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E3 Ubiquitin Ligases in Protein Quality Control Mechanism

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Abstract In living cells, polypeptide chains emerging from ribosomes and preexisting polypeptide chains face constant threat of misfolding and aggregation. To prevent protein aggregation and to fulfill their biological activity, generally, protein must fold into its proper three-dimensional structure throughout their lifetimes. Eukaryotic cell possesses a quality control (QC) system to contend the problem of protein misfolding and aggregation. Cells achieve this functional QC system with the help of molecular chaperones and ubiquitin–proteasome system (UPS). The well-conserved UPS regulates the stability of various proteins and maintains all essential cellular function through intracellular protein degradation. E3 ubiquitin ligase enzyme determines specificity for degradation of certain substrates via UPS. New emerging evidences have provided considerable information that various E3 ubiquitin ligases play a major role in cellular QC mechanism and principally designated as QC E3 ubiquitin ligases. Nevertheless, very little is known about how E3 ubiquitin ligase maintains QC mechanism against abnormal proteins under various stress conditions. Here in this review, we highlight and discuss the functions of various E3 ubiquitin ligases implicated in protein QC mechanism. Improving our knowledge about such processes may provide opportunities to modulate protein QC mechanism in age-of-onset diseases that are caused by protein aggregation.

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Introduction

A central dogma in cells and organism is the biogenesis of proteins by the conversion of genetic information into active proteins. Each minute in living cells, thousands of numerous proteins are synthesized by ribosomes. To make sure that cells do their function properly, rapid and efficient folding of each nascent polypeptide into mature functional protein is essential. Abnormal protein accumulation leads to impairment in UPS, and misfolded protein aggregation generates multifactorial toxic effects in cells [1–3]. Deregulation or inefficient folding leads to protein misfolding, aggregation, and accumulation in the various cellular compartments. Several studies and emerging evidences clearly suggest that protein misfolding is one of the possible causal factors of various neurodegenerative disorders and systemic diseases [4–6].

The correct cumulative function of a network of thousands of cellular proteins, simultaneous degradation, and clearance of aberrant proteins generate a cellular quality control (QC) system in cells. Ubiquitin–proteasome system (UPS) governs the selective intracellular protein degradation in eukaryotic cells [7]. Assembly of a polyubiquitin chain is marked for degradation of various cytosolic and nuclear proteins through UPS. The first step in ubiquitination process is covalent linkage of the small (7.6 kDa) ubiquitin protein to the target protein. Addition of a ubiquitin molecule to lysine residues of substrate is a multistep process. Ubiquitination of a protein is catalyzed by three classes of enzymes called ubiquitin-activating enzyme E1, a small group of ubiquitin-conjugating enzymes (UBCs) or E2s, and

ubiquitin-ligating enzymes (E3s). In this complex process, E3 ubiquitin ligase is the enzyme that determines substrate specificity to govern the ubiquitination process and exists with vast diversity [8].

A critical question is to identify and understand the molecular mechanism of the E3s ubiquitin ligase involved in QC system and misfolded protein degradation pathway. There are few QC E3s known for maintenance of proteostasis conditions under various biotic and abiotic insults. In this review, we summarize the current understanding of QC E3s and their associated molecular pathways implicated in protein misfolding, aggregation, and proteotoxicity in common neurodegenerative diseases. Here, we mainly focus on clarifying and understanding the QC mechanism of E3s during aberrant protein aggregation and during cellular insults and their connections with essential cellular functions.

E3 Ubiquitin Ligases and Quality Control System

In eukaryotic cells, generally, protein QC system includes post-translational modification processes by which cells govern folding of nascent polypeptide chains into mature proteins. The QC system is also essential for refolding of damaged stress proteins or suppression of accumulation of proteotoxic misfolded species with exposed hydrophobic surfaces [9–11]. Loss or imbalance in the QC system leads to inability of aberrant protein degradation and chiefly contributes to the molecular pathomechanism of protein-associated diseases such as Parkinson's disease (PD), Alzheimer's disease (AD), amyotrophic lateral sclerosis (ALS), and polyglutamine-associated neurodegenerative diseases. The main players, which provide specificity of aberrant protein degradation in the QC system, are E3 ubiquitin ligases of UPS [8, 12]. However, we are much far to understand how these QC E3 ubiquitin ligases solve the puzzle of discrimination between similar structural properties of useful folding intermediates and misfolded proteins. E3s implicated in several QC pathways have been studied, and it was found that these ligases respond against various stresses. Interestingly, a recent study suggests that QC E3s not only interact with modified proteins but interaction may also be possible during translation process; for example, Ltn1 is a ribosomal-associated E3 ubiquitin ligase, which promotes clearance of ribosomal stably, stall nascent nonstop proteins upon synthesis of a poly(Lys) tract [13]. Here in this section, we briefly describe the functional importance of few E3 ubiquitin ligases, which are entitled as QC E3 ligases. These QC E3 ubiquitin ligases generate a cellular defense mechanism against abnormal proteins. To maintain proper proteostasis, QC E3s interact with a target protein at distinct steps during their lifetimes (Fig. 1).

Endoplasmic Reticulum Stress and E3 Ubiquitin Ligases

Endoplasmic reticulum (ER) is an important cellular organelle for the correct folding and post-translational modifications of nascent polypeptides towards their right destiny in crowded milieu of cell. Accumulation of misfolded proteins generates ER stress, which is due to the disturbance in the structure and function of ER in cells and leads to cell death [14–16]. To protect cells against ER stress, numerous E3 ubiquitin ligases actively participate in the clearance of misfolded proteins through endoplasmic reticulum-associated degradation (ERAD) pathway. During ER stress exposure, numerous ER-linked E3 ubiquitin ligases facilitate degradation of ER-associated misfolded proteins. ERAD is an essential mechanism by which eukaryotes facilitate degradation of abnormal accumulated proteins in ER [17].

In humans, SMAD-specific E3 ubiquitin protein ligase 1 (*SMURF1*) gene encodes Smurf1 E3 ubiquitin ligase [18]. Recently, it was reported that Smurf1 targets ER-localized Wolfram syndrome protein (WFS1). Mutations in *WFS1* gene leads to Wolfram syndrome, an optic atrophy disease. Interaction of Smurf1 with WFS1 proteins promotes its proteasomal degradation. Depletion of Smurf1 endogenous level induces accumulation of WFS1. This finding clearly suggests that Smurf1 promotes ER-associated substrate degradation and that its endogenous level is induced by ER stress [19]. In *C. elegans*, Really Interesting New Gene (RING) finger protein 121 (RNF121) is localized into the ER membrane and retains E3 ubiquitin ligase activity. Inactivation of RNF121 generates sensitivity against ER stress and induces unfolded protein response (UPR) in cells. Surprisingly, ER stress treatment elevates RNF-121 protein level but not at the mRNA level of RNF-121 [20].

In general, major biomolecules, such as glycans and lipids, are synthesized in ER network. The ER network possesses a rigorous QC mechanism for final distribution of synthesized biomolecules into the right place in the cellular pool. This major function of Endoplasmic Reticulum Quality Control (ERQC) system is to sense ER stress and take an immediate action against various defective or aberrant proteins to avoid further accumulation of these proteotoxic species. Bifunctional apoptosis regulator (BAR) is one of the RING finger-type ER-associated E3 ubiquitin ligase; it was originally identified as an inhibitor of BAX-induced apoptosis [21]. ER-associated protein Bax inhibitor-1 (BI-1) is targeted by BAR-1 for proteasomal degradation. Association of BAR-1 with ER-resident proteins demonstrates a possibility of its involvement in ERAD pathway [22]. Due to ER stress exposure, when the local concentration of misfolded proteins exponentially elevates, the ERQC system releases various ER-associated E3s for clearance of ER-linked misfolded proteins. Membrane-associated

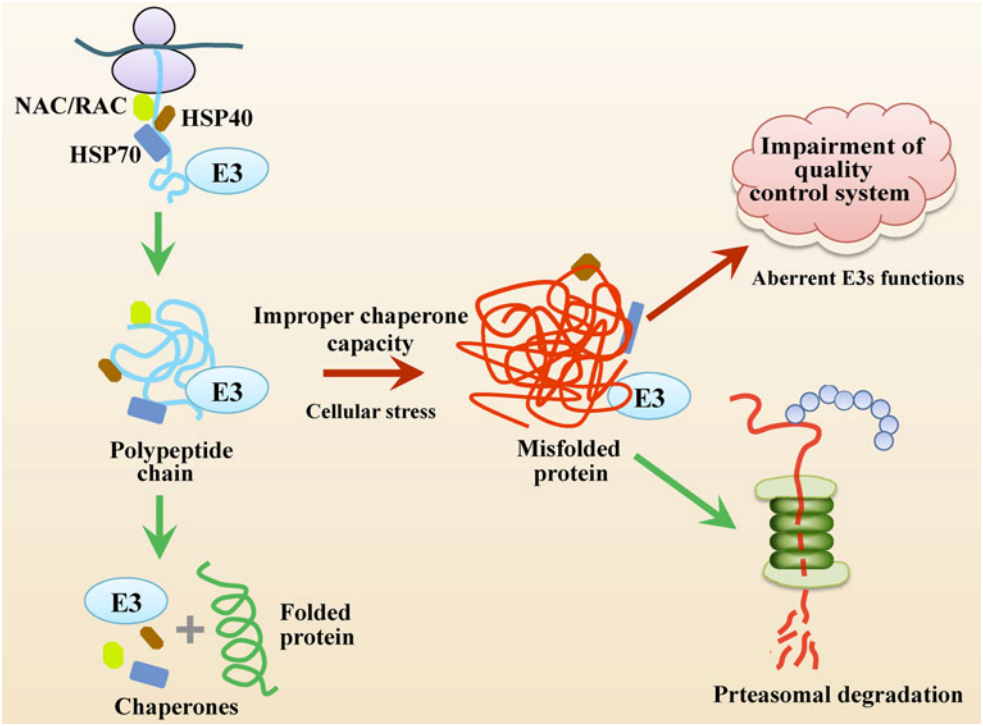

Fig. 1 Role of E3 ubiquitin ligases in various steps of quality control mechanism. Approximately 30 % of the newly synthesized proteins are misfolded because of inaccuracy during translation and folding mechanism or due to self-mutations. Predominant interaction of E3 ubiquitin ligase with ribosomes and nascent polypeptide chains contributes in protein quality control process. To ensure functional protein installation in cellular pool, molecular chaperones attempt to correct the folding defects, but an inefficient chaperone capacity leads to degradation via ubiquitin–proteasome system (UPS). Mutations in QC E3s initiate massive misfolded aggregation because of nondegradation. Loss of QC system accumulates misfolded proteins and finally impairs UPS. Incomplete degradation or inefficient clearance of aggregates can increase aggregate burden; therefore, reduced misfolded protein degradation generates proteotoxicity and plays a critical role in aggregate propagation in various neurodegenerative disorders

ring finger (C3HC4) (*MARCH*) gene encodes a novel RING finger-type ER-linked E3 ubiquitin ligase, TBE4. Surprisingly, ER stress treatment did not induce TEB4 endogenous level in cells. TEB4 localizes with chaperone calnexin and also promotes self-ubiquitination and proteasomal degradation [23].

Ubiquitination of misfolded ER proteins is an important process for clearance of accumulated proteins by ERAD system. Till now, various E3 ubiquitin ligases have been discovered to be involved in mammalian ERAD pathway. Here in this section, we discuss few very important E3 ubiquitin ligases, those that are mainly dedicated as ERQC E3s.

Gp78

The tumor autocrine motility factor receptor (AMFR), also known as gp78, is a transmembrane glycoprotein from murine melanoma cells and is implicated in tumor invasion and metastasis [24, 25]. Gp78 is a RING finger domain-dependent ubiquitin ligase mainly localizes in ER and is involved in ERAD of several substrates. ER membrane-anchored E3 gp78 specifically recruits murine ortholog of Ubc7p (MmUBC7), a ubiquitin-conjugating enzyme (E2) through a different region of RING finger domain. Gp78 targets and promotes proteasomal degradation of T cell antigen receptor (TCR) CD3 subunit "CD3-δ", a well-characterized ERAD substrate [26]. Gp78 specifically promotes the proteasomal degradation of superoxide dismutase-1 (SOD1) and ataxin-3 proteins, implicated in familial amyotrophic lateral sclerosis (FALS) and Machado–Joseph disease/spinocerebellar ataxia type 3 neurodegenerative diseases, respectively. Gp78 stimulates mutant SOD1 degradation, and this gp78-mediated ERAD loss of function elevates SOD1 accumulation [27]. Earlier, it has been observed that gp78 can target AAT deficiency disease protein Z variant of alpha-1-antitrypsin and normal 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMG CoA reductase) and also promotes self-ubiquitination [28, 29].

Doa10

Earlier observations have shown that Doa10, a transmembrane protein ubiquitin ligase governs ERAD function and is located in ER/nuclear envelope (NE). In cells, Ndc10 interacts with intranulcear spindle microtubules and acts as a subunit of DNA-binding CBF3 complex [30]. Doa 10 induces degradation of mutant Ndc10-2 kinetochore protein and a mutant NE membrane protein with the help of ubiquitin-conjugating enzymes, Ubc6 and Ubc7, as well as the Ubc7 cofactor Cue1 in *Saccharomyces cerevisiae* [31, 32]. Human Doa10 ortholog, TEB4 (MARCH-VI) is a member of the MARCH family E3 ubiquitin ligases. It

resides in ER and participates in ERAD pathway and also promotes degradation of Type 2 Iodothyronine Deiodinase [33, 34].

HRD1

During translocation, probable mislocalization may occur for a newly synthesized protein molecule from its final site in various cellular compartments. Human E3 ubiquitin ligase HRD1 influences the degradation of ER-linked two classic ERAD substrates, CD3-δ and TCR-α. It has also been observed that HRD1 endogenous level is elevated after the treatment with ER stress inducers suggesting that it may be implicated in ERAD pathway [35]. HRD1 is expressed in brain neurons but not in glia cells [36]. This E3 ubiquitin ligase is expressed against ER stress and generates cellular protective response against ER stress-induced apoptosis [37–40]. Earlier, it was reported that human HRD1 endogenous levels were changed after ER stress exposure, probably upon ER stress treatment HRD1 promotes degradation of ERAD-linked substrates and enhances clearance capacity of cell through ERAD process [35]. Recently, it has been shown that HRD1 induces ubiquitination and degradation of neurodegenerative disease-linked proteins such as huntingtin (Htt)-expanded polyglutamine proteins, Parkin-associated endothelin receptorlike receptor (Pael-R), and prion protein (PrP) [41]. HRD1 is involved in the degradation of immature nicastrin and regulates the production of amyloid beta-protein, thus showing indirect regulation in beta-amyloid levels [42].

Rfp2

Ret finger protein 2 (Rfp2), also known as tripartite motif-containing 13 (TRIM13) or LEU5, belongs to RING finger, B-box, coiled coil (RBCC) family of highly conserved group proteins [43]. It acts as a novel RING domain-dependent ERAD E3 ubiquitin ligase and colocalizes with distinct ER-resident proteins, including the T cell receptor subunits CD3-δ and Ubc6. Numerous ER-resident proteins interact with Rfp2, including valosin-containing protein (VCP). Functional interaction of Rfp2 with these ERAD substrates promotes their degradation, e.g., CD3-δ. Earlier studies suggest that E3 ubiquitin ligases can determine substrate selection specificity in UPS and, on other side, single substrates may be targeted by several different E3s [44, 45]. Most probably to cope against ER stress exposure and to suppress multifactorial toxic effects, E3s overlap in substrate specificity and possibly try to reduce overburden of misfolded proteins in ER.

RMA1

Multiprotein complex initiates ubiquitination of misfolded proteins and promotes degradation through ERAD system. It is a C′ terminus membrane-bound novel RING finger E3 ubiquitin ligase, which is conserved from *Arabidopsis* to human. RMA1 promotes ubiquitination of MBP–RMA1 fusion protein, but not free MBP in solution; this observation suggests that MBP recognition by Rma1 is due to its physical vicinity or localization [46]. ER membrane-linked RMA1 makes a complex that holds Ubc6e and the transmembrane QC factor Derlin-1 [47–49]. E2 Ubc6e and RMA1 facilitate proteasomal degradation of cystic fibrosis transmembrane conductance regulator (CFTR) protein. RMA1 sense folding defects coincident with translation, while carboxyl terminal Hsp70-interacting protein (CHIP) sense folding defects post-translationally. This study indicates that RMA1 and CHIP sequentially detect folding defects in both normal CFTR and CFTR ΔF508 in ER membrane and cytosol, respectively. This sequential stochastic interaction with various molecules generates a well-defined, highly regulated complex pattern to govern the correct folding of CFTR and promote degradation of CFTRΔF508 [49].

Oxidative Stress and E3 Ubiquitin Ligases

Protein oxidation leads to misfolding and requires higher UPS activity to maintain cellular homeostasis under oxidative insults. Earlier, it has been reported that UPS activity is markedly increased in cells during oxidative stress and recovery states [50]. Cells continuously tolerate proteotoxic threats from various kinds of stresses and always try to manage a proper cellular homeostasis. Mainly, intracellular cytosolic misfolded and aggregated proteins are targeted and degraded by UPS [51]. During oxidative stress exposure, cellular proteins suffer from several forms of post-translational protein modifications including oxidation of sulfhydryl groups and oxidation of amino acids residues [52, 53]. Oxidation of proteins may affect numerous cellular functions in cell, including deregulated cytoskeleton dynamics, aberrant protein synthesis, impairment in protein degradation, and lack of energy production, and this, finally, leads to apoptosis [54–58]. Various post-translational modifications help E3s in signal recognition process. RING finger E3 ubiquitin ligase heme-oxidized IRP2 ubiquitin ligase-1 (HOIL-1) sense the oxidized form of iron regulatory protein 2 (IPR2) protein. Probably, this function of HOIL-1 contributes to clearance of metabolized oxidized proteins [59, 60]. In this section, we review studies of few very important E3 ubiquitin ligases, those that are directly involved in oxidative stress, and discuss about their molecular mechanism and cellular events associated with human diseases.

CHIP

CHIP joins the two major cellular pathways of protein QC, the UPS and molecular chaperones. U-box domain family

member CHIP retains tetratricopeptide repeat (TPR) domains that interact with the Hsp chaperones [61, 62]. Proteasomal inhibition treatment induces colocalization of CHIP with proteasome, and this functional linkage of CHIP with chaperones facilitates the ubiquitination and degradation of chaperone-anchored substrates with the help of proteasome [63, 64]. E3-ubiquitin ligase activity of CHIP resides in the U-box domain. During stress conditions, CHIP can also control a chief heat shock transcriptional factor 1 (HSF1); by this function, it can actively contribute in protein QC system [65]. During stress conditions, CHIP mainly targets misfolded proteins for proteasomal degradation such as denatured luciferase protein, expanded polyglutamine proteins, CFTR, and tau [64, 66–69]. CHIP was earlier thought not to be a stress-induced E3 ligase, but Imai et al. have shown that ER stress affects CHIP activity [70]. Under oxidative load, CHIP can regulate senescence; a demonstration of CHIP (−/−) mouse fibroblast has been observed with impaired UPS in such condition [71]. It has also been observed that CHIP induces ubiquitylation of mutant SOD1-associated Hsc/Hsp70 molecules and thus facilitate proteasomal degradation of mutant SOD1 protein, too [72]. Recently, it was shown that CHIP endogenous levels elevate under various stress conditions to generate an adaptive cellular protective response against various stress conditions [73].

E3 Complexes Implicated in Oxidative Stress

Keap1–Cul3–Rbx1 E3 Ligase

Nuclear factor (erythroid-derived 2)-like 2 (Nrf2) transcription factor regulates the expression of many antioxidant genes [74]. Nrf2 is also involved in regulating a group of genes that protect cells against the harmful effects of environmental insults [75, 76]. In normal conditions, Nrf2 is regulated by complex cullin-based E3 ligase Keap1 (Kelch-like ECH-associated protein 1)–Cul3–Rbx1, and Keap1 acts as an adaptor protein that binds to Nrf2. So under normal condition, Nrf2 activity is repressed. Upon oxidative insults, Keap1 activity is inhibited, which activates Nrf2 [77].

Park–PINK–PARK7 Novel E3 Complex

Cumulative function of parkin, PTEN-induced putative kinase-1 (PINK1), and PARK7 most probably generates cellular protective response against oxidative stress. Functional interaction of these three proteins forms a novel E3 complex, which stimulates ubiquitination and proteasomal-mediated degradation of heat stress-stimulated parkin substrates, synphilin-1, and parkin. It has been reported that aberrant PINK1 or mutant parkin lost the proteasomal-dependent degradation ability for both parkin and synphilin-1 [78]. *PARK7* gene is ubiquitously expressed and linked to PD, and the end product of this gene is DJ1 protein; mutations in this gene cause early onset of the disease with autosomal recessive inheritance [79]. Oxidative stress exposure makes DJ1 a more acidic hydroperoxide-responsive protein, suggesting that it may act as an antioxidant protein [80]. DJ-1 generates cellular protective response both in cells as well as in *Drosophila* against oxidative stress [81–83]. PINK1 in *Drosophila* and parkin or DJ-1 inactivation in mouse leads to aberrant mitochondrial function and elevates sensitivity against by oxidative stress [84–86].

Cul2–VHL E3 Ligase Complex

Transcription factor hypoxia-inducible factor 1α (HIF-1α) has an important role in maintaining oxygen homeostasis. HIF-1α regulates various genes involved in reactive oxygen species (ROS) [87]. During normoxic conditions, HIF-1α endures prolyl hydroxylation; this change leads to its identification by Von Happel–Lindau (VHL) protein, a component of complex Cul2–VHL E3 ubiquitin ligase. Identification of HIF-1α by Cul2–VHL E3 promotes its proteasomal degradation. Hydroxylation of HIF-1α prevents its identification by Cul2–VHL E3 complex, and thus, it results in no degradation of HIF-1α under hypoxic condition [88].

E3 Ubiquitin Ligases Implicated in Neuroprotection

Accumulation of aberrant proteins induces various stress conditions that has major implication in neuronal dysfunction. Marinating the levels of functional proteins in neuronal cells is a highly regulated task. A well-controlled balance between protein synthesis and proper degradation of old proteins determine the life of cells. Deposition of misfolded proteins organized into insoluble aggregates is toxic for cells and leads them towards apoptosis or death in various protein conformational diseases [89–91]. During protein synthesis and up to various post-translational modification steps, cells continuously tolerate numerous cytotoxic potential extortions mediated by misfolded proteins. Eukaryotic cells maintain this delicate proteostasis balance with the help of an efficient QC system. Earlier, it has been reported in various studies that protein misfolding leads to generation of oligomers, aggresomes, fibrils, and inclusion body-like structures. UPS promotes the degradation of intracellular misfolded proteins [84]. In this complex, intracellular protein degradation process E3 ubiquitin ligases to determine the substrate specificity in UPS. Recently, few E3s are identified, which are directly implicated in the degradation and clearance of misfolded proteotoxic species. In the clearance of misfolded proteins by UPS, the key factor is to

understand how E3 ubiquitin ligases achieve the final recognition process of misfolded proteins as compared to normal substrates or proteins. It is a prime question to understand how these E3 ubiquitin ligases target the common structural hallmarks shared in misfolded proteins. In some cases, E3 QC ligases take help of few molecular chaperones in the identification process of misfolded proteins [92]. Here in this section, we discuss few important E3 ubiquitin ligases, which can actively manage normal cellular homeostasis state under various stress conditions (Fig. 2).

Parkin

Parkin is a RING finger E3 ubiquitin ligase and plays a key role in the molecular pathomechanism of PD [93]. Overexpression of parkin generates cellular protective response against oxidative stress through reduction in the intracellular load of oxyradicals [94]. Alteration in the cysteine residues of parkin by an oxygen radical impairs the function of parkin, and probably, this oxidative stress inactivates parkin and generates misfolded parkin protein [95]. In the context of PD, Lewy bodies are proteinaceous cytoplasmic inclusions that are well-characterized hallmark in PD patients [96, 97]. Parkin E3 ubiquitin ligase retains in Lewy bodies deposits of fibrous tissue found in patients with PD [98, 99]. Recently, molecular function of parkin in aggresome–autophagy pathway was reviewed by Chin et al. [100]. This report provides evidences of how parkin differentially contributes to both Lys63-linked polyubiquitination aggresome formation and Lys63-linked polyubiquitination autophagy pathways. α-Synuclein gene contains six exons; the end product of this gene is a 14-kDa phosphoprotein which was firstly identified as a presynaptic protein in rat brain [101, 102]. Two point mutations (Ala53Thr and Ala30Pro) in α-synuclein gene cause familial autosomal-dominant PD [103, 104]. α-Synuclein retains, as a chief protein, constituent of Lewy bodies in PD [105].

Mutant α-synuclein generates numerous cellular insults [106–108]. Several reports demonstrated that overexpression of mutant α-synuclein inhibits proteasomal activity in living cells and significantly induces cell death mediated by mutant α-synuclein [109, 110]. Overexpression of parkin alleviates cell death against toxicity directly linked with proteasome inhibition. Parkin is also involved in the ubiquitination of misfolded proteins derived from ER and protects against neurotoxicity stimulated by unfolded protein stresses [111]. Under normal conditions, parkin is diffusely distributed in the cytoplasm. During mitochondrial membrane depolarization stress conditions, PINK1 promotes recruitment of parkin towards the site of depolarized mitochondria. Subsequently, parkin governs the formation of two different polyubiquitin chains, linked through Lys 27 and Lys 63. Parkin mediates Lys 27-linked ubiquitin chains on voltage-dependent anion channel 1 (VDAC1) that is linked with mitophagy [112].

Malin

It is earlier reported that Lafora disease (LD) is caused by mutations in the protein laforin, encoded by *EPM2A* gene [113–115]. A mutated form of NHL repeat containing 1

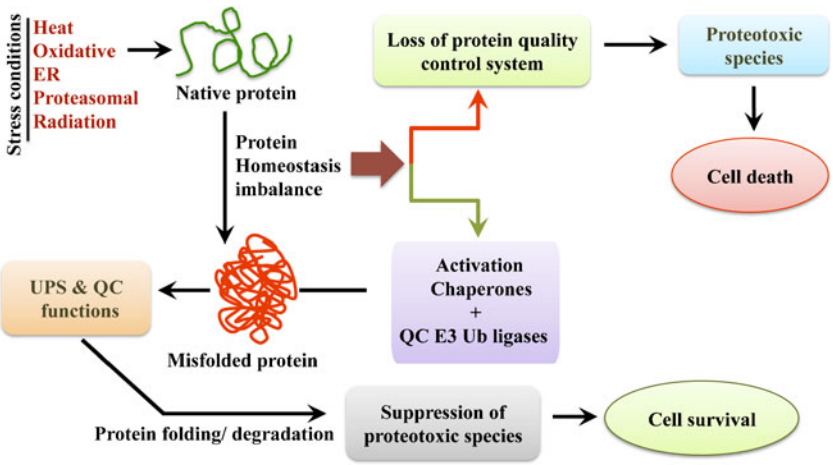

Fig. 2 Model for various cellular stress-induced E3-mediated quality control process in cells. Exposures of various biotic and abiotic stresses stimulate misfolded protein generation in cells. Imbalanced protein homeostasis mediated by aggregated proteins activates both chaperones and quality control (*QC*) E3 ubiquitin ligases for folding and degradation, respectively. Under stress conditions, continuous successful attempts of both ubiquitin–proteasome system (UPS) and QC system retains protein homeostasis balance and suppresses formation of proteotoxic species and leads cells towards normal survival conditions. Impairment in UPS and inefficient QC system due to mutations in QC E3s or overburden of misfolding proteins that aggravate multifactorial proteotoxic effects in cells probably lead towards cell death

(NHLRC1) encodes an aberrant malin, and a ubiquitin ligase may be one of the factor of LD pathogenesis [116, 117]. Malin is a RING finger ubiquitin ligase and laforin protein, a dual specificity phosphatase that is recruited towards aggresomes during proteasomal inhibition stress condition [118]. Malin also interacts with laforin and functionally promotes laforin degradation by UPS in cells [117]. Aberrant malin or laforin leads to the accumulation of misfolded proteins, suggesting the involvement of malin E3 ubiquitin ligase in the clearance of misfolded proteins with the help of UPS [119]. It may be possible that functional interaction of malin and laforin together contributes in the pathogenesis of LD [120]. Till now, malin is another ubiquitin ligase that is directly implicated in neurodegenerative diseases. The presence of ubiquitin-positive protein aggregates of malin suggests the dysfunction in UPS. Malin–laforin complex together with Hsp70 alleviates the cellular toxicity generated by misfolded proteins, and this functional complex could be targeted as a potential therapeutic strategy against neuronal cytotoxic proteins [119].

E6-AP

An end product of *UBE3A* gene is a homologous to E6-AP C terminus (HECT) domain E3 ubiquitin ligase known as E6-associated protein (E6-AP). Mutations in the *UBE3A* gene or aberrant form of E6-AP protein are considered as a prime factor for Angelman syndrome (AS) mental retardation neurodevelopmental disorders. Growing evidences suggest that some E3s are associated with chaperones and directly implicated in cellular QC system including regulation of neurogenesis [121, 122]. Recently, it has been reported that *UBE3A* is actively involved in synapse development and also plays an important role in experience-dependent synaptic plasticity [123]. An AS mice model study clearly demonstrates that E6-AP loss of function does not affect normal cellular architecture in the brain but leads to dendritic abnormalities related to shape, size, and density of spines. This study suggests that E6-AP probably contributes to the regulation of spine development and is actively involved in the development of synaptic plasticity [124]. *UBE3A* promotes the ubiquitination and degradation of synaptic protein Arc and regulates synaptic functions. Loss of function of *UBE3A* results in the accumulation of Arc synaptic protein in neurons. Accumulated Arc stimulates the excessive internalization of AMPA receptors at synapse and thus, finally, disturbs normal synaptic functions in neurons [125]. E6-AP also promotes the degradation of expanded polyglutamine proteins via UPS and suppresses protein aggregation-mediated cellular toxicity [126]. It is also reported that AS possesses PD-like symptoms [127]. E6-AP was also found to be a component of Lewy bodies linked with PD [128]. In our previous study, we observed that E6-AP interacts with Hsp70 molecular chaperone and promotes the clearance of misfolded proteins anchored by Hsp70 chaperone. Proteasomal inhibition induces recruitment of E6-AP at the site of microtubule organizing center (MTOC) and CFTR aggresomes. Under various cellular insults such as oxidative stress and ER stress, E6-AP endogenous levels are found to be induced [129]. To widely understand the AS pathomechanism, it is important to identify more pathogenic target proteins of E6-AP ubiquitin ligase.

QC E3 Ubiquitin Ligases and Neurodegenerative Diseases

Presence of misfolded and accumulated proteins is a chief pathological sign of various neurodegenerative diseases. Deposition of misfolded proteins affects neuronal signaling, as well as several cellular pathways and finally leads to cell death. It is a well-established thought that aggregated proteins stimulate UPS activity in these diseases either by enhanced ER stress or by oxidative stress [1, 130]. Loss of QC function due to aberrant QC E3 ubiquitin ligases aggravate protein homeostasis imbalance. QC E3 ubiquitin ligases promote clearance of accumulated misfolded proteins and reduce their cytotoxic potential. Still, we are so far to understand that how few E3 ubiquitin ligases function as QC E3s as well as target specific substrates. Overexpression of E3 ubiquitin ligases and/or chaperones ameliorates the cellular toxicity of misfolded proteins in both cellular and animal models [67, 131–134]. Hul5 is a HECT domain E3 ubiquitin protein ligase and interacts with proteasome with the help of Rpn2 subunit; this functional interaction leads to chain elongation of proteasomal substrates [135]. Hul5 is identified as a component of ERAD pathway and is involved in the degradation of specific protein fragments [136]. Hul5 is implicated in heat shock stress response, plays a chief role in the ubiquitin-mediated degradation of cytosolic misfolded proteins, and acts as a cytosolic protein QC E3 ubiquitin ligase [137]. Another cytosolic QC E3 ubiquitin ligase is Ubr1. Cytoplasmic proteostasis QC function of Ubr1 is independent of its "N-end rule." Ubr1 apply different cellular strategies against cytoplasmic misfolded proteins. Ubr1 possesses chaperone-assisted QC function [138]. Sir Antagonist 1 (San1) is an ubiquitin ligase; this QC nuclear ubiquitin ligase specifically targets abnormal cytotoxic proteins for degradation as compared to normal proteins with the help of conformational plasticity of disordered domains. San1 retains an exceptional capability to distinctly recognize abnormal toxic proteins [138–140]. Ubr1 and Ubr2 ubiquitin ligases stimulate the clearance of unfolded cytosolic proteins via UPS. Ubr1- and Ubr2-mediated clearance of toxic misfolded proteins leads to

cytoprotective response in cells and reduces cellular toxicity [141]. Here in this section, we summarize few E3 QC ubiquitin ligases, those that are chiefly involved in neurodegenerative diseases.

Recently, we investigated that E6-AP stimulates proteasomal degradation of misfolded polyglutamine repeats [126]. However, we still need to understand more about how E6-AP specifically responds against such aggregates. Apart from E6-AP, other E3 ligases such as parkin [133], mitochondrial ubiquitin ligase (MITOL) [142], CHIP [143, 144], and HRD1 [145] have been found playing active roles in clearance of polyglutamine-expanded aggregates and reducing cytotoxicity.

The role of E3 ubiquitin ligases in the pathophysiology of ALS has been implemented vastly. NEDL1 E3 ubiquitin ligase associates with mutant forms of SOD1 protein. NEDL1 tightly forms an ubiquitinated complex with translocon-associated protein-δ (TRAP-δ) and dishevelled-1 (Div-1); these cytotoxic protein aggregates potentially contribute in motor neuron death in FALS [146]. Dorfin [147], gp78 [27], MITOL [148, 149], and CHIP [72] have been reported for proteasomal-dependent degradation of mutant SOD1 protein and its inclusion-like structures. Dorfin, an in-between-ring-finger (RING-IBR), is an E3 ubiquitin ligase. Overexpression of dorfin reduces SOD1 inclusions in neuronal cells and generates cellular protective response against the toxic effects of mutant SOD1 proteins [147]. Chimeric complex of dorfin-CHIP proteins are the first chimera E3s, which effectively promotes the degradation of mutant SOD1 proteins and are to be strongly intended for the treatment of neurodegenerative disorders [150]. Studying the role of a vast range of E3 ubiquitin ligases significantly contribute to the pathophysiology of ALS diseases and suggests a more systemic approach towards understanding of this devastating motor neurodegenerative disorder.

E3 ubiquitin ligases also play a major role in pathobiology of AD. Hrd1 E3 ubiquitin ligase is capable of degrading tau protein and promotes neuronal survival under proteotoxic conditions [151]. In an earlier study, it is observed that CHIP regulates neurofibrillary tangle (NFT) formation with the help of Hsp70 chaperone. CHIP endogenous levels were found to be increased in AD samples as compared to normal tissue [152]. Ubiquitin ligase CHIP deletion only aggravates both phospho- and caspase-3-cleaved endogenous tau species [153]. SCF (Fbox2)-E3 ubiquitin ligase retains a potential to degrade beta-secretase which is involved in AD pathology [154]. Heterodimer of amyloid precursor protein binding protein (APP-BP1) and Uba3 act as neural precursor cell expressed, developmentally downregulated 8 (NEDD8)-specific E1-activating enzyme [155–157]. APP-BP1 is also found to be implicated in apoptosis; its overexpression causes death

Table 1 A unified list of several E3 ubiquitin ligases (shaded in green) that actively interact or recruit with various misfolded proteinaceous bodies and implicated in diseases caused by protein aggregation

E3 ubiquitin ligase	Aggregated proteins	Aggresomes	Misfolded proteins	Amyloids	Inclusion bodies	References
RNF146						(171)
Parkin						(100,111, 167-169,172,175)
E6-AP						(126,128-129)
Malin						(118-119)
Rfp2						(43)
Dorfin						(147, 150, 174,179)
CHIP						(64, 67, 176-178,181)
RNF5/RMA1						(173)
Ubr2						(141)
Ubr1						(138, 141)
Hul5						(137)
NEDL1						(146)
Tul1						(180)
BAR						(22)
TRAF6						(165)
Gp78						(27)
San1						(138,140)
Doa10/TEB4						(32,34)
HRD1						(35, 40, 37-38, 42, 170)

of neuronal cells [158]. A monomer of APP-BP1 is the substrate of TRIP12, a HECT domain containing E3 ligase, but a heterodimer of APP-BP1 and Uba3 is not a substrate of TRIP12, suggesting a potential role of TRIP12 in protecting cell from apoptosis [159]. SEL-10, a member of the Skp1-Cdc53/CUL1-F-box protein (SCF) and a homologue of yeast Cdc4 has also been reported to degrade another product of amyloid precursor protein called presenilin [160].

Mutated parkin gene encodes its aberrant form of E3 ubiquitin ligase protein, which is one of the responsible factors of PD. Parkin also acts as ubiquitin ligase for several other proteins including synphilin-1, α/β tubulin, and cyclin E [98, 161, 162]. CHIP enhances ubiquitin ligase activity of parkin [70]. Phosphorylation is an important biological process, which plays a critical role in pathogenesis of PD. In this context, two protein kinases have been reported importantly—leucine-rich repeat kinase 2 (LRRK2) and PINK1 [163]. The complexity becomes much prominent when such regulatory kinase is found to be a substrate of another E3 ubiquitin ligase, e.g., LRRK2 is a substrate of CHIP [164]. Tumor necrosis factor receptor-associated factor 6 (TRAF6) E3 ubiquitin ligase has been found associated with Lewy bodies in PD. TRAF6 ubiquitinates both mutant DJ-1 as well as α-synuclein. TRAF6 protein acts as ubiquitin ligase for both mutant DJ-1 and α-synuclein. TRAF6 induces the accumulation of misfolded and polyubiquitinated DJ-1 into cytoplasmic aggregates and also colocalizes with α-synuclein in Lewy bodies of human postmortem brains of PD patients [165]. TRAF6 also colocalizes with tau in the samples of AD patients [166]. Involvement of other E3 ubiquitin ligase makes our understandings much clear towards a comprehensive approach where not only one but rather various E3 ligases work together in coordination, so that they can alleviate pathological conditions in cells or tissues. Here in Table 1, we enlist various E3 ubiquitin ligases with probable association with various abnormal misfolded proteinaceous species implicated in protein aggregation events. Most of such E3 ligases are in periphery of cell's QC mechanism. The E3 ligase activity of ubiquitin ligases plays an important role in the clearance of abnormal protein and probably generates cytoprotective

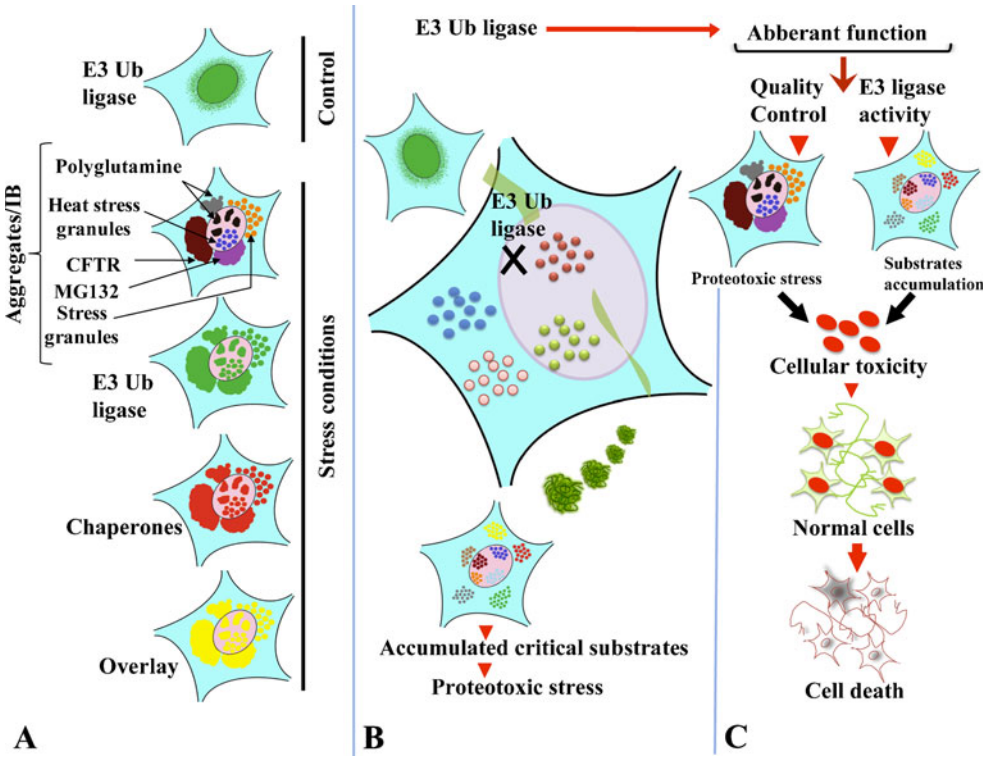

Fig. 3 Proposed schematic overview of a general QC E3 ubiquitin ligase protective function against protein misfolding and aggregation-mediated cell death. **a** Several neurodegenerative diseases are associated with the formation of disordered and ordered intracellular metastable aggregates by toxic proteins. Under normal conditions, QC E3 ubiquitin ligases localize in various cellular compartments, such as nucleus, and when cellular chaperone capacity does not cope under stress conditions, abnormal protein aggregates can be targeted by QC E3 ubiquitin ligases for degradation in various cellular compartments. **b** Previous studies suggest that QC E3 ubiquitin ligases are actively involved in the ubiquitination of both cytoplasmic and nuclear substrate proteins. Possibly, QC E3 ubiquitin ligases' loss of function mediates accumulation and sequestration of these substrates with preformed aggregates that chiefly contribute in proteotoxicity. **c** In the current model, on the basis of emerging evidences, most likely QC E3 ubiquitin ligases' aberrant function leads to accumulation of critical substrates in cells. Loss of QC function may be responsible for imbalance in protein quality control mechanism of cells generated by proteotoxic stress. Deregulated stress cascade may aggravate endogenous cellular toxicity induced by cumulative aggregation of misfolded proteins and critical substrates; most probably this proteostasis imbalance finally progresses cells towards death

response against various proteotoxic species. On the basis of previous reports, here we propose a general QC E3 model, which describes the significance of QC E3s in protein QC mechanism as shown in Fig. 3.

Conclusions and Perspectives

Recent studies have reported the role of E3 ubiquitin ligases in various neurodegenerative diseases. Here in this review, we summarize that most probably, QC E3s are mainly neuroprotective in nature. QC E3s retain an ability to promote the degradation of misfolded or aberrant proteins that trigger pathogenic conditions in neurodegenerative diseases. However, the mechanism by which QC E3s facilitates crucial clearance of misfolded proteins and generates neuroprotection remains poorly understood. It is important to investigate which other proteins are associated with QC E3s and how the loss of this novel interaction initiates neurodegeneration against various stress conditions. Functional implication of E3 ubiquitin ligase in QC system provides hope for common therapeutic strategies against these diseases. Still, we are so far to know how these QC E3s distinctly target abnormal toxic proteins as compared to certain critical substrates in the same cell. Perhaps the most exciting direction for future research is to investigate an unidentified sequence in the functional domains of various QC E3s to facilitate a unique mechanism to differentiate in between normal and misfolded proteins associated with neurodegenerative diseases.

Understanding the effects and consequences of QC E3s' loss of function and their role in the neuronal dysfunction would anticipate the possibility of treatment of neurodegenerative diseases. How QC E3s sense both protein misfolding signature and aggregation in cell and target them for further degradation is an exciting area of future research. Lowering the burden of protein aggregation, QC E3s get recruited towards the site of aggregation in the cell, and most probably, sequestration of these QC E3s and their association with aggregates lead to more disastrous situation in the cell. Depletion of QC E3s at the site of origin may lead to accumulation of respective substrate proteins and probably aggravate the phase of aggregation in the same cell and eventually collapse essential cellular functions. It is with interest that we can consider the next challenges in this field which include the search of more ribosomal-associated QC E3s as first line defense mechanism partners in cells against proteotoxicity. It is also important to understand the effects of protein misfolding in cells and clarify the mechanistic principles of misfolded protein-associated E3s into the degradation process. The prospect is that QC E3s upregulation could be one of the best possible therapeutic value to various neurodegenerative diseases caused by aggregate-prone proteins.

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