.*, 2015; Ohsumi, 2013]. In general terms, autophagy refers to macroautophagy (MA), which involves a bulk degradation process of a whole cytosolic region, by the formation of autophagosome, followed by fusion with lysosomes and in turn its digestion [Mizushima *et al.*, 2008]. But, some specialized and selective forms of lysosomal degradation of proteins also exist inside the cells which are: chaperone mediated autophagy (CMA), and chaperone-assisted selective autophagy (CASA). Similar to E3 ubiquitin ligases, chaperones are also involved in the recognition of aberrant cytosolic proteins, which are not acted upon by UPS, for their clearance from the cell [Feder and Hofmann, 1999]. Chaperones also redirect a large number of substrates for lysosomal degradation [Kettern *et al.*, 2010].

One characteristic feature of such proteins which are targeted by chaperones for autophagic degradation is the presence of a pentapeptide motif 'KFERQ', without which their recognition is abolished [Cuervo, 2011; Dice *et al.*, 1986]. This degradation mechanism does not require the formation of vesicle; instead the substrate is picked from crowded cytosol, by Hsc70 chaperones and attached to the outer lysosomal membrane, through the cytosolic tail of lysosome-associated membrane protein type 2A (LAMP-2A) [Cuervo, 2010]. After binding, substrate unfolds its three-dimensional structure and gets internalized into the lysosome in a linear form, where degradatory enzymes cleave them with the help of lysosomal chaperones [Kaushik and Cuervo, 2012]. The rate limiting step of this system is the substrate binding with the transiently multimerized LAMP-2A, which are later disassembled by membrane bound Hsc70 molecules [Arias and Cuervo, 2011]. The consequences of CMA dysfunction in various

neurodegenerative diseases have been investigated in past; which is indicative of its active involvement in clearance of misfolded proteins [Cuervo and Wong, 2013].

CASA is another mechanism of selective autophagy of protein aggregates, which is orchestrated by chaperones Hsc70 along with few associated co-chaperones [Arndt *et al.*, 2010; Upadhyay *et al.*, 2015]. Hsc70 and HspB8 bind to target cargo protein, which is later ubiquitinated by CHIP, a co-chaperone, whereas B-cell lymphoma 2 (BCl2)-associated athanogene 3 (BAG3), another co-chaperone mediates this association [Kaushik and Cuervo, 2012]. Upon ubiquitination, this cargo becomes identifiable to an adapter protein p62, which, through its interaction with phagophore protein light chain 3 (LC3), mediates formation of autophagosome [Gamerdinger *et al.*, 2009; Pankiv *et al.*, 2007]. Bulky aggregates of huntingtin and SOD1 are targeted for degradation through this autophagy pathway. Unlike CMA, where Hsc70 is dissociated before engulfment, CASA consumes chaperones, through which cargo transport is mediated [Kaushik and Cuervo, 2012].

Protein synthesis takes place on ribosomes, present at the membranes of rough endoplasmic reticulum (ER) and the nascent polypeptides are cotranslationally transferred through translocon complexes, into the lumen of the ER [Ellgaard, 1999]. Most of the proteins undergo proofreading, put up by ER itself, before being forwarded to secretory pathways [Wang and Hebert, 2003]. ER lumen contains a high proportion of glycan-dependent chaperones, folding sensors and enzymes [Gething and Sambrook, 1992]. Ion concentrations and redox conditions are also very different from cytosol. This provides a suitable *milieu* to covalently modify new polypeptide chains, which is also essential for their proper folding [Ellgaard and Helenius, 2003]. Synthesis and maturation processes of native polypeptide chains sometimes results in generation of misfolded or incorrectly folded proteins, which unlike normal proteins, are retained inside the ER. Chaperones and folding enzymes like BiP, protein disulfide isomerase (PDI), calnexin, calreticulin, and GRP94 etc. provides the retention capacity to ER [Ellgaard, 1999]. These obnoxious ER-retained proteins are prone towards aggregation, and hence need a proper clearance from ERAD, as well as cytoplasm [Klausner and Sitia, 1990; Lippincottschwartz, 1988].

2.2.4 Protein Quality Control in Endoplasmic Reticulum

For selective degradation of ER retained proteins, few E3 ubiquitin ligase complexes are present on ER membranes [Hirsch *et al.*, 2009]. Hrd1-Derlin is one such well-known E3 ubiquitin ligase complex, present in yeasts as well as human, which along with its associated proteins, is found to be involved in selective ubiquitination and translocation of misfolded proteins to cytosol through Sec61 translocation system [Bays *et al.*, 2001; Wiertz *et al.*, 1996]. Yeast Cdc48 or mammalian AAA-ATPase p97 proteins play crucial roles in these retrotranslocation steps [Römisch, 2006]. Sel1 or Ubx2 proteins are also required to link Cdc48 complex with the Hrd1-Derlin or Doa1 E3 ubiquitin ligase complexes [Neuber *et al.*, 2005; Schuberth and Buchberger, 2005]. Ubc1 and Ubc7 are E2 ligases that are recruited to membrane bound E3 ubiquitin ligase complexes with the help of another protein Cue1 (coupling of ubiquitin conjugation to ER degradation 1) to selectively ubiquitinate ER substrates [Biederer *et al.*, 1997; Hirsch *et al.*, 2009]. Another E3 ubiquitin ligase identified in yeast ERAD system is Doa10, which has been less explored. Similar to Hrd1, it also has Ubc6 and Ubc7 as E2 partners [Kreft *et al.*, 2005]. Transmembrane protein autocrine motility factor receptor (AMFR) is also an E3 ubiquitin ligase, which has been characterized with ERAD for ubiquitinating misfolded proteins [Fang *et al.*, 2001]. It is believed that ER lumen itself does not contain proteasome or other UPS components. The misfolded proteins, once ubiquitylated are retrotranslocated to cytoplasm; and then targeted to proteasome for their degradation [Christianson and Ye, 2014].

2.2.5 Protein Quality Control in Mitochondria

Mitochondria are the another subcellular systems, homeostasis of which is very critical for overall cellular health [Tatsuta and Langer, 2008]. Aging could also be associated with decrease in mitochondrial health and proteostasis, which may later lead to many age-related diseases [López-Otín *et al.*, 2013]. Cellular proteostasis systems continuously monitor mitochondrial health, and if found any dysfunctional mitochondria, it is engulfed by lysosomal vesicles, which subsequently removes mitochondria from cytosol by a process, known as mitophagy [Youle and Narendra, 2011]. Recent advances have shown that Parkinson's related genes kinase PTEN-induced putative kinase protein 1 (PINK1) and parkin play major roles in mitophagy. PINK1, an outer mitochondrial membrane (OMM) kinase detects any damage in mitochondria with some yet to be elucidated mechanism, followed by phosphorylation of cytosolic parkin, a well known E3 ubiquitin ligase [Scarffe *et al.*, 2014]. Parkin translocates to mitochondria, where it ubiquitinates mitochondrial substrates in Lys-63 manner and delivers the damaged mitochondria to autophagosomes for their clearance [Narendra *et al.*, 2010]. A broader term, mitochondrial quality control (mtQC), is used to describe various aspects and mechanisms involved in maintaining functional health of the cellular power house [Rugarli and Langer, 2012]. Functions of mtQC range from controlling production of reactive oxygen species to degradation of proteinaceous inclusions inside mitochondria [Baker and Haynes, 2011]. To meet all these requirements, mitochondria have
a set of proteases, which are involved in regulated maturation of mitochondrial proteins (with the help of chaperones), as well as their proteolysis, if required [Hamon *et al.*, 2015; Voos and Röttgers, 2002]

ATPases associated with diverse cellular activities (AAA) are major proteases involved in mtQC [Langer *et al.*, 2001]. Intermembrane space and matrix AAA (iAAA and mAAA) proteases, Lon protease homologue (LONP), Clp protease proteolytic subunit (CLPP) are ATP dependent proteases which assemble themselves to form multimeric complexes having proteolytic compartments [Quirós *et al.*, 2015; Rugarli and Langer, 2012]. Damaged electron transport chain (ETC) components are also removed by these AAA proteases. LONP and CLPP are serine proteases which specifically identify and degrade misfolded proteins of the matrix [Quirós *et al.*, 2015]. HTRA2 enzyme has also been identified with important proteolytic roles in maintaining mitochondrial homeostasis. A trimer formed by HTRA2 in mitochondrial intermembrane (IM) space where it chiefly regulate many misfolded proteins [Clausen *et al.*, 2011]. ATP23, a metalloproteases is found in intermembrane space and helps iAAA in maintenance of ETC component proteins [Osman *et al.*, 2007]. ATP-binding cassette (ABC) transporters on mitochondrial membranes release broken small peptides from mitochondria matrix to IM space and then they are delivered to cytosol, where further degradation takes place to release free amino acids [Young, 2001]. Under stress conditions, or when damaged proteins are accumulated inside the mitochondria, it may confer another combating response to provide protection from stress-like conditions [Haynes and Ron, 2010]. Such a coordinated response is known as mitochondrial unfolded protein response (UPR), which mount signals to the nucleus to synthesize new proteases and chaperones which can take over the load of unfolded proteins accumulated inside the mitochondria [Zhao, 2002]. Interestingly, accumulation of K48 and K63 polyubiquitinated proteins has been associated with mitochondrial mediated cell death under the state of proteasomal dysfunction [Sun *et al.*, 2009] .

2.2.6 Protein Quality Control in the Nucleus

In order to properly regulate the expression of genetic material, the state of proteostasis inside the nucleus is an obvious requisite for a cell. However, as compared to cytoplasm and endoplasmic reticulum the understanding of proteostasis mechanism in nucleus is lacking [Jones and Gardner, 2016]. According to current understanding, mostly nuclear proteins are synthesized and imported from cytoplasm through nuclear pores, which is expandable and aqueous in nature [Gallagher *et al.*, 2013]. The aqueous nature of nuclear pore provides a path to

import nuclear proteins in a fully folded conformation, reducing the burden of nucleus to fold these proteins. Still, the need of restructuring cannot be excluded for some large molecules due to constriction occurred while passing through these pores [Alberts B, 2002]. So far, different studies have provided clues of involvement of chaperone machinery, ubiquitin proteasome system and autophagy in maintaining nuclear proteostasis. Chaperones such as Hsp26 [Willsie and Clegg, 2002], Hsp70 [Chughtai *et al*., 2001], and Hsp90 [Tapia and Morano, 2010] have been found to relocalize in nucleus in response to stress which might be a protective response. Interestingly, chaperones or co-chaperones like Hsp70 and Hsp40 have been found to colocalize with aggregates or inclusions formed by TDP43 and polyglutamine expansions of huntingtin and ataxin proteins [Gallagher *et al*., 2013; Udan-Johns *et al*., 2013], which might have protective roles.

The involvement of ubiquitin proteasome system in removing aberrant proteins from nucleus was initially observed in *Saccharomyces cerevisiae* [Gardner *et al*., 2005]. They identified nuclear E3 ubiquitin ligase, San1p along with E2s Cdc34p and Ubc1p, that ubiquitinates misfolded nuclear protein and targets for proteasomal degradation. Further report also indicated that this E3 ubiquitin ligase with the help of a cytosolic chaperone Hsp70 (Ssa1p), recognizes and interacts with nucleotide binding domain 2 (NBD2) domain of Ste6p (an ERAD substrate) leading to its proteasomal degradation [Guerriero *et al*., 2013]. This study can be considered as an example of the crosstalk that exists between nuclear and cytoplasmic protein quality control components in a cell. Another E3 ubiquitin ligase, Mahogunin ring finger 1 (MGRN1) was recently shown to redistribute in nucleus from cytoplasm as a result of proteasome impairment in aging neurons, aiding in protective response against proteotoxic stress [Benvegnù *et al*., 2017]. Previously, MGRN1 nuclear presence with Huntingtin protein has been reported [Chhangani *et al*., 2014]. Similarly, it has also been observed that Degradation of alpha2-10 (Doa10), an ER situated E3 ubiquitin ligase also localizes to inner nuclear membrane and takes part in nuclear protein quality control [Boban *et al*., 2014].

A form of autophagy, termed as nucleophagy also aids in maintaining integrity of nucleus by digesting unwanted nuclear regions or material [Mijaljica and Devenish, 2013]. The role of autophagy in maintaining nuclear homeostasis was also confirmed from the study by [Park *et al*., 2009] that showed nuclear components in autophagosomes and increase in nuclear abnormalities on inhibition of autophagy. Additionally, the autophagy protein light chain 3/Autophagy-related protein 8 (LC3/Atg8) has been recently shown to have nuclear presence. This study also observed the role of LC3 in restricting tumorigenesis through senescence induction by degrading nuclear lamina protein lamin B1 in response to oncogenic insults [Dou *et al*., 2015]. Thus, the above mentioned studies provide considerable evidence that like ER and mitochondria, proteostasis mechanism is also present for nucleus that work along with cytoplasmic proteostasis components in maintaining a stable nuclear protein environment. The association of different neurodegenerative diseases including Huntington's disease and spinal cerebellar ataxias with nuclear protein aggregates or inclusions [Gallagher *et al*., 2013] shows critical role played by nuclear proteostasis mechanism in maintaining homeostasis at organism level. Despite these studies further research is still required to unravel signaling mechanisms involved in sensing nuclear proteostasis. It would be interesting to further elucidate how crosstalk between different proteostasis pathways both inside and outside nucleus works. Also, finding downstream effects of nuclear protein aggregates or inclusions along with their affects on other vital nuclear processes will be crucial to gain insights of link between nuclear proteostasis and disease occurrence.

2.3 UTILITY OF CELLULAR PROTEOSTASIS MECHANISMS IN THERAPEUTIC INTERVENTIONS

At many instances, the quality control pathways get interrupted by various cellular or environmental factors and lead to multiple disease conditions, like neurodegenerative disorders, cancer and aging [Cook *et al.*, 2012; Kabashi and Durham, 2006]. Hence, the question arises, how to induce the clearance capacity of cellular quality control system to compensate the loss of physiological functionality. The organisms defense system includes molecular chaperones, heat shock proteins, UPR, integrated stress responses, and adaptive protein degradation mechanisms play a key role in cellular survival [Schneider and Bertolotti, 2015]. Apart from the efforts made by components of cellular quality control mechanism, sometimes it is important to provide few external stimuli to maintain the intracellular proteome balance [Harper and Bennett, 2016]. Upcoming sub-sections provide the detailed description about the cellular pathways and their molecular partners, which are directly related to the maintenance of functional proteome. Figure 2.3 is a systematic representation of possible components of cellular quality control mechanisms that have the ability to remove aberrant proteins from cell, which can be used for therapeutic purpose.

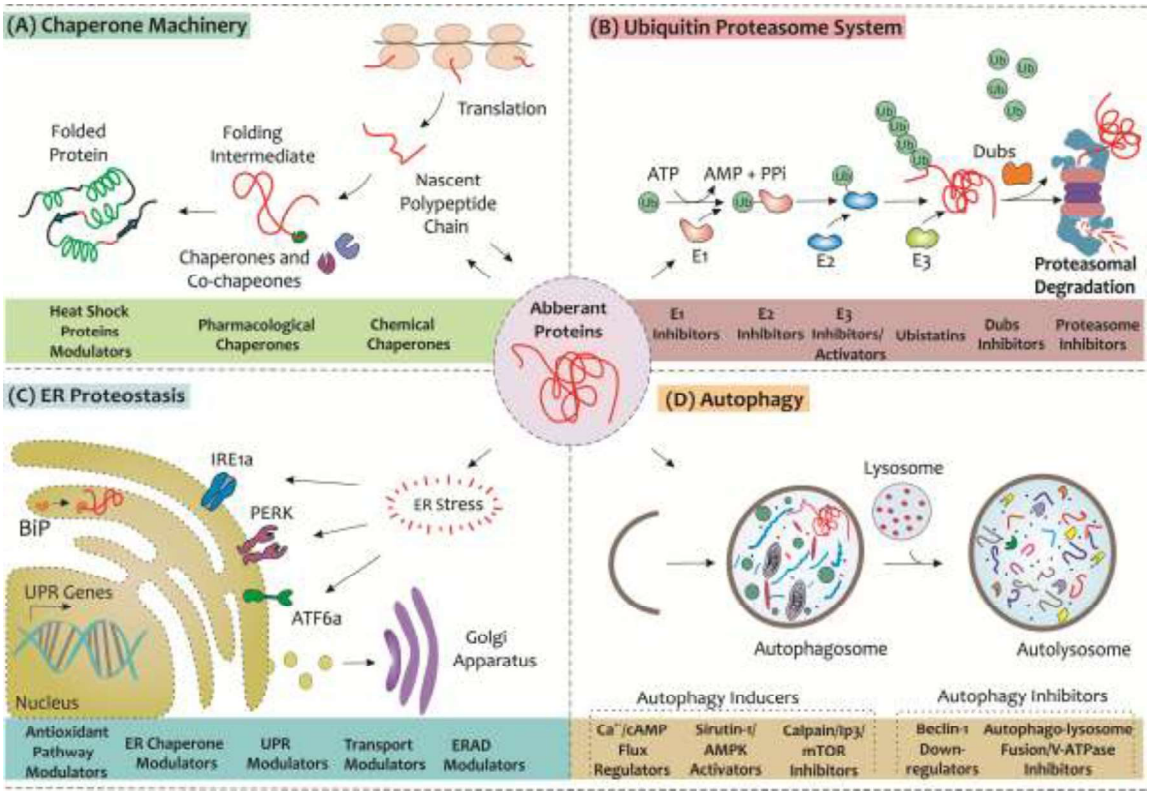

Figure 2.3 : *Diagrammatic representation of target mechanisms for faster clearance of multifactorial proteotoxic load generated by aberrant protein for therapeutic implication :* Various components that have been utilized and identified in four key molecular mechanisms, i.e. chaperone machinery, ubiquitin proteasome system, endoplasmic reticulum stress response and autophagy, involved in proteostasis maintenance are represented as therapeutic targets. Panels below each mechanism mentions those targets i.e. (A) Induction or inhibition of heat shock proteins and synthesis or screening of molecules that can work as chaperones (pharmacological and chemical chaperones). (B) Activation or inhibition of different components of UPS including E1, E2, E3, ubistatins, DUBs and proteasome. (C) Modulation of ER stress response by modulating antioxidant pathway, ER chaperones, UPR and ERAD and (D) Various upstream and downstream signaling molecules involved in autophagy, regulated by natural or synthetic molecules have shown beneficial outcomes to counter diseases, like cancer and neurodegeneration and hence need to be explored more for therapeutic purposes

Following subsections provides an overview of different proteostasis mechanisms that has been looked upon to be utilized as therapeutic targets:

2.3.1 Chaperone Machinery

To deal with the disaster of proteotoxic load of aberrant proteins, chaperones or heat shock proteins are the first point, which are responsible for folding of nascent polypeptide chains into properly folded functional proteins [Wang and Segatori, 2013]. Work on modulation of heat shock proteins has started with the regulation of heat shock transcription factor 1 (HSF1) [Anckar and Sistonen, 2011]. There are numerous chemicals like MG132 and natural compounds including celastrol etc., which regulate the HSF1 activity in multiple manners, have a high potential to be used as therapeutic agents for diseases linked with the intracellular misfolded protein accumulation and aging [Westerheide and Morimoto, 2005]. Recently, apoptozole was also discovered as small molecule which binds specifically to Hsp70 and inhibits its ATPase activity, and leads to induction of apoptosis in cancer cells [Berman *et al.*, 2014]. A new 4, 5-diaryloxazole adenosine triphosphate-binding site inhibitor, NVP-AUY922, of another specific heat shock protein Hsp90 has been exploited to induce apoptosis and give beneficial outcomes in oral squamous cells cancer [Okui *et al.*, 2013]. Other strategy developed to reduce the proteotoxicity was, use of pharmacological chaperones such as nicotine, γ-aminobutyric acid (GABA) etc. that modulate the activities of lysosomal enzymes, ion channels, G-protein-coupled receptor (GPCR) to stabilize the target protein and increase folded protein population inside the cells in disease conditions like epilepsy, cystic fibrosis and many more [Wang *et al.*, 2014]. The same effect was also observed in pharmacological retromer chaperones and others such as galactose for neurological diseases including Alzheimer's and non neurological diseases like lysosomal storage and endosomal disorders [Berman *et al.*, 2014; Parenti *et al.*, 2015]. Chaperones based methods or chaperones mediated autophagy have also been used as a better alternate of enzyme replacement therapy for neurodegenerative, lysosomal and other diseases, such as treatment of cystic fibrosis by molecular stabilizers like glycerol [Suzuki, 2014; Xilouri and Stefanis, 2015]. More efforts need to be done in future before using pharmacological chaperones for treatment purposes to provide specificity and to obtain maximum outcomes.

2.3.2 Ubiquitin Proteasome System

Ubiquitin proteasome system efficiently functions to recognize specifically aberrant proteins for their elimination and maintain a cellular proteostatic balance between synthesis and degradation of proteins. The exploration of different components of UPS protein cascade for therapeutic purposes involves E1 activating enzyme inhibition by natural compounds including panepophenanthrin and himeic acid obtained from mushroom and mould respectively, whereas E2 conjugating enzyme UbcH10 has been inhibited by RNA interference for treating cancer cells [Berlingieri *et al.*, 2006; Eldridge and O'Brien, 2010]. But both these strategies showed less impact, as E1 and E2 enzymes have a global degradatory function over UPS inside the cell; hence can affect the whole system. Another class of proteins involved in the UPS, deubiquitinase (DUBs), were also targeted to fight cancer by chemical compound like b-AP15, which inhibits DUBs; USP14 and UCHL5, which are directly associated with proteasome and hence shows some possibilities for drug development [D'Arcy *et al.*, 2011; D'Arcy and Linder, 2012].

E3 ubiquitin ligases that are known for providing specificity to this system in the selection of aberrant proteins have been modulated, activated or inhibited according to need of diseases and pharmacology to treat that particular disease [Edelmann *et al.*, 2011]. Nutlin-3a, act as a competitive inhibitor of HDM2, which was further found to be inhibited by HLI98 and RITA [Issaeva *et al.*, 2004; Vassilev, 2004; Yang *et al.*, 2005]. Other examples of E3 ubiquitin ligase inhibition are SCF skp2 by CpdA, cereblon by thalidomide, MuRF1 by P013222 etc., which show some positive effect in neurodegeneration and cancer [Chen *et al.*, 2008; Eddins *et al.*, 2011; Ito *et al.*, 2010]. The inhibition of the proteolytic machinery of the UPS, 20S proteasome is not a new

thought, as it was observed that its inhibition, by small molecules induces apoptosis in cancer cells [Voorhees and Orlowski, 2006]. The most studied chemical example of this mechanism is bortezomib, a well known proteasome inhibitor proved to be beneficiary in both animal models and patients suffering with multiple myeloma [Hideshima *et al.*, 2005]. Other than bortezomib, carfilzomib, MLN9708, CEP-18770 and few others are proteasome inhibitors under phase-I or phase-II clinical trials [Crawford *et al.*, 2011]. Interestingly, proteasome inhibitors have also been found to have applications in diseases like malaria, microbial infection and autoimmune disorders [Crunkhorn, 2016; Hsu *et al.*, 2015; Lin *et al.*, 2009]. However, they have been approved clinically in the treatment of multiple myeloma and mantle cell lymphoma [Holkova and Grant, 2012; Moreau *et al.*, 2012]. Utilization of UPS system for drug development is increasing leaps and bounds, and has reached up to conjugation of protein with ubiquitin molecules [Bedford *et al.*, 2010; Huang and Dixit, 2016]. But still a huge gap between research and drug development is needed to be filled in upcoming years.

2.3.3 Endoplasmic Reticulum Stress Response Pathway

Various steps in protein quality control system not only help in maintenance of proteome balance inside the cells, but they also play a significant role in the generation of proteome equilibrium in all the cellular compartments [Wolff *et al.*, 2014]. Most of the ER resident proteins are translocated there at the time of translation and folding, therefore to maintain the ER proteostasis, it is essential that ER chaperones function properly to avoid an ER stress linked conditions [Wang and Kaufman, 2016]. Chaperones and E3 ubiquitin ligases residing inside ER, such as HRD1, are induced by ER stress, and this property of induction makes both these molecules target for neurodegenerative diseases like Alzheimer's and Parkinson's [Kostova *et al.*, 2007; Nomura *et al.*, 2016]. Gp78, the ERAD E3 ubiquitin ligase has also been explored for reducing ER stress [Chen *et al.*, 2014]. There is a long list of natural compounds like vitexin, which increase chaperone Hsp90 during ER stress and studied for drug development [Liu *et al.*, 2016]. Few researchers suggested that antioxidant pathway modulation is one of the solutions for ER stress, e.g., a lipid soluble antioxidant butylated hydroxyanisole has been found to reduce ER stress under *in vitro* conditions [Malhotra *et al.*, 2008]. ER itself have an UPR system to fight against a load of unfolded proteins and activation of UPR by MG132, radicicol and 17-AAG (17-N-allylamino-17-demethoxygeldanamycin) etc. are under preclinical trials for cancer treatment [Hetz *et al.*, 2013]. ERAD pathway specifically works for QC of ER proteins, which makes it a good therapeutic target for treatment of ER stress mediated pathologies [Kim *et al.*, 2008]. Some protein targets found, do not only function to control ER protein homeostasis, but also do similar function for Golgi complex [Wlodkowic *et al.*, 2009].

2.3.4 Autophagy

In cellular conditions, where other proteostasis mechanisms get ineffective or inefficient, the catastrophic event of the cell known as autophagy, takes charge of bulk removal of unwanted proteins from cells to keep proteome balanced [White, 2012; Williams *et al.*, 2006]. The whole process is carried out at two different levels: the smaller one microautophagy and massive one macroautophagy; both of these pathways could be selective or non-selective in bulk removal [Glick *et al.*, 2010]. The feature that shows the difference between these two processes are autophagosome formation in macroautophagy and vacuole invagination in microautophagy [Feng *et al.*, 2013]. Modulation of various signaling molecules of autophagy pathway, such as Ca2+/cAMP flux regulation by verapamil, AMPK activation by metformin or reduction by clonidine and rilmenidine, help in fast recovery of cells from aberrant proteins and various diseases [Puri and Chandra, 2014]. Targeting proteins involved in autophagy like mTOR inhibited by rapamycin in cell and animal models of neurodegenerative diseases and use of autophagy inducers such as trehalose, resveratrol etc. gives new hopes in therapeutic implications for aging and multiple neurological disorders [Tan *et al.*, 2014; Vidal *et al.*, 2014]. In lymphoproliferative disorders, targeting autophagy components with drugs, like chloroquine, causes p53-mediated cell death [Pujals *et al.*, 2015]. Some others like sorafenib reached up to

phase II clinical trials [Guidetti *et al.*, 2012]. PI3K and mTOR inhibitors including CAL-101, ridaforolimus, respectively, were also used to regulate autophagy and control disease conditions [Pierdominici *et al.*, 2014]. Beclin-1, which is involved in the autophagy stimulating mechanism, is downregulated to treat breast cancer, which gives new hopes in the direction of drug development [Rubinsztein *et al.*, 2007].

As discussed in the above section, formation of autophagosome, a vacuole with double membrane around the cytoplasmic bulk of aberrant proteins, which eventually merge with lysosome, represent macroautophagy [Feng *et al.*, 2015; Mehrpour *et al.*, 2010; Yang and Klionsky, 2010]. It is mainly considered as a cellular bulk clearance pathway of the cell, but also plays a cytoprotective role in some conditions like starvation or nutrient deprivation, and removes unwanted load of cytoplasmic misfolded proteins [Mizushima *et al.*, 2008; Moreau *et al.*, 2010; Yang and Klionsky, 2010]. The loss of autophagy genes like Atg7 in neurons cause neurodegeneration and acute condition lead to death, hence focusing autophagy is important for developing therapeutics against neurodegenerative diseases [Komatsu *et al.*, 2006]. Recently, some RNA and their complexes with ribonucleoproteins (RNPs) were also found as a part of phagophore for lysosomal degradation [Fujiwara *et al.*, 2013]. Similar studies suggest that RNA and RNPs serve as a regulator of macroautophagy and hence can be targeted in future for therapeutic implications [Frankel *et al.*, 2016]. Xenophagy, a special kind of selective autophagy where intracellular pathogens like *Mycobacterium tuberculosis* are targeted, is a novel method against infections caused by antibiotic resistance bacteria [Kimmey and Stallings, 2016]. The involvement of autophagy in various neurodegenerative, immune disorders and cancers is already well described; however, studies also elaborate its involvement in various oral diseases [Mizushima *et al.*, 2008; Tan *et al.*, 2016]. Understanding the complete mechanism of macroautophagy and associated pathological conditions may give a new direction in modulation of this pathway for the establishement of healthy cellular environment.

In case of microautophagy there is direct engulfment of cytosolic components as compared to multistep process for recruitment in macroautophagy [Li *et al.*, 2011]. Microautophagy could be non selective or selective microautophagy [Kiššová *et al.*, 2007; Kraft *et al.*, 2009]. This form of autophagy is normally observed in mammals, which includes engulfment of cytosolic mass of misfolded proteins and can be divided into multiple stages, i.e., invagination of lysosomal membrane and formation of autophagic tubes, vesicle formation, vesicle expansion, vesicle scission, vesicle degradation and recycling [Kunz *et al.*, 2003; Sahu *et al.*, 2011]. The selective microautophagy found in yeasts targets aberrant proteins of organelles viz. peroxisomes (micropexophagy), nucleus (piecemeal microautophagy) and mitochondria (micromitophagy) for quality control of cell [Farré and Subramani, 2004; Krick *et al.*, 2009; Lemasters, 2014]. A recent study has reported the functional importance of endosomal microautophagy in controlling neurotransmission by regulating synaptic protein turnover [Uytterhoeven *et al.*, 2015].

The mechanism of microautophagy has also been involved in maintaining organelle shape, in this mechanism a large tubular invagination is formed by vacuoles membrane that gives rise to vesicle bud and this inverse budding is similar to microautophagocytosis [Müller *et al.*, 2000]. Therefore, involvement of autophagy in such type of housekeeping functions makes this process one of the center to study different pathologies [Todde *et al.*, 2009]. Autophagy could be induced by nutrient limitation or by carbon and nitrogen starvation. Rapamycin, which inhibit TOR signaling pathway is one of the known inducer of piecemeal autophagy [Roberts *et al.*, 2003]. Other than rapamycin, many FDA approved drugs like clonidine, lithium etc. and nutritional supplements such as caffeine were studied as an inducers of autophagy [Levine *et al.*, 2015]. But still, there is a need to search more efficient autophagy inducers to obtain therapeutic importance of this process. Further, increased understanding of

microautophagy induction and its regulating mechanisms from drug discovery perspective can prove to be beneficial for cancer, neurodegenerative disorders and aging.

2.4 PROTEOSTASIS MECHANISMS AS TARGETS OF KNOWN NATURAL AND PHARMACEUTICAL MOLECULES

Folding and degrading mechanisms including trafficking pathways constitutes significant part of proteostasis network [Sklirou *et al.*, 2015]. Molecules isolated from natural resources have been shown to produce beneficial outcomes such as anti-aging properties [Argyropoulou *et al.*, 2013]. These naturally obtained molecules can also be used in cancer pathology as they shows less side effects and improved treatment for chemo-resistant tumors [Reddy *et al.*, 2003; Tan *et al.*, 2011]. Different classes of natural molecules, such as vitamins [Schaeffer *et al.*, 2014], isothiocyanates [Powolny *et al.*, 2011], flavonoids [Jinwal *et al.*, 2009] , alkaloids [Tsukamoto *et al.*, 2010], and fatty acids [O'Rourke *et al.*, 2013] have been reported to regulate proteostasis mechanisms showing potential in cancer and protein conformation disorders treatment. Natural compounds with their roles in the induction or inhibition of autophagy have also been studied, they control autophagic pathways by different mechanisms, and can cause apoptosis in cancerous cells [Ding *et al.*, 2014].

Interestingly, these molecules are also found to have an impact on the other protein degradatory pathway, i.e., UPS, such as by inhibition of proteasomal activity [Tsukamoto and Yokosawa, 2010]. However, the effectiveness of these molecules at clinical level is needed to be established [Bent, 2008]. For conversion of these naturally derived molecules into disease specific medications, a deep investigation of the affected molecular mechanisms is required. Despite limitations in terms of understanding due to their chemical complexities [Dias *et al.*, 2012], use of natural products in traditional medications over generations provides a strong evidence of their potential in therapeutic applications. Availability of advanced chemical approaches and computational techniques have regained interest of researchers in identifying new and potentiating efficacy of existing available natural molecules [Rodrigues *et al.*, 2016].

Apart from the naturally obtained proteostatic regulators, various synthetic chemical compounds utilized and developed for different applications have also been largely studied and screened for their roles in proteostatsis modulation. Pharmaceutical drug such as aspirin (NSAID) has been shown to affect proteostasis through inhibition of proteolytic degradatory activity of the proteasome [Dikshit *et al.*, 2006]. Furthermore, another NSAID ibuprofen downregulates the expression of Hsp70 chaperone enhancing antitumor activity of cisplatin [Endo *et al.*, 2014]. Drugs designed to target proteasome as a strategy to develop antitumor agents have been approved for treatment of multiple myeloma [Kane, 2003]. To reduce the undesirable side effects, such as protein aggregation in non cancerous cells other targets in ubiquitin proteasome system are also being investigated [Buckley and Crews, 2014]. Similarly, several other medications have also been reported to affect proteostasis which will be discussed in detail in further sub-sections

2.4.1 Natural Compounds

A small description of recent studies exploring beneficial roles of natural products as proteostatic regulators is given below.

(a) Vitamins

Vitamins are vital components of various biological processes occurring in the cell. They have been reported to modulate various mechanisms involved in proteostasis [Cao *et al.*, 2009; Høyer-Hansen *et al.*, 2010]. A recent study has shown Vitamin K3 as an inhibitor of Siah2 ubiquitin ligase leading to blocking of melanoma tumorigenesis which may be due to attenuation of hypoxia and MAPK signaling [Shah *et al.*, 2009]. It has been observed in past that vitamin D3 increases the levels of its nuclear vitamin D3 receptor molecules by inhibiting its

proteasome mediated degradation [Li *et al.*, 1999]. Role of vitamin D3 in autophagy regulation has also been studied extensively [Wu and Sun, 2011]. Similarly, members of vitamin E family like delta- tocopherol and alpha-tocotrienol have been shown to regulate proteasome activity and elevated levels of p27Kip1 and p53 [Munteanu *et al.*, 2007]. Mechanism elucidating role of vitamins in providing protein stability and activity has also been postulated such as in case of riboflavin [Henriques *et al.*, 2008]. Riboflavin therapy has been proved to be beneficial in patients with altered level of flavin adenine dinucleotide (FAD) [Vergani *et al.*, 1999], which inturn has pharmacological chaperone like effects on mitochondrial electron transfer flavoprotein enzyme [Henriques *et al.*, 2008].

Recently, role of vitamin k3 in inhibition of amyloid fibrils aggregation in hen egg white lysozyme (HEWL) and Aβ-42 peptide was reported [Alam *et al.*, 2016]. Despite evidences of therapeutic potential vitamins possess there are also limitations at various levels which pose challenges to use them for clinical purpose. As in case of vitamin D3 short half life and rapid catabolism limits its availability at the target site [Ramalho *et al.*, 2015]. Similarly, reduced stability due to oxidation caused by presence of excess oxygen in cell culture environment and lack of adequate animal models are barrier in investigating possible role of ascorbic acid in disease treatment [Michels and Frei, 2013]. However, use of advanced approaches like nanotechnology to increase bioavailability [Ramalho *et al.*, 2015] and genetically modified animals [Maeda *et al.*, 2000] for specific studies has helped in providing a step further to counter such problems and making vitamins clinically usable natural product.

(b) Natural Isothiocyanates

Natural isothiocyanates produced by hydrolysis of glucosinolates via myrosinase enzyme in cruciferous vegetables have been reported to have useful properties that can be utilized in treatment of cancer, cardiovascular diseases, neurodegeneration, diabetes and as an antibacterial agent [Dufour *et al.*, 2015; Fimognari *et al.*, 2012]. They contain N=C=S functional group, which gives them ability to act as an electrophile and react with nucleophilic moiety in protein and hence causing their modification [Fimognari *et al.*, 2012]. Isothiocyanates, such as benzyl and phenyl isothiocyanates inhibit deubiquitinating enzyme (DUBs) USP9x and UCH37, which protect degradation of anti-apoptotic proteins Mcl-1 and oncogenic fusion protein Bcr-Ab [Lawson *et al.*, 2015]. Sulforaphane, a well studied isothiocyanate, protects cells from hydrogen peroxide induced oxidative stress by enhancing expression level of proteasomal catalytic subunit and its peptidase activity in neuroblastoma cells [Kwak *et al.*, 2007]. Sulforaphane was also found to inhibit heat shock proteins such as Hsp70, Hsp90 and transcription factor HSF1, and upregulated expression level of apoptotic proteins such as Bad and Bax [Sarkar *et al.*, 2012].

Synergistically with arsenic trioxide sulforaphane increases apoptotic induction in multiple myeloma cells, as shown by increase in cleavage of caspase-3, caspase-4, reactive oxygen species (ROS) production, depletion of glutathione, Hsp90 expression and PERK activation in UPR pathway of ER stress [Doudican *et al.*, 2012]. Effect of phenethyl isothiocyanate on autophagy has also been reported as a result of suppression of phoshphorylation of Akt, mTOR and triggers Atg5-dependent autophagic and apoptotic cell death in prostate cancer cell line [Bommareddy *et al.*, 2009; Zhang *et al.*, 2014]. Sulforaphane also modulate proteolytic degradatory machinery of autophagy, by inducing the levels of LC3-II and increasing ROS production in pancreatic cells [Naumann *et al.*, 2011]. These studies on different isothiocyanates provide a view of influence of isothiocynates on various proteostatic pathways. Further research is also needed to provide sufficient evidences on establishing beneficial role of isothiocyanates for a particular disease, such as sulforaphane (a known antiproliferative agent) has also been shown to promote cell proliferation in human mesenchymal stem cells [Bao *et al.*, 2014]. Thus further studies leading to proper optimization and in depth characterization of these useful natural products can provide us with novel ways to counter various pathologies.

(c) Natural Phenols, Flavanoids and Flavonols

Naturally occurring alkyl phenol such as ginkgolic acid extracted from the leaves of *Ginkgo biloba L*, directly binds to E1 ubiquitin activating enzyme which disrupts formation of E1-SUMO complex, a critical modulator of cellular proteostasis [Fukuda *et al.*, 2009]. Polyphenol rich propolis extracts regulates the activation of transcription factor nuclear factor kappa B (NF-κβ) by inhibiting the autoubiquitination of TNF receptor associated factor 6 (TRAF6), hence can control the transcription of important anti-inflammatory cytokines, which shows inflammatory properties [Wang *et al.*, 2015]. Moreover, previous studies also provides an evidence of role of polyphenolic compound such as curcumin in inhibiting proteolytic activity of proteasome and cellular deubiquitinating activity leading to dysregulation in UPS (Jana *et al.*, 2004; Si *et al.*, 2007). Isolated caffeic acid, a plant polyphenol shows protective effect in cells from the toxicity induced by fluoride through modulation of Nox4, p38alpha, MAPK and restoring levels of heat shock proteins Hsp60, and Hsp27 [Kanagaraj *et al.*, 2015]. Another plant phenol resveratrol (RES) has also been investigated for its functional importance in ER proteostasis. Resveratrol was found to induce UPR response by elevating levels of GRP78 and CHOP resulting in proliferation inhibition of myelogenous leukemia cells [Liu *et al.*, 2010].

Resveratrol and hydroxytyrosol (a phenol isolated from olive) can induce SIRT1 signaling mediated autophagy, showing protective properties in pathologies of hepatic steatosis and osteoarthritis [Cetrullo *et al.*, 2016; Zhang *et al.*, 2015]. Flavonoids extracted from herb *Orostachys japonicus* have an inhibitory effect on calcium dependent enzyme, calpain, and thus may play regulatory roles in autophagy and related turnover rate of proteins having significance in diseases such as Alzheimer's and cataract [Je Ma *et al.*, 2009]. Despite actively studied for their roles in various proteostatic pathways, understanding bioavailability and bioefficacy of these molecules will support in further investigation of their health benefits [Manach *et al.*, 2005]. As indicated in study by [Lotito *et al.*, 2011] that flavonoids gets metabolized into derivatives that may produce different outcomes from the parent one. Similarly another study in past has shown flavonols induced chromosomal aberrations in Chinese hamster ovary cells [Carver *et al.*, 1983] which could be a deleterious side effect in considering them fo drug designing.

(d) Alkaloids

Alkaloids are another class of naturally occurring nitrogen containing bioactive compounds that are present in diverse range of distinctive structures produced by plants, marine species, fungi etc. [Qiu *et al.*, 2014]. Alkaloids like spongiacidin C, purified from marine sponges have shown to inhibit de-ubiquitinating enzyme USP7 activity with high potency, affecting cellular proteostasis [Tsukamoto, 2016]. Lissoclinidine B a pyridoacridine alkaloid from *Lissoclinum cf. badium* can stabilize the level of p53 by inhibiting Hdm2 E3 ubiquitin ligase activity, which can be used to develop anti-cancer therapy [Clement *et al.*, 2008]. The protective functions of berberine, an isoquinoline alkaloid, isolated from Chinese herb *Rhizoma coptidis* on intestinal epithelial cells is illustrated by its ability to decrease GRP78 expression and the splicing of xbp-1 mRNA, ameliorating ER stress *in vitro* [Hao *et al.*, 2011]. Similarly, another alkaloid kifunensine can restore activity of destabilized lysosomal enzymes prone to degradation by inhibition of ERAD [Wang *et al.*, 2011]. Another well studied alkaloid capsaicin, have property to induce autophagy by AMPK mediated SIRT1 activation [Lee *et al.*, 2015].

Alkaloid based drugs like paclitaxel and vinblastine have been used in cancer therapy showing the potential of alkaloids in pharmaceutical applications [Amirkia and Heinrich, 2014]. However, alkaloid like tylocrebrine were terminated in Phase I studies due to its side effects like CNS toxicity which can be overcome by introducing modifications that reduces its capability to cross blood brain barrier [Chemler, 2009]. Further studies targeted to understand the structure and regions that are actually pharmaceutically relevant and thereby designing mimetics of these valuable molecules will prove to be extremely beneficial for diseases like cancer and neurodegeneration that have limited treatment options.

(e) Fatty Acids and Their Derivatives

Fatty acids modulate cellular proteostasis through different mechanisms. Lower levels of polyunsaturated fatty acid, docosahexaenoic acid (DHA) in brain and serum of Alzheimer's patient and reduction in amyloid burden on DHA intake shows importance of fatty acids in proteostasis maintenance [Lim, 2005]. 4-phenylbutyrate a fatty acid derivative modulates the trafficking of CFTR mutant protein, by modifying the expression level Hsp70 chaperone [Suaud *et al*., 2011]. Similarly, oleic acid (OA), an unsaturated fatty acid decreases protein level of ER chaperone, GRP78, which confers higher insulin resistance in hepatocytes, a mechanism important in type-2 diabetes development [Yamagishi *et al*., 2012]. OA, further have critical functions in regulating ER proteostasis, as it can suppress UPR induced apoptosis by palmitate in INS-1E β-cells [Sommerweiss *et al*., 2013]. Palmitic acid (PA), a saturated fatty acid, has also shown ability to modulate autophagy by regulating levels of different amino acids [Enot *et al*., 2015]. These studies have shown that fatty acids like docosahexaenoic acid, OA and their derivatives like 4-phenylbutyrate have important roles to play in proteostasis maintenance and thus may be useful in diseases like diabetes type-2, Alzheimer's and cystic fibrosis therapeutics.

(f) Bacterial Isolates

Hoiamide D, a peptide based p53/MDM2 interaction inhibitor isolated from a Papua New Guinea marine cyanobacteria *Symploca sp.,* is an useful therapeutic option in treatment of cancer [Malloy *et al*., 2012]. Using same cyanobacteria *Symploca sp.,* another molecule named largazole was isolated which inhibits activation of E1 ubiquitin activating enzyme and consequently leads to reduction in ubiquitination and an increase in level of p27 in the cells [Ungermannova *et al*., 2012]. Bacterial metabolite like Geldanamycin, isolated from *Streptomyces hygroscopicus* increases association of ER chaperone BiP with nascent proteins [Lawson *et al*., 1998]. Rapamycin, another isolate from *Streptomyces hygroscopicus* is a macrolide compound that have shown ability to reduce cardiac hypertrophy by inducing autophagy and decreasing the levels of important autophagic regulator beclin-1 by MEK/ERK signaling pathway [Gu *et al*., 2016]. Well known autophagy inhibitor like bafilomycin A1, a macrolide antibiotic isolated from *Streptomyces sp.* is an inhibitor of lysosomal acidification and protein degradation [Yoshimori *et al*., 1991]. These above studies demonstrate that bacterial isolates provide a vast source of molecules that can be used for various chemotherapeutic purpose. Further studies targeted towards understating properties and specificity of these molecules will add significantly in direction of developing drug targeting mechanisms involved in proteostatic pathway .

(g) Fungal Isolates

Since the discovery of penicillin from fungus *Penicillium notatum* by Fleming, researchers have identified various new fungal isolates, some of which exert their effects by modulating proteostasis mechanisms. Molecules such as panepophenanthrin isolated from mushroom and hexylitaconic acid, obtained from marine derived fungus *Arthrinium sp.* modulates ubiquitin proteasome signaling by targeting E1 and E3 enzymes respectively [Tsukamoto *et al*., 2006]. Versipelostatin, isolated from *Streptomyces versipellis* 4083-SVS6, downregulates ER associated chaperone GRP78, which can be used as strategy in controlling the pathologies of neurodegeneration and cancer [Park *et al*., 2002]. Another recently identified fungal isolate, SD118-xanthocillin X, from *Penicillium commune* has shown to induce autophagy via inhibition of MEK/ERK Pathway [Zhao *et al*., 2012]. Parasitic fungus, *Cordycepis miltaris* derived molecule cordycepin (3'-deoxyadenosine), activates AMPK pathway of autophagy and causes reduction in level of mammalian target of rapamycin (mTOR), affecting the activity of Akt and causing autophagy [Wong *et al*., 2009]. Identification of approximately 100,000 species from around 5.1 million estimated fungal species [Blackwell, 2011] and much less commercially cultivable fungi gives an idea of limitations we have in research targeting fungi as source for natural molecules.

(h) Terpenes/Terpenoids

Terpenes are another class of natural molecules involved in organism's defense and interactions have also been shown to have proteostasis modulation properties. Betulinic acid, a

pentacyclic triterpenoid and its C-3 position modification can activate or inhibit proteasome respectively [Huang *et al.*, 2007]. Similarly, a diterpenoid derivative 15-oxospiramilactone inhibits an UPS deubiquitinase enzyme USP30 and thus regulates the ubiquitination of mitofusin, which modulate mitochondrial fusion [Yue *et al.*, 2014]. Oridonin, a diterpenoid from *Rabdosia rubescen*, has anti-oncogenic properties; it induces autophagy in HeLa cell line by increasing the protein expression of beclin-1, JNK and MAP-LC3 [Cui *et al.*, 2007]. Carnosic acid, a benzenediol diterpene causes degradation of androgen receptor by increasing the ER stress response proteins BiP and CHOP and induces androgen receptor degradation by the proteolytic machinery of UPS [Petiwala *et al.*, 2016] Being the largest group of natural molecules of various reported structures, functional knowledge of these precious class of molecules is lacking due to limitations in testing at natural settings and their presence as complex mixtures [Gershenzon and Dudareva, 2007]. However, methods like increasing terpene production by employing molecular biology and genetics tools thereby modulating metabolism are providing helpful solutions for studying these compound for industrial and therapeutic applications [Wu *et al.*, 2006].

(i) Other Natural Agents

Other than all of the above mentioned naturally derived products there are also other studies that have identified molecules obtained from varied sources the nature provides, which affects proteostasis mechanism. Leucettamols, a sphingoid isolated from marine sponge *Leucetta aff. microrhaphis* inhibits interaction between E2 ubiquitin conjugating enzyme Ubc13 and Uev1A, which can be used to increase the cellular levels of tumor suppressor gene p53 and may be developed in a potential anti-cancer therapy [Tsukamoto *et al.*, 2008]. Furthermore, siladenoserinols A-L (a serinol derivative) obtained from tunicate inhibits interaction between p53-Hdm2 [Nakamura *et al.*, 2012]. Additionally, Marchantin M, obtained from plant liverwort inhibits chymotrypsin-like and peptidyl-glutamyl peptide-hydrolyzing activities of proteasome, induces ER stress and autophagic death in human prostate cancerous cells [Jiang *et al.*, 2013]. Similarly, another plant product allicin, an active ingredient in *Allium sativum* (or commonly known as garlic) induces autophagy in hepatocellular cancer cell line as evident from decreased level of PI3K/mTOR induction in AMPK/TSC2 and Beclin-1 signaling pathways in Hep G2 cells [Chu *et al.*, 2012]. Recently, *Acanthostrongylophora ingens* (porifera) obtained manzamine A, an amine derivative, was reported to effectively inhibits proteasome [Tsukamoto, 2016].

2.4.2 Pharmacological Compounds

Following sub-sections provide some information on existing pharmaceutical drugs that effect important signaling events of proteostasis.

(a) Anti-Inflammatory Drugs

Anti-inflammatory drug like celecoxib upregulate E3 ubiquitin ligase Casitas in B-lineage lymphoma B cells (Cbl-b), which results in inhibition of Akt activation through rapamycin thereby attenuating gastric cancer cell resistance to rapamycin [Cao *et al.*, 2015]. Another example of NSAID induced proteostasis modulation include aspirin, that inhibits proteasome activity leading to cellular apoptosis [Dikshit *et al.*, 2006]. Derivative of salicylic acid, diflunisal acts as pharmacological chaperone, in transthyretin (TTR) amyloidosis, as it stabilizes the tertiary configuration of this protein [Sekijima *et al.*, 2006]. Furthermore, anti-inflammatory drug indomethacin has been reported to induce cytoprotective lipophagy in enterocytes [Narabayashi *et al.*, 2015]. Thus these few studies provide evidence that anti-inflammatory drugs have role in regulating the proteostasis pathways thus can be used for therapeutic purpose of the same. However, common side effects of anti-inflammatory drugs like ulceration, gastrointestinal bleeding should be kept in mind in designing therapeutic strategies. Also, any links of the original targets of the drug with disease mechanism must be well investigated.

(b) Cancer Drugs

Tyrosine kinase inhibitors such as nilotinib and bosutinib used in leukemia treatement alleviates proteotoxic load in Alzheimer's disease animals by enhancing interaction between parkin and beclin-1 [Lonskaya *et al.*, 2013]. Erlotinib another tyrosine kinase inhibitor used to treat non small lung cancer induces autophagy by activation of AMPK pathway and mTOR suppression, a mechanism that could be protective and provides resistance to cancer cell against erlotinib [Li *et al.*, 2013]. Similarly, another study reported resistance of cancer cell to anti-cancerous chimeric antibody cetuximab due to activation of autophagy [Li and Fan, 2010]. Bortezomib, a well known drug to treat multiple myeloma inhibits proteasome [Kane, 2003] causing disturbed proteostasis in cancerous cells. It has also been observed that bortezomib can induce ER stress mediated apoptosis in pancreatic cancer cells [Nawrocki *et al.*, 2005]. As evident from these studies different research work has used several proteostatic modulation strategies to control cancer cell growth. However, usage of anti-cancerous drugs have caused serious side effects, like doxorubicin (Dox) have shown to cause muscle atrophy, as it activates important cellular enzyme calpain in skeletal muscle [Smuder *et al.*, 2011], similarly molecule like cisplatin have been shown to have side effects like nephrotoxicity, renal failure and cardiotoxicity in cancer patients [Florea and Büsselberg, 2011]. Therefore, developing anti-cancerous drugs as agents to treat ailments of proteostatic disorders needs a detailed investigation on the chemical properties of these molecules and thought should be put on to reduce their serious side effects.

(c) Cardiovascular Drugs

Drugs used in treatment of cardiovascular diseases have potential therapeutic properties in modulating pathways of proteostasis, as drugs like thalidomide, lenalidommide and pomalidomide, binds protein cereblon (CRBN) within the DNA damage binding E3 ubiquitin ligase complex and inhibits CRBN autoubiquitination, leading to reduction in CRBN level and increased p21 level in cancerous cells [Lopez-Girona *et al.*, 2012]. Cardio-protective drug, diazoxide, also shows therapeutic effect in metabolic disorder, as it activates KATP channel activity in case of persistent hyperinsulinemic hypoglycemia of infancy, a disease of pancreatic β-cells [Molinari, 2007; Shyng *et al.*, 1998]. It can also regulate proteostatic pathway by interacting with Hsp90 and inhibiting cleavage of Bid, a pro-apoptotic protein [Yang *et al.*, 2011]. The ability of cardiovascular drug to modulate proteostatic pathway in ER, is used to produce therapeutic effect in disease of ALS, as guanabenz, inhibit the ER chaperone GRP78/Bip, AIF6α reduces ER stress [Jiang *et al.*, 2014]. Cardiovascular drug, lacidipine can be used in form of a pharmacological chaperone, as it improves folding, trafficking and lysosomal activity of the destabilized glucocerebrosidase via ERAD inhibition in fibroblasts isolated from patients of Gaucher's disease [Wang and Segatori, 2013]. Compounds identified in treating disorders of cardiovascular system are also observed to regulate proteostatic pathway of autophagy, as drugs like minoxidil and clonidine show their therapeutic potential by reducing protein aggregation in a Huntington's disease model by inducing autophagy through the inhibition of Ca^{2+} channels, reducing cAMP levels, normalizing enhanced calpain activity and suppressing IP3 [Williams *et al.*, 2008]. Fenofibrate, can also cause autophagy in a diabetic mouse cardiac muscles by different mechanism through upregulation of sirutin-1 signaling pathway [Zhang *et al.*, 2016]. Above mentioned studies have shown promising evidences of some cardiovascular drugs which can be used for therapeutic purpose in proteostasis associated pathologies.

(d) Neurological Disorder Drugs

Antidepressant drug, clomipramine was reported to inhibit E3 ubiquitin ligase ITCH autoubiquitination which leads to ITCH functional inhibition and autophagy reduction in cancer cells [Rossi *et al.*, 2014]. Anti-depressants drugs of various types (serotonin selective, norepinephrine selective, and nonselective reuptake inhibitors, monoamine oxidase inhibitor) treating neurological disorders are shown to increase ubiquitylation and degradation by proteasomal pathway of β-arrestin 2 [Golan *et al.*, 2009]. Ubiquitin specific proteases have shown activity against proliferation of non-small cell cancerous cells by acting synergistically

with cisplatin [Chen *et al.*, 2011]. Aβ-specific β-sheet breaker peptide H102 has shown pharmacological activity to reverse the misfolding and aggregation of Aβ which can be used to treat neurodegeneration caused by aggregation of Aβ in Alzheimer's disease [Bessis and Breton-Gorius, 1965]. Neurological drug, valproic acid used to treat epilepsy increases mRNA levels of Hsp70 in rat cortical neurons [Marinova *et al.*, 2009]. The antioxidant edaravone protects against ER stress in autoimmune rats by causing an inhibitory effect on ER chaperone GRP78, which is a putative marker of ER stress [Shimazaki *et al.*, 2010]. Lithium, a mood enhancer, alters the proteostasis of ER in galactose fed cells, by modifying the XBP, XBPS levels and UPR signaling pathway in ER stress mechanism, they can also suppress the release of apoptosis associated proteins and inhibit neuronal apoptosis via down-regulating autophagy through reducing the calpain activation in neonatal rat different brain regions [Li *et al.*, 2010; Nagy *et al.*, 2013]. In SH-SY5Y cells, zonisamide, at low doses upregulate ER E3 ubiquitin ligase HRD1, which may ameliorate ER stress conditions and ultimately results in decreased neuronal cell death in Parkinson's disease [Omura *et al.*, 2011]. Together, drugs used to treat various neurological disorders can be further investigated for neurodegenerative diseases like Alzheimer's and Parkinson's which currently have very limited treatment options.

(e) Drugs Used in Other Therapies

Molecules utilized in treatment of diseases other than proteostatic disorder, have shown further ability to affect proteostatic pathways, N-acetylglucosamine thiazoline enzyme activity regulator of β-hexosaminidase A is currently being developed as pharmacological chaperone to treat disease of lysosomal storage disorder, where it restores the defective enzyme's functional activity [Tropak and Mahuran, 2007]. Ritonavir and Metformin, used in treating pathological disorders of HIV and type-2 diabetes, respectively are also used with bortezomib for inducing ER stress (via UPR pathway) and suppressing GRP78-mediated autophagy, to increase the apoptotic function of bortezomib in cancerous cells [Jagannathan *et al.*, 2015; Kraus *et al.*, 2008]. Antidiarrheal medication, such as loperamide, causes autophagy in alveolar cells of a mouse animal model infected with *Mycobacterium tuberculosis* (Mtb) by inducing localization of autophagic protein LC3 with Mtb [Fleming *et al.*, 2011; Juárez *et al.*, 2016]. Antibiotics are normally used to treat microorganisms infections, but they have also shown ability to modulate proteostasis, like azithromycin inhibits autophagy by preventing lysosomal acidification [Renna *et al.*, 2011], whereas, another antibiotic tigecycline induces autophagy with activation of AMPK pathway and inhibition of downstream effectors mTOR and p70S6K [Tang *et al.*, 2014]. The scientific research work on compound, metformin, have shown that this molecule can cause re-establish autophagy in various disorders, via restoring disturbed AMPK signaling using different pathways of mTOR activation [Ravindran *et al.*, 2016; Song *et al.*, 2014].

Identifying and characterizing compounds derived from either natural resources or from synthetic origin for utilizing in modulating major pathways and machinery of proteostasis i.e. UPS, autophagy, chaperone machinery requires a detailed investigation of the molecule with respect to its bio-availability, pharmacokinetics, pharmacodynamics, chemistry, side-effects and also its clinical suitability. Thus finding specific modulators of the proteostasis is still a challenge and in this context steps are already being taken as can be understood from example of protacs. Proteolysis targeting chimeras (protacs) are ternary complex, which contains an E3 ubiquitin ligase, linked to another ligand for protein to be targeted for degradation. The E3 ubiquitin ligase, causes polyubiquitination of ligand binded protein, leading to their degradation [Buckley and Crews, 2014]. Thus using such chimeric molecules developed by advanced molecular biology and chemical techniques could alleviate problems of specificity.

2.5 ROLE OF PROTEASOMES IN NONSTEROIDAL ANTI-INFLAMMATORY DRUGS MEDIATED APOPTOSIS

The Nonsteroidal anti inflammatory drugs basically works on cyclooxygenases (COX) enzymes that are responsible to convert arachidonic acid released from cell membranes in response to stimuli into eicosanoids such as prostaglandins [Rao and Knaus, 2008]. With respect to NSAIDs mechanism of action, COX-1 (the constitutive form) and COX-2 (the inducible form) are two isoforms that are found to be crucial [Agyin *et al.*, 2009]. Traditional NSAIDs such as diclofenac and ibuprofen were non-selective in nature, i.e. they inhibited both COX-1 and COX-2 enzymes leading to gastrointestinial complications [Hochwald *et al.*, 2003; Moreau *et al.*, 2012]. Eventually, COX-2 selective NSAIDs were designed such as rofecoxib and celecoxib. But later it was found that they too have side effects leading to cardiovascular abnormalities [Bunn, 2004]. Previously, different mechanisms underlying NSAIDs induced anti-proliferative and pro-apoptotic outcomes have been identified [Concannon *et al.*, 2006; Hideshima and Anderson, 2012; Hsu *et al.*, 2015; Sohn *et al.*, 2006]. The effect on proteostasis pathways such as ER stress response and autophagy have also been shown as one of the possible mechanisms of NSAIDs mediated reduced cellular viability [Hampton, 2007; Wakabayashi, 2000]. Further, NSAIDs such as meclofenamate sodium and aspirin were also found to inhibit protein degrading component of UPS system, the proteasome [Gurpinar *et al.*, 2014; Latham *et al.*, 2014].

The proteasome is a multi subunit, highly complex 2.5 MDa enzymatic structure that have protease activity [Livneh *et al.*, 2016]. It basically contains two subcomplexes, the 20S core which has the catalytic activity and the 19S regulatory particle. The 20S core particle is composed of two outer α and two inner β rings. Each α ring is composed of seven identical α subunits and similarly each β ring is composed of seven identical β subunits which are stacked together forming a barrel like structure having a lumen inside where the catalytically active subunits face [Latham *et al.*, 2014]. So far three sites having protease activity in 20S catalytic core has been identified, the β1 subunit (caspase like activity), β2 (trypsin like activity) and the β5 subunit (chymotryspsin like activity). The 19S regulatory particle is composed of lid and base regions. The lid region is made up of non ATPase subunits that are involved in deubiquitlyation of ubiquitin chains tagged to proteins. The base region is composed of both ATPase and non ATPase subunits that recognizes ubiquitin chains and unfold the proteins to make them available for degradation in 20S catalytic core [Mishra *et al.*, 2018; Tanaka, 2009].

Inhibition of proteasome by small molecules has been observed to induce apoptosis in cancer cells as mentioned in previous sections. Different apoptosis pathways have been associated with proteasome inhibition [Concannon *et al.*, 2006; Hideshima and Anderson, 2012; Sohn *et al.*, 2006]. Interestingly, NSAIDs have been found to have protective role in various types of cancers [Cuzick *et al.*, 2009; Hampton, 2007; Pantziarka *et al.*, 2016; Wakabayashi, 2000]. Different pathways have been found to be associated with NSAIDs mediated apoptotic and anti proliferative properties [Cecere *et al.*, 2010; Gurpinar *et al.*, 2014; Luciani *et al.*, 2007]. However, with respect to proteasome as target and its underlying mechanism involved in induction of apoptosis, few studies involving NSAIDs have been conducted and the information available is quite limited. Thus, more studies in this direction are required to identify NSAIDs that can affect the proteasome activity, and what are the mechanisms involved, so that their potential therapeutic advantages can be explored. Further, such studies may also help in understanding NSAIDs exact potential and limitations as proteasome inhibitors. In addition, studies involving inhibition of proteasome activity may also provide insights into the key role proteasomes play, as a regulatory unit in maintaining protein homeostasis along with the consequences that may occur, when their functions get compromised giving insights of mechanisms involved in disease occurrence. Interestingly, such studies might also unravel the hidden mechanisms underlying the side effects of the NSAIDs that may occur during the course of their long term usage.