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Review

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Review

The ubiquitin codes in cellular stress responses

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Abstract

Ubiquitination/ubiquitylation, one of the most fundamental post-translational modifications, regulates almost every critical cellular process in eukaryotes. Emerging evidence has shown that essential components of numerous biological processes undergo ubiquitination in mammalian cells upon exposure to diverse stresses, from exogenous factors to cellular reactions, causing a dazzling variety of functional consequences. Various forms of ubiquitin signals generated by ubiquitylation events in specific milieus, known as ubiquitin codes, constitute an intrinsic part of myriad cellular stress responses. These ubiquitination events, leading to proteolytic turnover of the substrates or just switch in functionality, initiate, regulate, or supervise multiple cellular stress-associated responses, supporting adaptation, homeostasis recovery, and survival of the stressed cells. In this review, we attempted to summarize the crucial roles of ubiquitination in response to different environmental and intracellular stresses, while discussing how stresses modulate the ubiquitin system. This review also updates the most recent advances in understanding ubiquitination machinery as well as different stress responses and discusses some important questions that may warrant future investigation.

Keywords ubiquitin, E3 ligase, environmental stresses, intercellular stresses, stress response, homeostasis

Introduction

Living organisms are never free of the stresses induced by internal or exogenous factors, as constant changes are intrinsic to all live cells that have dynamic reactions ongoing within. The changes in various environmental effectors, such as temperature, oxygen availability, salinity, pH, toxic chemicals, and infectious reagents, or the physiological alterations, including DNA damage/lesions and accumulated biomolecules [oxidative molecules, ubiquitin (Ub), and misfolded proteins], can result in the disruption of the relatively balanced status of biological or chemical molecules or cellular networks, causing stresses at cellular, tissue, or organismal levels (Galluzzi et al. 2018). Maintaining homeostasis is fundamental for cells to survive under different stresses. Cells possess complicated and effective responses to defend against and recover from stresses. Once the noxious stress is prolonged and unresolved or the corresponding cellular response is disrupted, stressed cells could face severe damages even death. Regulation of gene transcription and translation is one of the most basic strategies that cells have evolved to maintain homeostasis (Spriggs et al. 2010). Moreover, post-translational modifications (PTMs) (including phosphorylation, ubiquitination, acetylation, etc.) are dynamic and reversible strategies that cells employ to alter the functionality of specific signaling

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pathways in response to different stresses, and direct cells to different fates. It is noteworthy that PTMs also play critical roles in controlling gene transcription and expression. Thus, understanding the roles of the PTMs in stress responses and developing proper interventions, if possible, would therefore provide an increased number of avenues to maintain cellular homeostasis, and may ultimately bring about beneficial clinical outcomes.

Ubiquitination is one of the most ubiquitous and crucial PTMs in normal homeostasis and diseases, targeting thousands of substrates and controlling the majority of physiological processes, such as gene transcription, cell growth and death, DNA replication, chromatin assembly, molecule trafficking, metabolism, immune response, and development (Pickart 2001; Weissman 2001). By covalently labeling a Ub molecule or Ub chains to substrates, ubiquitination shows the ability to control stability, activity, localization, or binding partners of targeted substrates. Disruption of ubiquitination could lead to mislocalization of proteins, accumulation of damaged or misfolded proteins, improper complex assembly, aberrant enzymatic activities, or inaccurate signal transductions, contributing to the development of human diseases, including cancers, autoimmune diseases, developmental disorders, metabolic syndromes, and neurodegeneration (Popovic et al. 2014; Rape 2018).

Although ubiquitination has displayed regulatory roles in different cellular stress responses, it is still not been systematically summarized. In the following sections, we will introduce the ubiquitination system first, and discuss the important roles of ubiquitination in regulating cellular responses to various environmental stressors (hypoxia, heat or cold shock, and osmotic stress) or intercellular stressors (DNA damage, Ub stress, ER stress, and oxidative stress). Notably, one environmental stressor may induce one or more intercellular stresses, and an intercellular stress can lead to other intercellular stresses. As one stress is usually associated with others, some cellular responses could regulate and resist multiple stresses.

The ubiquitination system

Ubiquitin

Ub, a small and highly stable protein with 76 amino acids, is named for its extremely ubiquitous distributions in all eukaryotic cells. Ub was first isolated from the thymus in 1975 by Goldstein et al. and was found to form an isopeptide linkage with histone 2A in 1977 by Goldknopf and Busch (Goldknopf and Busch, 1977; Hershko and Ciechanover 1998). There are four genes in human encoding Ub molecules (Fig. 1A). Gene *UBA52* and *RPS27A* encode two N-terminal Ub moieties that are fused to ribosomal protein L40 and S27a. The monomeric Ub that is cleaved off from fusion proteins by deubiquitinating enzymes (DUBs) sustains the total Ub pool in normal conditions. Moreover, gene *UBB* and *UBC* encode PolyUb precursors with 3 and 9 repeats, respectively, rapidly increasing Ub concentration in cells under stress (Finley et al. 1987; Rape 2018). These two fusion precursors are also cleaved by specific DUBs to release free Ub molecules. Apart from being a modifier, Ub can be targeted by multiple PTMs (Fig. 1B) (Swatek and Komander 2016). In particular, conjugation of Ub to lysine residues (K6, K11, K27, K29, K33, K48, and K63) or N-terminal methionine of Ub itself generates different PolyUb chain linkages, which determines the destiny of substrates (Mansour 2018). SUMO, ISG15, and NEDD8 can also modify the lysine residues of Ub (Liao et al. 2022). Moreover, small chemical groups can covalently attach Ub to form phosphorylation, acetylation, deamidation, ADP ribosylation, and phospho-ribosylation of Ub (Mattiroli and Penengo 2021). While all the Ser, Thr, and Tyr residues on Ub can be phosphorylated, 6 out of 7 Lys residues of Ub undergo acetylation (Mattiroli and Penengo 2021). Recent reports established that a specific arginine of Ub can undergo phospho-ribosylation (Bhogaraju et al. 2016; Qiu et al. 2016). Modified Ub could act as different signaling molecules to regulate cellular activities. The new layers of Ub modifications exponentially increase the complexity and functionality of the Ub system, which is part of the so-called Ub codes.

The structure of Ub includes an β-grasp fold with a C-terminal flexible tail and several hydrophobic surfaces, which is highly conservative from yeast to human (Vijay-Kumar et al. 1987). A variety of ubiquitin-binding domains (UBDs) are presented in numerous cellular proteins (>150) and recognize the hydrophobic surfaces, especially Ile44 patch, of Ub, triggering functional events (Dikic et al. 2009). Noteworthy, UBB + 1, a frameshift Ub mutant, bearing a 19-amino-acid extension at the C-terminus, emerges in aging cells and is readily utilizable by the Ub machinery. The presence and accumulation of UBB + 1 can ultimately impact the activity of the Ub-proteasome system (UPS) (Van Leeuwen et al. 1998; Lam et al. 2000), and probably other cellular processes as well. UBB + 1 expression leads to long-term proteasome dysfunction and memory deficits in mice, suggesting a pathogenic role in the etiology of Alzheimer's disease (AD) (Fischer et al. 2009; Tank and True 2009).

Ubiquitin-like proteins

Ubiquitin-like proteins (UBLs) belong to a protein family whose members shares a conserved globular β-grasp conformation similar to Ub. UBLs can modify substrates via a similar enzymatic cascade (Kerscher et al. 2006). As UBLs members, SUMO conjugates substrates for sumoylation, NEDD8 for neddylation, ISG15 for Isgylation, UFM1 for ufmylation, URM1 for urmylation, FAT10 for FATylation, and ATG8 or ATG12 for ATGylation. UBL

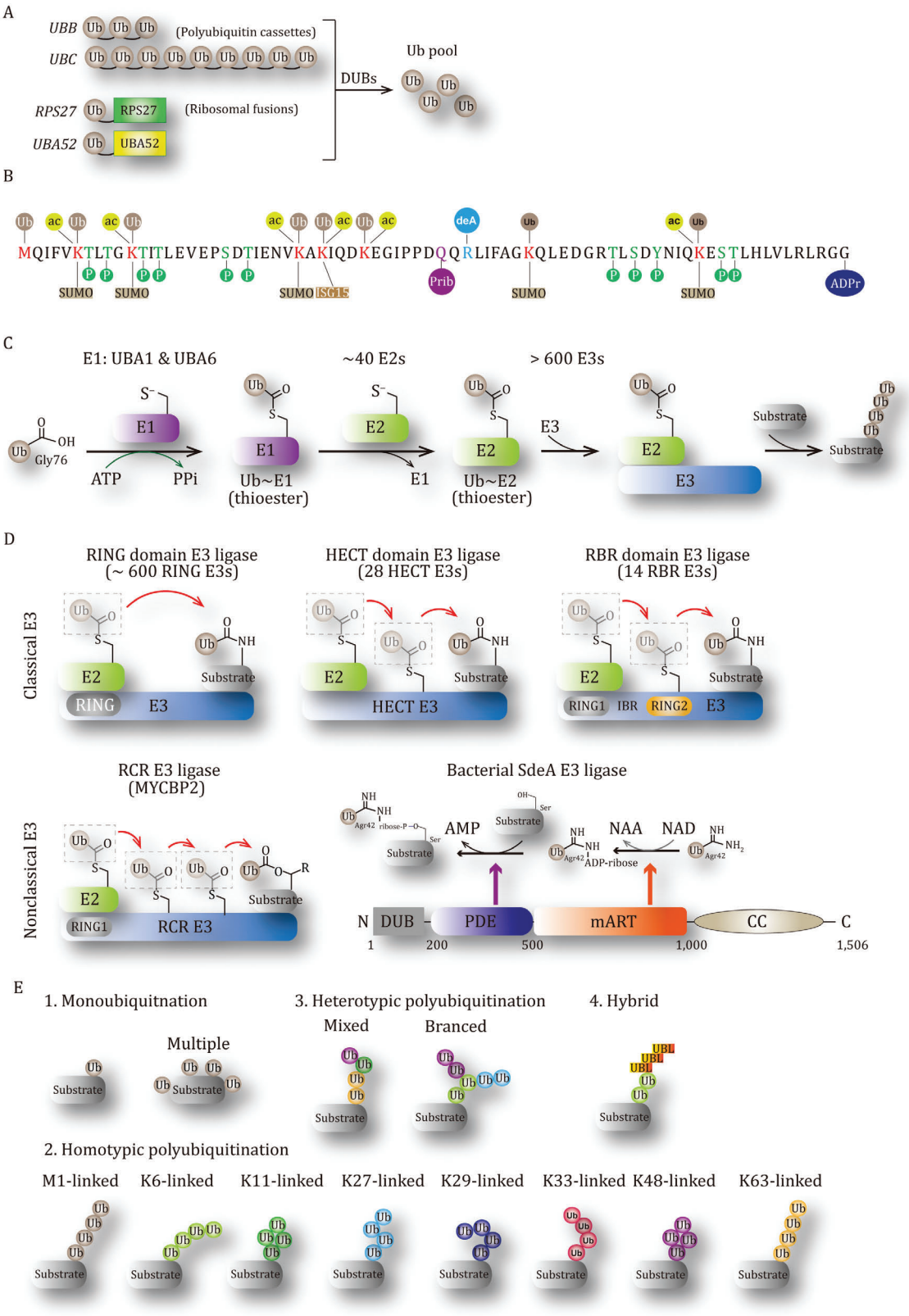


Figure 1. Ub and ubiquitination system. (A) Genes encoding Ub molecule. (B) PTMs on ubiquitin. (C) The ATP-dependent ubiquitination process. (D) E3 Ub ligases having different ubiquitin-transfer mechanisms. (E) Linkages of Ub chains.

conjugations regulate the stability, activity, molecular interactions, or localization of target substrates, which greatly increases the diversity and dynamics of proteome (Cappadocia and Lima 2018).

Ubiquitination

Ubiquitination is a specific three-step enzymatic cascade that covalently attaches Ub to substrates. Typically, the C-terminal glycine residue of Ub is conjugated to the ε-amino group of lysine residue(s) of substrate protein by forming an isopeptide bond (Komander 2009). The process of labeling substrate with Ub is cooperatively performed by at least three types of enzymes: Ub-activating enzyme (E1), Ub-conjugating enzyme (E2), and Ub ligase (E3) (Weissman 2001) (Fig. 1C). First of all, E1 activates free Ub via catalyzing the formation of a high-energy thioester bond between its catalytic cysteine residue and the C-terminal carboxyl group of Ub, in an ATP-dependent manner. In the second step termed E1–E2 thioester transfer, E1 submits the activated Ub to the catalytic cysteine residue of an E2. Finally, E3 ligases act as adaptors that specifically recruit both the Ub-charged E2 and substrate, leading to Ub transfer and the formation of isopeptide bond between Ub and the lysine residue(s) of the substrate (Olsen and Lima 2013; Liao et al. 2022). Once the ubiquitination process is finished, the stability or biological function of the target will be altered. In humans, there are eight E1 enzymes, but only two E1s (UBA1 and UBA6) are known to initiate the conjugation of Ub (Schulman and Wade Harper 2009). To date, around 40 E2 proteins and more than 600 E3 ligases are encoded by the human genome (Liu et al. 2019a; Liao et al. 2022). Besides the Ub carriers that mainly exist as E2~Ub conjugates (Ye and Rape 2009), E2s are now considered to play critical roles in controlling Ub chain assembly in the ubiquitination process [for a special review of E2, see (Stewart et al. 2016)]. E3 ligases have the capacity to selectively recognize substrates, determining the specificity of ubiquitination; E3 contains a large number of members and catalyzes different Ub linkages on substrates, which increases the diversity of ubiquitination.

Nonclassical ubiquitination

The lysine residue is the canonical site for ubiquitination. Although lysine was previously thought to be the only ubiquitination site for decades, no-lysine ubiquitination on other amino acids has been firmly demonstrated (McClellan et al. 2019). Nonclassical ubiquitination on cysteine, serine, and threonine residues of special substrates has been reported to modulate a variety of physiological activities (Squair and Virdee 2022). Moreover, the free amino group of the translation-initiating methionine or the amino acid at the second position (in the case of methionine removal) of substrate can act as the site of N-terminal ubiquitination (Akimov et al. 2018).

Protein has been considered as the only substrate for ubiquitination all the time, but two recent studies extend ubiquitination substrates beyond protein. Otten et al. first reported the ubiquitination of a non-proteinaceous substrate (Otten et al. 2021). During *Salmonella* infection, host E3 ligase RNF213 catalyzes the ubiquitination of the lipid A moiety of bacterial lipopolysaccharide (LPS), which relies on the RZ-finger domain but not the RING domain of RNF213, thus triggering cell-autonomous immunity. During the preparation of this review, a new research was published to show that eukaryotic phospholipids, mainly phosphatidylethanolamine (PE) presented in endosomes and lysosomes, were ubiquitinated by an E3 ligase Tul1 and deubiquitinated by Doa4, which regulates recruitment of ESCRT components (Sakamaki et al. 2022). Therefore, lipid is emerging as a new class of substrates beyond protein for ubiquitination.

DUBs

As a reversible reaction, ubiquitination is counteracted by DUBs. DUBs cleave the isopeptide bond and catalyze Ub removal from substrates. Mammalian cells express nearly 100 putative DUBs that are classified into two classes, cysteine proteases and zinc metalloproteases, according to different catalytic mechanisms they possess (Trulsson et al. 2022). Most mammalian DUBs (~90) are the members of cysteine proteases class harboring Cys-His-Asp catalytic motif, and these cysteine protease DUBs can be further classified into six families based on their sequence similarity: UCHs, USPs, OTUs, Josephins (MJDs), and two nearly identified families MINDYs and ZUFSP/ZUP1 (Kwasna et al. 2018; Clague et al. 2019; Liu et al. 2022a). Only the 12-member JAMM family DUBs are zinc-dependent metalloproteinases (Clague et al. 2019). DUBs have the selectivity to recognize and cleave particular Ub linkages and maintain ubiquitome homeostasis in cells. DUBs are well known for their antagonistic roles in regulating ubiquitination under stressful conditions, but they will not be discussed in detail here because of space limitations.

E3 Ub ligases

E3 ligases specifically recognize substrates and control the modification process, which makes it extraordinarily critical in the ubiquitination system. As the largest group in the ubiquitination process, E3 ligases have different E2-binding structures and catalytic mechanisms of Ub transfer, and they are generally divided into three major categories: RING (really interesting new gene) domain E3 (~600 members), HECT (homologous to E6AP carboxyl terminus) domain E3 (28 members), RBR (RING-between-RING) domain E3 (14 members) (Fig. 1D). While RING E3s catalyze the direct Ub transfer from E2 to substrate, HECT and RBR E3s deliver Ub to substrate via a two-step reaction.

RING domain E3 ligases

Freemont *et al*. first reported the conserved RING motif in 1991 (Freemont et al. 1991). RING domain was originally thought to mediate DNA binding, but numerous studies since 1999 unambiguously establish that most RING domain proteins possess E3 activity (Deshaies and Joazeiro 2009). It is worth noting that not every single RING domain can catalyze ubiquitination. Some RING proteins, such as MDMX, BARD1, and BMI1, do not display E3 activity alone, but they can form heterodimer with other RING E3 proteins to regulate E3 activity (Linares et al. 2003). The canonical RING domain is a Zn^{2+}-binding "cross-brace" structure that contains several uniquely spaced Cys/His residues: C-X_2-C-X_{9-39}-C-X_{1-3}-H-X_{2-3}-C/H-X_2-C-X_{4-48}-C-X_2-C (where X is any residue), responsible for E2~Ub recruitment (Deng et al. 2020). A conserved "U-box" structure is a modified version of RING domain that lacks Zn^{2+}-chelating Cys/His residues, but retains the similar RING structure stabilized by hydrogen bonds and salt-bridges (Aravind and Koonin 2000). RING E3 does not form an intermediate thioester bond with Ub, but acts as a scaffold to simultaneously recruit E2 and the substrate by different domains, finally facilitates Ub transfer from E2 to substrate.

Nearly 600 E3s, which is ~95% of total human E3s, are RING domain E3s. RING E3s can be categorized into four types according to their oligomeric patterns for function: monomers, homodimers, heterodimers, and multi-subunit complexes (Rennie et al. 2020). Some RING E3s can recruit E2 and substrate in the monomeric form, e.g., CBL-B, EL5, RBX1, RNF168, and E4B (U-box) (Dou et al. 2013). Many RING E3s often form dimer via the RING domain or surrounding motifs, which generate homodimers, such as BIRC7, RNF4, cIAP (BIRC2), TRIM5α, TRAF2, and CHIP (U-box), or heterodimers in which only one subunit binds to E2, such as RING1b-BMI1, BRCA1-BARD1, and Mdm2-MdmX (Morreale and Walden 2016; Balaji and Hoppe 2020). Moreover, Some RING E3s function as a multiple-subunit complex, such as the cullin-RING ligases (CRLs) that share a similar architecture consisting of several specific subunits: a cullin protein as scaffold, a RING-box protein for E2 binding at scaffold N-terminus, an adaptor protein, and a receptor protein for substrate recognition at scaffold C-terminus (Petroski and Deshaies 2005). Anaphase-promoting complex/cyclosome (APC/C), the largest E3 Ub ligase (1.2 MDa) ever described, is a multi-subunit RING E3 comprised of total 20 subunits from 15 proteins, including a RING E3 Apc11 and a cullin-like adaptor Apc2 (Barford 2020). Some covalent modifications, Ub binding, adaptors, cofactors, or ligands can modulate the catalytic activity of RING E3s.

HECT domain E3 ligases

E6-associated protein (E6AP or UBE3A) has a ~350 aa C-terminal domain that catalyzes polyUb chain formation (Huibregtse et al. 1995). Homologous related to E6AP carboxyl terminus are referred to HECT E3 family, containing 28 E3 members in human (Wang et al. 2020b). All HECT E3 have a conservative C-terminal catalytic HECT domain and structurally distinct N-terminus. The HECT domain is composed of two lobes: a larger N-terminal lobe (N-lobe) responsible for E2 binding and a smaller C-terminal lobe (C-lobe) that contains the catalytic cysteine. A highly flexible hinge region links these two lobes, allowing the catalytic C-lobe to move around to transfer Ub to the substrate (Weber et al. 2019). In contrast, the N-terminal part of HECT E3s is structurally variable and primarily mediates substrate recognition. HECT E3 has three subfamilies based on the similarity of N-terminal domains. The most famous one is nine-member NEDD4 family that is characterized by the existence of a C2 domain and 2–4 WW domains at the N-terminus: NEDD4, NEDD4L, SMURF1, SMURF2, NEDL1, NEDL2, WWP1, WWP2, and ITCH (Wang et al. 2020b). The second subfamily is the HERC family that owns RCC1-like domain (RLD). This family has four small HECR E3s (HERC3, HERC4, HERC5, and HERC6) containing a single RLD domain, and two large HERCs (HERC1 and HERC2) that possess more RLD domains (Sánchez-Tena et al. 2016). Last, the remaining 13 HECT E3s are categorized as "other" HECTs because they lack WW or RLD domains and show no specific N-terminal domains, such as an AZUL domain for E6AP, an ANK domain for HACE1, and a DOC domain for HECTD3; and these different N-terminal domains are able to recognize numerous substrates (Li et al. 2020a; Singh et al. 2021). HECT E3s catalyze an intermediate thioester bond between its catalytic cysteine and the N-terminus of the transferred Ub, which is the most significant feature of HECTs (Rotin and Kumar 2009). Specifically, HECT E3s first receive Ub from E2 by its active cysteine to further form an E3~Ub covalent intermediate, and subsequently transfer Ub to a specific residue of substrate. HECT E3s have intrinsic capacity to generate linkage-specific polyUb chains, especially the heterotypic chains with mixed linkages (Sheng et al. 2017; French et al. 2021).

RBR domain E3 ligases

RBR E3 ligases are characterized by a RING-HECT hybrid pattern (Wang et al. 2020a). Two research groups in 1999 first identified a highly conserved triple-RING/zinc finger motif in RBR proteins (Morett and Bork 1999; Van Der Reijden et al. 1999). RBRs share a structurally similar catalytic triad, consisting of a RING1 domain, a RING2 (or Rcat) domain that does not show canonical RING E3 fold, and an IBR (In-Between-RING) domain in the middle of the RING1 and RING2 (Reiter and Klevit 2018). While RING1 is responsible for the recruitment of Ub-changed E2 and RING2 offers a catalytic cysteine, the function of the IBR domain is still under investigation. The RBR

E3s-mediated ubiquitination is a sequential reaction: the E2-Ub conjugate is recruited to RING1, and Ub is subsequently transferred to the active cysteine of RING2 to form a covalent intermediate, and finally, RBRs catalyze Ub delivery from RING2 to the substrate (Wang et al. 2020a). To our knowledge, human genome encodes total 14 different RBR proteins. The best-known RBR member is Parkin whose ligase activity is associated with neurodegeneration (Pickrell and Youle 2015). Two RBR E3s (HOIL-1L and HOIP) and an adaptor SHARPIN form an E3 enzyme complex, the liner Ub chain assembly complex (LUBAC), which generates linear polyUb chains and regulates apoptosis, inflammation, angiogenesis, and immune diseases (Fu et al. 2021; Ning et al. 2022). The remaining 11 RBRs are Ariadne E3s (ARIH1, ARIH2, CUL9, and ANKIB1), RNF14, RNF144A, RNF144B, RNF19A, RNF18B, RNF216, and RNF217. All RBR E3s possess auto-inhibitory mechanisms. Structure studies reveal that the non-RBR domains occlude the catalytic cysteine on RING2, and isolates the RING2 far from the RING1-IBR, thus suppressing RBR activity (Duda et al. 2013; Trempe et al. 2013). The highly disordered linkers that connect RING1 to IBR and IBR to RING2 provide conformational flexibility, allowing structural rearrangements to expose the catalytic cysteine and fully exhibit RBR activity (Dove and Klevit 2017).

Nonclassical E3 ligases

Besides the classical E3 types discussed above, several atypical E3 Ub ligases have been established. UBC domain-containing BIRC6 (BRUCE/Apollon) and UBE2O have been identified as two special E2/E3 chimera, exhibiting dual E2 and E3 activities (Bartke et al. 2004; Nguyen et al. 2017; Yanagitani et al. 2017). Interestingly, several recent studies synchronously revealed a horseshoe-shaped anti-parallel dimeric architecture of BIRC6 that structurally facilitates SMAC engagement and antagonizes caspase-binding of BIRC6 (Liu et al. 2022c; Dietz et al. 2023; Hunkeler et al. 2023). Based on this, BIRC6/SMAC complex can efficiently modulate apoptosis and autophagy as a stress-induced hub (Ehrmann et al. 2022).

Notably, no human E3 enzymes targeting non-lysine residues had ever been identified until 2018. RING-Cys-relay (RCR) ligase MYCBP2/PHR1, a neuron-associated large protein that regulates axon maintenance, is a new class of E3 ligase that possesses esterification activity and intrinsic selectivity for threonine residues (Pao et al. 2018). Furthermore, the esterification activity of MYCBP2 modulates neurodevelopment and axon integrity (Mabbitt et al. 2020). Inhibition of the RCR may be a promising therapeutic strategy for mitigating neurologic diseases linked to axonal degeneration. However, whether MYCBP2 could target non-protein substrate by its high esterification activity needs further investigation.

Finally, several members of a bacterial SdiE effector family from *Legionella pneumophila* function as an E3 ligase and catalyze NAD-dependent ubiquitination on serine of multiple host GTPases, which is independent of ATP, E1, and E2 enzymes (Qiu et al. 2016). Unlike other E3s, SdiE proteins have multiple enzyme activities to complete a unique ubiquitination process. SdiE cleaves NAD, and then delivers NAD-derived ADP-ribose onto arginine 42 of Ub to generate ADP-ribosylated Ub via its mono-ADP-ribosyl transferase (mART) domain; the phosphodiesterase (PDE) domain of SdiE then converts ADP-ribosylated Ub into phosphoribosyl Ub by the cleavage of phosphodiester bond; the PDE simultaneously catalyzes a covalent ligation of phosphoribosyl Ub to substrate serine through a two-step transfer reaction (Bhogaraju et al. 2016; Akturk et al. 2018). SdiE-modified Ub can prevent the activation of host E1 and E2 and thus disturb numerous host cellular events. The DUB domain of SdiE does not disrupt its E3 activity but can cleave host Ub chains. Recently, two bacterial deubiquitinases (DupA and DupB) that harbor a catalytic PDE domain were reported to cleave phosphoribosyl-Ub conjugates catalyzed by SidE (Wan et al. 2019; Shin et al. 2020). Effector protein SidJ was also proposed to be a deubiquitinase for targeting phosphoribosyl-linked ubiquitination (Qiu et al. 2017). However, SidJ was further demonstrated to be a calmodulin-dependent enzyme that suppresses SidE E3 activity via a glutamylation reaction (Bhogaraju et al. 2019; Black et al. 2019; Gan et al. 2019). It will be extremely interesting to conduct investigations to explore SdiE-like mammalian E3 ligase in mammalian cells. However, so far, no such kind of human E3 has been identified. Given that mammalian cells can express abundant E1 and E2 proteins, a potential E1/E2-independent E3 could be easily ignored. It is almost certain that, with the continual development of research perceptions and experimental methods, more nonclassical E3 ligases should be found and reported in the near future.

Ubiquitination linkages

A single Ub is conjugated to one or multiple residues of the substrate, resulting in monoubiquitination or multi-monoubiquitination, respectively; multiple Ub molecules can be polymerized to generate various Ub chains by a single linkage type or different linkage types, which refer to the homotypic or heterotypic polyubiquitination (Fig. 1E). Seven lysine residues (K6, K11, K27, K29, K33, K48, and K63) and the first methionine (M1) of Ub can serve as acceptor sites for next Ub, which leads to eight kinds of homotypic linkages if all the Ub molecules in a chain offer the same residue to link another one (Komander 2009; Liao et al. 2022). Mixed linkages can be formed when Ub chains having one type of linkage is extended by a different type; ubiquitination targeting multiple residues of a Ub molecule in chains will

generate branched linkages (Liu et al. 2022b). Different linkages usually lead to distinct conformations, different protein recognitions, and changed functions of polyUb chains, extremely increasing the complexity and diversity of ubiquitination.

K48 and K63 linkages are probably the two best studied linkage types, while the other linkages were previously considered to be unconventional types with yet incomplete understanding but significant roles. A quantitative proteomics had revealed the relative abundances of seven individual polyUb linkages in eukaryotic cells (Xu et al. 2009). K48 and K11 linkages, which are 29% and 28% of all Ub linkages, represent two most abundant types, whereas two minimal linkage factions are K29 (3%) and K33 (3.5%); the remaining linkages K63, K6, and K27 have 16%, 11%, and 9% abundances, respectively. K48-linked Ub chains that show a compact fold structure generally target substrates for proteasomal degradation (Eddins et al. 2007). Different from K48 chains, K63-linked chains adopt an open linear conformation, controlling protein recruitment, trafficking, and activity, which plays roles in signal transduction, DNA damage repair, immune response, and other processes (Liao et al. 2022). Linear M1-linked chains have a similar structure to K63 chains and regulate NF-κB signaling (Spit et al. 2019). Interestingly, other atypical Ub linkages show distinct structural status between "open" K63 linkages and "close" K48 linkages (Liao et al. 2022). K6 linkage is proposed to have non-degradative roles, which may be involved in DNA damage response associated with E3 complex BRCA1/BARD1 or in Parkin-mediated mitochondrial homeostasis (Wu-Baer et al. 2003; Martinez et al. 2017). K11-linked chains were originally considered to be another proteolytic signal independent of K48 linkages; however, a recent study found that homotypic K11-linkages disrupts proteasomal degradation, while the heterotypic K11-linked chains are readily recognized by the proteasome in cell cycle regulation (Grice et al. 2015). As one of the noncanonical types, K27-linked ubiquitination exhibits diverse effects on DNA damage response (DDR), gene transcription, innate immune response, and T cell signaling, which has drawn more attention (Gatti et al. 2015; Li et al. 2020b, 2021; Zhou and Zhang 2022). K29-linked chains have been found to be enriched after proteasome inhibition in mammalian cells, proposing a role in regulating protein degradation; K29 linkages also modulate neuronal protection or pathogen infection (Nucifora et al. 2016; Karim et al. 2020; Sheng et al. 2020). Finally, K33-linked typical ubiquitination controls intracellular trafficking and autophagy (Heath et al. 2016; Feng et al. 2019). Although emerging insights of nonclassical Ub linkages have been established, more follow-up investigations are still needed to further elucidate the biological functions of these linkages. Several recent literature had reviewed atypical ubiquitination in detail (French et al. 2021; Squair and Virdee 2022; Zhou and Zhang 2022).

Ubiquitination controls cellular responses to environmental stresses

Hypoxia, temperature stresses, and osmotic stress are three most common environmental stressors for cells. They could disrupt regular signaling pathways and trigger cellular responses. Ub-associated events were frequently observed in these stresses-induced responses, which will be fully discussed as follows.

Hypoxia stress

Oxygen is fundamental for most metazoan organisms on Earth because cells utilize O_2 to fuel aerobic respiration and maintain ATP production. In particular, vertebrates develop multiple complex systems to efficiently capture and distribute oxygen to support living cells. Cells will experience hypoxia, a state of insufficient oxygen levels, and cannot perform cellular respiration to execute normal functional events if oxygen concentrations drop below a certain extent (Schödel and Ratcliffe 2019). Once hypoxia occurs, the hypoxia signaling pathway is activated to sense O_2 levels, initiating anaerobic glycolysis and maintaining cellular homeostasis for adaptation to oxygen starvation (Lee et al. 2020). Notably, the hypoxia response is predominantly controlled by the ubiquitination and degradation of hypoxia-inducible factor (HIF), a critical transcription factor (Muz et al. 2015).

HIF ubiquitination predominantly regulates hypoxia response

HIF accumulates under hypoxia and its stability governs hypoxia response. HIF is a heterodimer that consists of a HIF-α subunit and a HIF-β subunit (Wang et al. 1995). Three HIF-α proteins (HIF-1α, HIF-2α, and HIF-3α) are Class 1 bHLH-PAS proteins, which can heterodimerize with Class 2 bHLH-PAS protein HIF-1β (ARNT) or tissue-specific expressed HIF-2β (ARNT2), via their basic bHLH-PAS domains (Wu and Rastinejad 2017; Albanese et al. 2020). In hypoxia, HIF-1α, HIF-2α, or HIF-3α interacts with HIF-1β to form HIF-1, HIF-2, or HIF-3 complex, respectively, which can bind to hypoxia response elements (HREs) and activate the transcription of various target genes, modulating cell proliferation, metabolism, migration, apoptosis, and DNA repair (Ke and Costa 2006). While β subunits are constitutively expressed in nucleus and not affected by the oxygen levels, α subunits are highly sensitive to cellular O_2 tension and thus determine the oxygen sensitivity of HIF complexes (Lee et al. 2004). As an oxygen sensor and a critical regulator, HIF-α stability is precisely manipulated by a PTM cascade, including hydroxylation and ubiquitination (Fig. 2).

In normoxia, three prolyl hydroxylase domain (PHD) enzymes (PHD1, PHD2, and PHD3) sense and utilize O_2 as

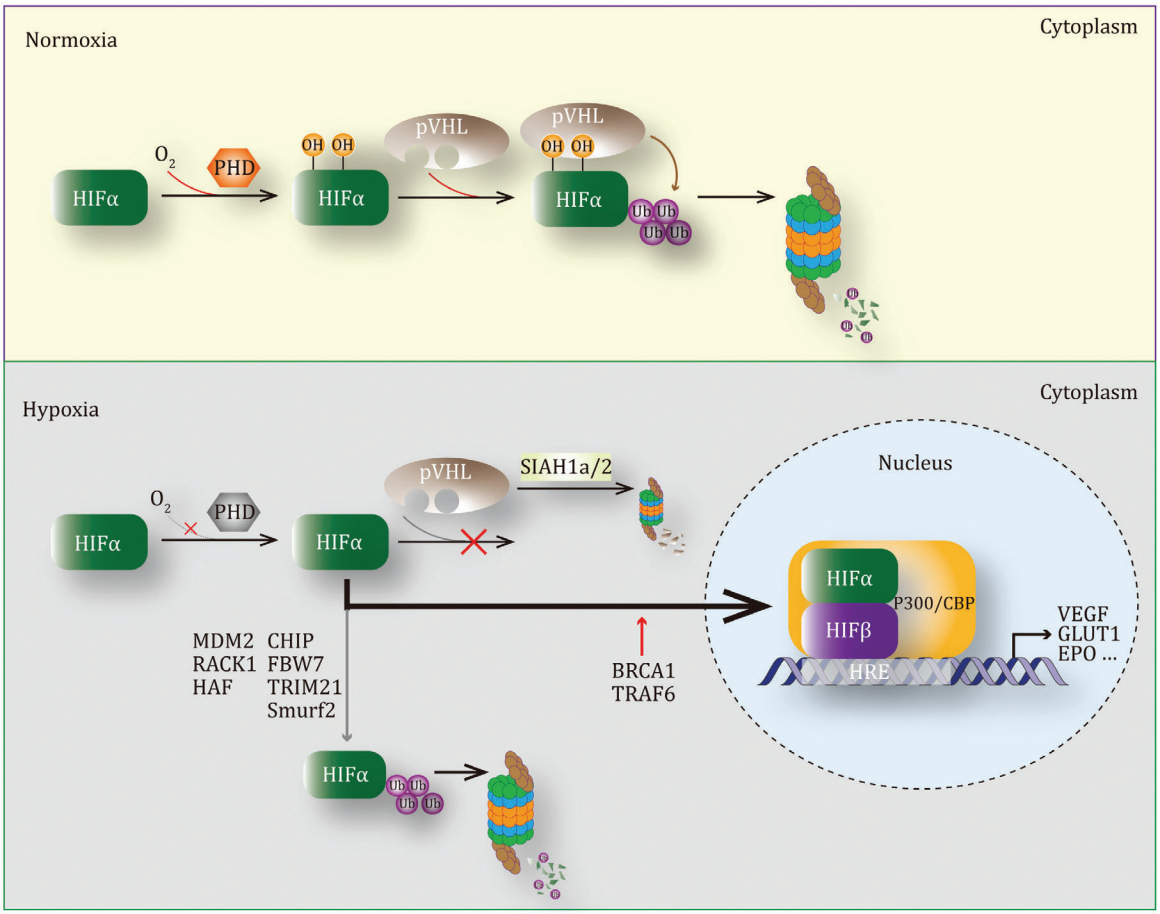


Figure 2. Regulation of HIF by ubiquitination. While VHL complex destroys HIFα in normoxia, some other E3 ligases can modulate HIFα accumulation under hypoxia.

a substrate to catalyze prolyl hydroxylation of conserved proline residues in HIF-α proteins (Pro402/564 of HIF-1α; Pro405/531 of HIF-2α; Pro492 of HIF-3α), which is the first step to destroy HIF (Bruick and McKnight 2001; Epstein et al. 2001; Ke and Costa 2006). Once hydroxylated, HIF-α proteins have more than a 1,000-fold increase in binding affinity of the von-Hippel-Lindau protein (pVHL), which functions as the recognition component of a multi-subunit RING E3 ligase (VHL complex) containing Cullin-2, Elongin-1, Elongin-2, and Ring-Box 1 (RBX1) (Hon et al. 2002; Min et al. 2002). Subsequently, the VHL complex ubiquitinates several specific lysine residues on HIF-α (K532/K538/K547 on HIF-1α, K497/503/K512 on HIF-2α) and triggers HIF-α proteasomal degradation (Maxwell et al. 1999; Ohh et al. 2000; Paltoglou and Roberts 2007). Apart from PHDs, factor inhibiting HIF (FIH), another hydroxylase, binds HIF-1α and uses molecular oxygen to catalyze asparagine hydroxylation of HIF-1α, which controls HIF-1α transcriptional activity under normoxia (Lando et al. 2002). These studies provided two different mechanisms to completely block HIF-α function in normoxia: PHD-mediated hydroxylation together with ubiquitination dramatically destabilizes HIF-α, and FIH-catalyzed hydroxylation further inhibits its activity. However, under hypoxia, PHDs show a decreased activity to catalyze HIF-α hydroxylation as available molecular oxygen is not enough, which leads to reduced interaction between HIF-α and VHL complex, therefore inhibiting HIF-α ubiquitination and promoting HIF accumulation in nucleus (Schödel and Ratcliffe 2019). Thus, controlling HIF-α abundance by oxygen levels is primarily mediated by ubiquitination and proteasomal degradation.

Although the activity of the VHL E3 complex is attenuated and HIF-α is accumulated in hypoxia, many other E3 ligases could regulate HIF-α abundance under hypoxia, which prevents the excessive accumulation of HIF-α. For example, E3 ligases MDM2, Parkin, and hypoxia-associated factor (HAF) had been shown to ubiquitinate and degrade HIF-1α irrespective of oxygen levels (Chen et al. 2003; Koh et al. 2008; Joshi et al. 2014; Liu et al. 2017). Recently, it is demonstrated that TRIM21 also physically interacts with HIF-1α and promotes its K48-linked ubiquitination and degradation (Chen et al. 2021; Zheng et al. 2021). Moreover, there are several E3 ligases that have been involved in HIF-1α ubiquitination in some diseases. Hyperglycemia had previously been proved to impair hypoxia-induced stabilization of HIF-1α protein without known mechanisms (Catrina et al. 2004). A subsequent

study showed that a glycolysis byproduct methylglyoxal (MGO) can be accumulated in high-glucose conditions and promote CHIP-mediated HIF-1α ubiquitination and degradation under hypoxia (Bento et al. 2010). Furthermore, glycogen synthase kinase 3β (GSK3β) catalyzes HIF-1α phosphorylation in hypoxia, facilitating the interaction of HIF-1α and an E3 ligase FBW7 (Cassavaugh et al. 2011). FBW7 deletion promotes HIF-1α accumulation, while FBW7 overexpression ubiquitinates and degrades phosphorylated HIF-1α, thus regulating angiogenesis in cancer. Because hyperglycemia can result in GSK3β activation (Mathur et al. 2018), these studies suggested that CHIP and FBW7 may cooperate to mediate the degradation of HIF-1α in hypoxia. Additionally, a proteomics screen identified Smurf2 (SMAD-specific E3 Ub protein ligase 2) as a HIF-1α interactor that can lead to the degradation of HIF-1α in hypoxic colorectal cancer cells (Zhao and El-Deiry 2021). Therefore, the stability of accumulated HIF-1α in hypoxia can be manipulated by multiple E3 ligases, which suggests that these E3 ligases have comparable but limited capacities to degrade HIF protein.

On the other hand, some E3 ligases can stabilize HIF-1α in hypoxia. Ub ligase BRCA1 was found to interact with HIF-1α and increase HIF-1α half-life, in which the RING domain of BCRA1 is required (Kang et al. 2006). TRAF6 also associates with HIF-1α and mediates K63-linked ubiquitination of HIF-1α, which stabilizes HIF-1α independent of oxygen; but TRAF6 does not target HIF-2α (Sun et al. 2013). BRCA1 or TRAF6-induced HIF-1α stabilization is probably resulted from non-proteolytic Ub linkages conjugated on HIF-1α. Taken together, while the VHL E3 ligase complex primarily mediates HIF-1α degradation in normoxia, the regulation of HIF-1α stability in hypoxia needs ubiquitination induced by different E3 ligases, which probably maintains an appropriate accumulation of HIF-1α in hypoxia.

Ubiquitination of other proteins supports hypoxia response

Besides HIF-α proteins, other proteins could undergo ubiquitination in response to oxygen changes. RING E3 SIAH proteins are important regulatory proteins in the hypoxic response (Nakayama et al. 2009). When hypoxia occurs, SIAH1a/2 can target HIF-1α hydroxylases, PHD1, PHD3, and FIH, for ubiquitination and proteasomal degradation (Nakayama et al. 2004; Fukuba et al. 2008). Moreover, hypoxia increases the interaction between SIAH2 and a kinase HIPK2, a negative regulator of gene expression, leading to HIPK2 polyubiquitination and degradation (Calzado et al. 2009). Therefore, although SIAH proteins do not target HIF-1α directly, they can regulate the hypoxic response by ubiquitinating some negative regulators of hypoxia. Additionally, hypoxia deactivates Hippo signaling in a SIAH2-dependent manner. In response to low cellular O_2 level, SIAH2 interacts with LATS2, a critical Hippo pathway component, and mediates LATS2 ubiquitination and degradation, which causes YAP nuclear translocation and tumorigenesis (Ma et al. 2015). In addition, E3 ligase Pellino-3 mediates TRAF6 ubiquitination and suppresses TRAF6 ability to ubiquitinate and stabilize HIF-1α (Siednienko et al. 2012; Yang et al. 2014). Interestingly, hypoxia can upregulate an Ub E2 UBE2M that cooperates with DJ-1/Parkin ligases to ubiquitinate and degrade another E2, UBE2F, and this event could be a negative regulatory mechanism that inhibits the growth of lung cancer cells under hypoxia (Zhou et al. 2018). In summary, ubiquitination of other proteins in hypoxia could further modulate cellular hypoxia response, via indirect ways to control HIF stability and functions.

Temperature stresses

Sudden temperature changes can alter biochemical features of biological molecules and interfere with cellular functions. Heat shock or cold shock response can be activated when cells undergo different temperature stresses. Ubiquitination events happen in the heat/cold shock response.

Heat shock

Heat is the major temperature stressor, which usually causes the aggregation of damaged proteins. A temperature increase of just a few degrees can trigger the heat shock response, inducing the expression of a group of highly conserved heat-shock proteins (HSPs) that promote protein folding, trafficking, and complex assembly (Sonna et al. 2002; Richter et al. 2010). These HSPs are molecular chaperones and can alleviate protein aggregation. The expression of HSPs is generally determined by the activation of heat shock transcription factor 1 (HSF1). HSF1 is usually inactivated in the non-stressful cells but is activated upon heat stress. Heat shock leads to the activation of many protein kinases, including MAPK and GSK, which catalyze HSF1 phosphorylation and trimerization (Guettouche et al. 2005; Kmiecik et al. 2021). Activated trimeric HSF1 can bind to heat shock elements (HSE) DNA sequence of gene promoters and recruits transcriptional machinery to initiate genes transcription and expression, including HSPs and various other proteins. Ubiquitination has shown critical roles in regulating the heat shock response (Fig. 3).

First, Ub itself is a heat shock protein. In the *UBC* gene promoter, there are at least three HSEs with different configurations (Bond and Schlesinger 1985, 1986; Crinelli et al. 2015), which dramatically increase Ub expression and accumulation in the heat-shock cells, leading to secondary Ub stress, an intercellular stress that will be discussed later. Moreover, an increase of high molecular weight Ub conjugates is accompanied by a dramatic fall of free Ub molecules in the heat-shock cells, suggesting an elevated ubiquitination activity (Carlson et al. 1987; Parag et al. 1987). The increase of Ub and ubiquitination

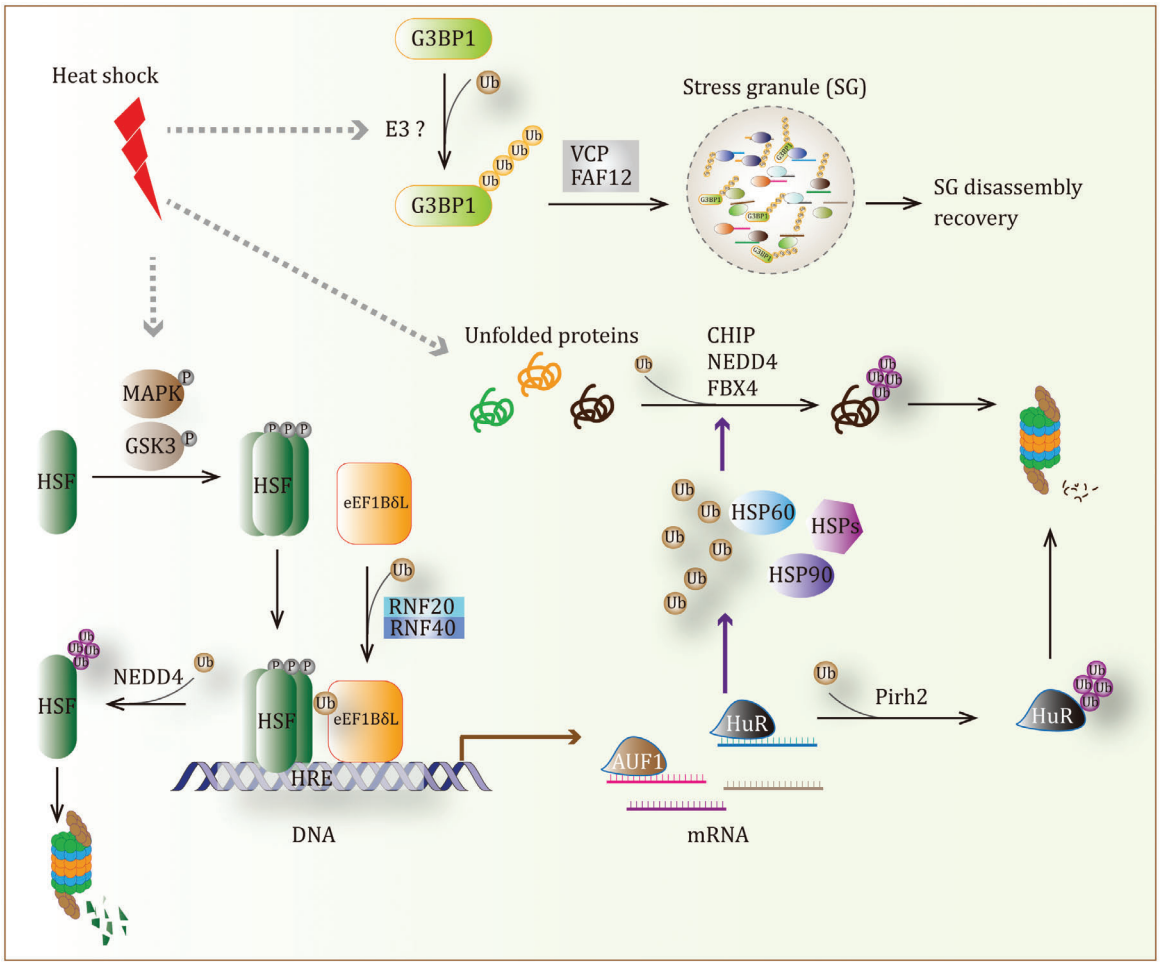


Figure 3. Role of ubiquitination in the heat shock response. Ubiquitination regulates the stability of HSF, the expression of heat shock-responsive genes, the degradation of unfolded proteins, and cellular recovery from heat shock.

has been proposed to play roles in eliminating misfolded proteins and preventing excessive synthesis in heat shock. Indeed, lots of proteins are synthesized and readily accumulated upon heat shock, but newly synthesized proteins could be sensitive to Ub-dependent degradation (Medicherla and Goldberg 2008). Heat shock also triggers ubiquitination-mediated proteolysis of some RNA-binding proteins, including AUF1 and HuR, inhibiting the expression of target genes (Laroia et al. 1999; Abdelmohsen et al. 2009). Moreover, ubiquitinated nascent proteins can facilitate the recruitment of negative transcription elongation factors to gene promoters, leading to transcriptional downregulation of relevant genes (Aprile-Garcia et al. 2019). According to these studies, it is reasonable to speculate that the Ub system is a powerful strategy cells utilized to control heat-induced protein synthesis and alleviate the burden of misfolded proteins, by either direct degradation of synthesized proteins or inhibition of gene expression.

Second, several E3 ligases have shown important effects on the removal of misfolded proteins. The E3 Ub ligase CHIP is a co-chaperone of heat shock protein Hsp70/Hsp90 (Ballinger et al. 1999; Connell et al. 2001). The TPR domain of CHIP interacts with the C-terminal GPTIEEVD motif and the α-helical lid subdomain of Hsp70 (Graf et al. 2010; Zhang et al. 2015). This bipartite interaction is necessary for CHIP to ubiquitinate and degrade chaperone-bound substrates, eliminate misfolded proteins, and maintain homeostasis (Petrucelli et al. 2004; Qian et al. 2006; Soss et al. 2015). FBX4, an F-box containing E3 ligase, specifically interacts with a small HSP αB-crystallin to mediate the ubiquitination of unknown proteins (den Engelsman et al. 2003). *Drosophila* Linear Ubiquitin E3 ligase (LUBEL), an orthologue of mammalian HOIP, controls linear ubiquitination upon heat shock and promotes survival of flies (Asaoka et al. 2016). Moreover, NEDD4 was reported to be an E3 ligase that promotes ubiquitination and degradation of cytosolic misfolded proteins in the heat-shock cells (Fang et al. 2014; Kim et al. 2016). In addition, E3 ligase Pirh2 ubiquitinates HuR protein and facilitates its degradation in response to heat stress, controlling cell survival under elevated temperatures (Daks et al. 2021). These E3 ligases had shown negative roles in protein synthesis,

probably providing quality control to reduce the hazard of damaged proteins in heat shock. However, some other E3 ligases have the ability to increase specific responsive proteins under heat stress. For example, RNF20/40 E3 complex monoubiquitylates Lys382 of a heat shock transcription factor eEF1BδL and promotes its accumulation, increasing the transcription of heat shock-responsive genes (In et al. 2019). Notably, CHIP could induce trimerization and transcriptional activation of HSF1 to protect against heat shock-induced cell death (Dai et al. 2003). Although ubiquitination generally limits excessive protein synthesis in heat shock, some E3 ligases may facilitate protein expression of specific response factors to execute critical functions, which needs to be explored by further research.

Finally, two recent studies from a research group have addressed a new function of ubiquitination in recovery from heat stress (Gwon et al. 2021; Maxwell et al. 2021). The total ubiquitination is dramatically increased at the beginning of heat stress and then remained at an elevated level when the stress was prolonged; but the accumulation after heat shock was temporary, rapidly returning to baseline. Specifically, heat shock leads to an increase in the ubiquitination of stress granule proteins. Surprisingly, heat stress-induced ubiquitination is not required for the assembly of stress granules, but is essential for their rapid disassembly and subsequent recovery of cellular activities following heat shock (Maxwell et al. 2021). Furthermore, the ubiquitination of the G3BP1 protein, the central protein of the stress granule induced by heat shock, was fully addressed (Gwon et al. 2021). G3BP1 undergoes K63-linked ubiquitination under heat stress within its NTF2L domain. Ubiquitinated G3BP1 next forms a complex with VCP and FAF2, which is required for stress granules disassembly during recovery from heat shock. These studies demonstrated an important role of ubiquitination of stress granule proteins in the recovery of cellular activities after heat shock; however, the E3 Ub ligases responsible for this ubiquitination remain unknown. Although a proteome analysis performed in this study did not reveal any stress-induced E3 ligases, potential E3 ligases may exhibit altered binding affinity to G3BP1 upon heat stress. Notably, TRIM25 could be a critical candidate. Yang *et al.* recently found that TRIM25 can interact with G3BP1/2 and modify them with K63-linked Ub chains (Yang et al. 2022). Whether TRIM25 is responsible for stress-induced G3BP1 ubiquitination and stress granules disassembly deserves future investigations. Knockout of *TRIM25* gene in stressed cells and exploring the changes of G3BP1 ubiquitination and granules disassembly could be a useful strategy to validate the functions of TRIM25.

Cold shock

Compared with that in heat shock, cellular response to cold stress in animals has attracted far less attention except in some research areas such as cell or organ preservation, cold tolerance, adaptive thermogenesis, and protein production (Fujita 1999). Generally, cold easily reduces physiological activities and leads to the alterations in supramolecular organizations. Temperature up-shift to 37°C from cold stress, as a form of heat shock, leads to dramatic cellular changes, and even cell death. However, only a limited number of genes could be upregulated during moderate hypothermia (25–33°C), and few gene expression or protein modifications has been reported to be induced by severe cold stress (below 5°C) without recovery at 37°C (Danno et al. 1997; Nishiyama et al. 1997; Sonna et al. 2002).

Adaptive thermogenesis has usually been investigated after exposing humans or rodents to cold temperature, in which several E3 Ub ligases have been found to be involved. RNF34 is a cold-regulated E3 ligase responsible for the ubiquitination of PGC-1α, a master regulator of thermogenesis, and negatively controls brown fat cell metabolism (Wei et al. 2012). Another E3 ligase Parkin is also a key protein in mitochondrial homeostasis and facilitates brown adipose tissue plasticity in response to thermogenic challenges (Cairó et al. 2019). However, it should be noted that exposing intact non-hibernating animals to cold may not lower the body temperature as expected. As shown in one study, incubating single mice at 2–3°C for 8 h only decreased the body temperatures from 36.5°C to 34.0°C, giving an average decrease of 2.5°C (Cullen and Sarge 1997). Moreover, for human, core body temperature lower than 29°C is actually life-threatening. Thus, non-hibernating animals may be not good models to study cellular responses to severe cold stress.

However, similar to bacteria or plants, ectothermic animals (fish, amphibians, reptiles, and insects), heterotherms (some species of birds, small rodents, marsupials, and bats), and some endothermic mammals (bears, hamsters, dwarf lemurs, and ground squirrels) show robust ability to tolerate cold or freezing even if the core body temperature decreases to an extremely low level (close to 0°C) in hibernation (Mohr et al. 2020). For example, the arctic ground squirrel can drop its body temperature to below 0°C and enter a hibernating status (Barnes 1989). In plants, under cold conditions (4°C), RING E3 ligase HOS1 remains highly active and mediates the cold-induced ubiquitination and degradation of a transcription factor ICE1, which controls freezing tolerance and flowering time (Dong et al. 2006; MacGregor and Penfield 2015). Mechanically, cold stress can initiate membrane rigidification, which results in a Ca^{2+} influx and MAPK cascade activations, probably causing ICE1 phosphorylation and relative conformation changes. Especially, Ser185 phosphorylation of ICE1 may promote its binding to HOS1 and subsequent degradation, because the S158A mutant of ICE1 was reported to lose the capacity to interact with HOS1 in a yeast two-hybrid assay (Cheng et al.

2020). However, no protein ubiquitination in response to such severe cold stress has been reported in animals and humans. It should be very interesting to explore whether protein PTMs happens or functions in mammalian cells upon extremely low temperature.

Osmotic stress

The equilibrium osmolality is highly crucial for maintaining normal cellular functions and is tightly controlled by a balance of hydration and solute concentrations. While most cells are usually maintained in constant osmotic environment, some body cells are exposed to a dynamic osmotic environment under physiologic or pathologic conditions (Finan et al. 2011). Increasing or decreasing in the external osmolarity beyond normal range, termed hypertonic or hypotonic stress, respectively, triggers water fluxes across semipermeable cytoplasmic membrane. Some adaptive mechanisms have been developed in cells to compensate changes in extracellular osmolarity (Brocker et al. 2012). Osmotic changes trigger the alterations of cell volume, which simultaneously activates cell volume recovery mechanisms and membrane channel proteins (Sadowska et al. 2018). Channel proteins that transport water or ions across plasma membrane regulate cellular osmolarity under osmotic stress. Many Ub E3 ligases have been reported to be involved in plant osmotic stress response, but the roles of ubiquitination under osmotic stress in animal cells are still poorly studied. The roles of ubiquitination in osmotic regulation are summarized as follows (Fig. 4).

Channel proteins could be functionally modulated by ubiquitination. First, the ubiquitination and stability of aquaporin-1 (AQP-1), a water channel protein, are altered in response to hypertonic stress (Leitch et al. 2001). The hypertonic stress decreases AQP-1 ubiquitination and promotes AQP-1 accumulation, contributing to the regulation of water transport and osmolarity. Second, Ub E3 ligase Nedd4-2 binds to the proline-rich PY motifs of ENaC, an amiloride-sensitive epithelial sodium channel protein, via its WW domains, and triggers ENaC ubiquitination and degradation and regulates salt and water balance (Staub et al. 1996, 1997; Kamynina et al. 2001). WWP2, another member of the Nedd4 family, also targets ENaC (McDonald et al. 2002). Moreover, Nedd4-2 and WWP2 may have a role in regulating a chloride channel ClC-5 via the interaction between WW domains and PY motifs (Schwake et al. 2001; Hryciw et al. 2004). Thus, the Ub system regulates osmosis via ubiquitinating some channel proteins and modulating their capacities to transport water or ions.

RNF183 may be the most important E3 ligase in osmosis regulation. The mRNA of RNF183 was reported to be specifically expressed in human and mouse kidney (Kaneko et al. 2016). In particular, by using *RNF183-GFP* knock-in mice, a recent study shows that RNF183 predominantly locates in the renal medullary collecting ducts and colocalizes with water channel aquaporin-2 (Maeoka et al. 2019a). Interestingly, the renal medulla of kidney is the only tissue that is continuously under a hypertonic environment (Bankir et al. 1989). These studies suggest that RNF183 may be involved in cellular hypertonic response. Indeed, the promoter of gene *RNF183* is bound directly by nuclear factor of activated T cells 5 (NFAT5), a transcription factor that drives gene expression for adaption to the hypertonic stress (Neuhofer 2010; Maeoka et al. 2019b). Moreover, hypertonic stress specifically promotes RNF183 expression, which is consistent with the activation of NFAT5; on the other hand, NFAT5 knockdown decreases the expression level of RNF183. Consistently, furosemide, a loop diuretic that has been proved to efficiently suppress NFAT5 level (Sheen et al. 2009), can dramatically decrease RNF183 expression (Maeoka et al. 2019a). Thus, NFAT5 mediate the expression and increase of RNF183 in response to hypertonic stress. Furthermore, RNF183 has been shown to be crucial to inhibit caspase-3 activation and maintain cell viability under hypertonic stress (Maeoka et al. 2019b). Together, RNF183 is involved in cellular response to osmotic stress.

Moreover, Na, K-ATPase, an ion transporter responsible for active transport of Na^+ and K^+ across cell membrane, has been recently identified as a specific target of RNF183 (Okamoto et al. 2020). As a ubiquitously expressed transmembrane complex, Na, K-ATPase contains a large catalytic α1 subunit and a small β1 subunit, maintains ionic homeostasis in the cytoplasm, and thus contributes to cell volume regulation and osmotic adaption (Kaplan 2002; Matchkov and Krivoi 2016). Although RNF183 binds both α1 and β1 subunits, RNF183 only ubiquitinates the β1 subunit, leading to translocation and degradation of both α1 and β1 subunits in lysosomes (Okamoto et al. 2020). Taken together, hypertonicity-responsive E3 ligase RNF183 functions for hypotonic environment adaptation through modulating Na, K-ATPase activity, and maintaining ionic homeostasis.

Additionally, some specific E3 ligases may also have roles in regulating osmotic stress. under hypertonic stress, a major transcription factor c-Jun undergoes ubiquitination-dependent degradation by the PHD/RING finger domain of MEKK1, which exhibits E3 ligase activity toward c-Jun and kinase activity toward several MKKs (McCabe and Burrell 2001; Xia et al. 2007). Shop21, a homolog of Ub ligase Rbx1, had been reported to be increased upon hyperosmotic stress in salmon (Pan et al. 2002). Although transferring salmon to seawater leads to Shop21 accumulation, the role of Shop21 under hyperosmotic stress remains unclear. Rapid correction of chronic hyponatremia can result in intense osmotic stress in brain cells, which induces ubiquitination and insoluble aggregation of unfolded proteins and contributes to demyelination

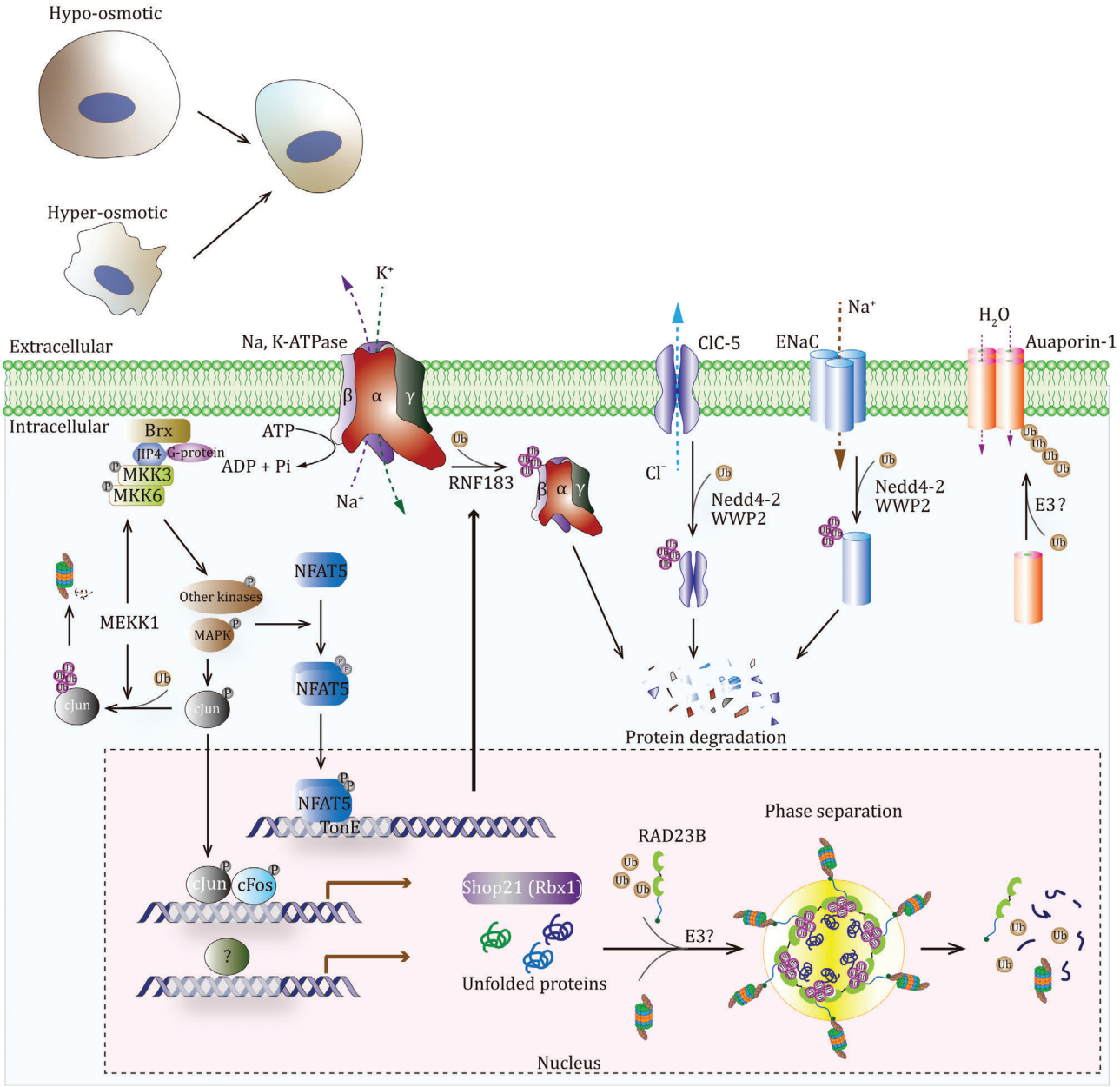


Figure 4. Ubiquitination regulates cellular osmolality by targeting multiple channel proteins. RNF183, Nedd4-2, WWP2, and other E3 ligases are involved in the regulation of osmotic stress.

syndrome development, suggesting osmotic stress may be a potent protein aggregation stimuli in mammalian brains (Gankam-Kengne et al. 2017). However, the functions of this ubiquitination in protein aggregation were still unexplored. Interestingly, a recent study demonstrated that hyperosmotic stress can trigger ubiquitination-dependent liquid–liquid phase separation (LLPS) of proteasomes in the nucleus (Yasuda et al. 2020). Following hyperosmotic stress, some ribosomal proteins that failed to properly fold can undergo ubiquitination, and RAD23B, a shuttle factor that has ubiquitin-associated domains, further drives LLPS of ubiquitinated ribosomal proteins and nuclear proteasomes, leading to proteasomal removal of misfolded ribosomal proteins. Notably, the mechanism by which the proteasome-containing structures induced by hyperosmotic stress were prominently formed in the nucleoplasm, but not the cytoplasm, remain unclear. Potential E3 ligases responsible for LLPS of ubiquitinated proteins and proteasomes also need to be elucidated in the future. In summary, these available, although limited, studies have highlighted the critical roles of ubiquitination in cellular responses to osmotic stress.

Ubiquitination regulates responses to intercellular stresses

Besides triggering adaptive responses to recover from disruption, these exogenous stresses we have discussed

above could result in multiple intercellular stresses if the initial stressful situation is prolonged. Hypoxia or heat shock induces several intercellular stresses, including DNA strand breaks, generation of reactive oxygen species (ROS), and endoplasmic reticulum (ER) stress (Møller et al. 2001; Bettaieb and Averill-Bates 2015; Wang et al. 2019; Akman et al. 2021). Heat shock also leads to Ub accumulation, also named Ub stress. Moreover, many studies had demonstrated that hyperosmolarity elevates ROS and causes ER stress (Burgos et al. 2019; Dai et al. 2019). In addition, there are many cross-talks between different intercellular stressors. Similar to environmental stresses, intercellular stresses, such as DNA damage stress, Ub stress, ROS stress, and ER stress, can initiate further cellular responses that are modulated by the ubiquitination system.

DNA damage stress

Large amounts of environmental or physical stressors can generate thousands of DNA lesions per cell per day, which causes many types of damages, such as DNA-protein crosslinks, base damage (BD), single-strand breaks (SSB), and double-strand breaks (DSB) (Table 1) (Jackson and Bartek 2009; Barghouth et al. 2019; Hindi et al. 2021). Genomic instability causes devastating effects that could result in various diseases and threaten the viability of cells or organism (Lord and Ashworth 2012). To maintain genomic integrity, cell have evolved complex and accurate repair mechanisms that counteract DNA damages, termed DNA damage response (DDR). DDR is tightly regulated by multiple PTMs, among which ubiquitination plays a central role and coordinates other PTMs (Mattiroli and Penengo 2021). Given the fact that DSB is the major and most dangerous DNA lesion (Podhorecka et al. 2010), this review mainly focused on ubiquitination-associated signaling cascades in DSB response. Once DSBs occur, these broken DNA sites can be recognized quickly by either MRN protein complex or Ku heterodimer, which subsequently guides repair signals via homologous recombination (HR) or non-homologous end-joining (NHEJ), respectively (Fig. 5).

NHEJ repair response

Ku-mediated NHEJ is a faster but more error-prone repair pathway than HR. Although NHEJ may create base deletions/insertions at damage sites, it plays important roles in safeguarding genomic integrity upon acute repair at any stages of the cell cycle. NHEJ is usually initiated by K70/80 protein heterodimer and glues two broken DNA ends at a DSB site, regardless of the original DNA sequence. While Ku70/80 heterodimer has less affinity to single-strand DNA ends and circular DNA, it shows a very high affinity to double-strand DNA ends and thus recognizes DSB sites within 5 s after DNA damage (Fell and Schild-Poulter 2015). Once being recruited to DSB site, Ku70/80 encircles and stabilizes the two ends of the DSB site. Next, Ku70/80 dimer works as a scaffold to recruit a transducer kinase DNA-PK$_{CS}$, inducing its autophosphorylation and activation. Active DNA-PK$_{CS}$ complex further attracts multiple factors, including Artemis, polymerases, kinases, and nucleases, to remove damaged DNA bases and finally form compatible sites for the ligation complex (Ligase IV, XRCC4, XLF, and PAXX) that directly heals the DNA break (Hammel et al. 2011; Ochi et al. 2015).

While the NHEJ process maybe not require ubiquitination as described above, it is regulated by ubiquitination. First, ubiquitination of Ku protein facilitates its release from the ligated sites. RNF8, RNF138, and a Fbxl12-containing SCF complex mediate K48-linked ubiquitination and degradation of Ku80 protein, which promotes Ku removal and NHEJ repair (Feng and Chen 2012; Postow and Funabiki 2013; Ismail et al. 2015). Another E3 ligase RNF126 was also found to promote Ku80 ubiquitination and dissociation from DSB sites (Ishida et al. 2017). Moreover, RING E3 RNF144A had been identified to physically interact with DNA-PK$_{CS}$ and trigger DNA-PK$_{CS}$ ubiquitination and degradation, which promotes apoptosis upon DNA damage (Ho et al. 2014). Additionally,

Table 1. Reasons and repair mechanisms of different DNA lesions.

Damaging agents	Replication errors	Ultraviolet light Chemicals	Ultraviolet light Alkylating agents	Oxygen radicals (ROS) Chemotherapeutics X-rays	Ionizing radiation Chemotherapeutics X-rays	
DNA lesions	Base mismatch Deletions Insertions	Bulky adducts Intrastrand Crosslinks	Small adducts Reverse/ Release	Single-strand breaks Abasic sites Base deamination 8-Oxoguanine lesions	Double-strand breaks Single-strand breaks Inter/intra-strand crosslinks	
Repair pathways	Mismatch mediated repair (MMR)	Nucleotide excision repair (NER)	Direct damage reversal	Base excision repair (BER)	Non-homologous end-joining (NHEJ)	Homologous recombination (HR)

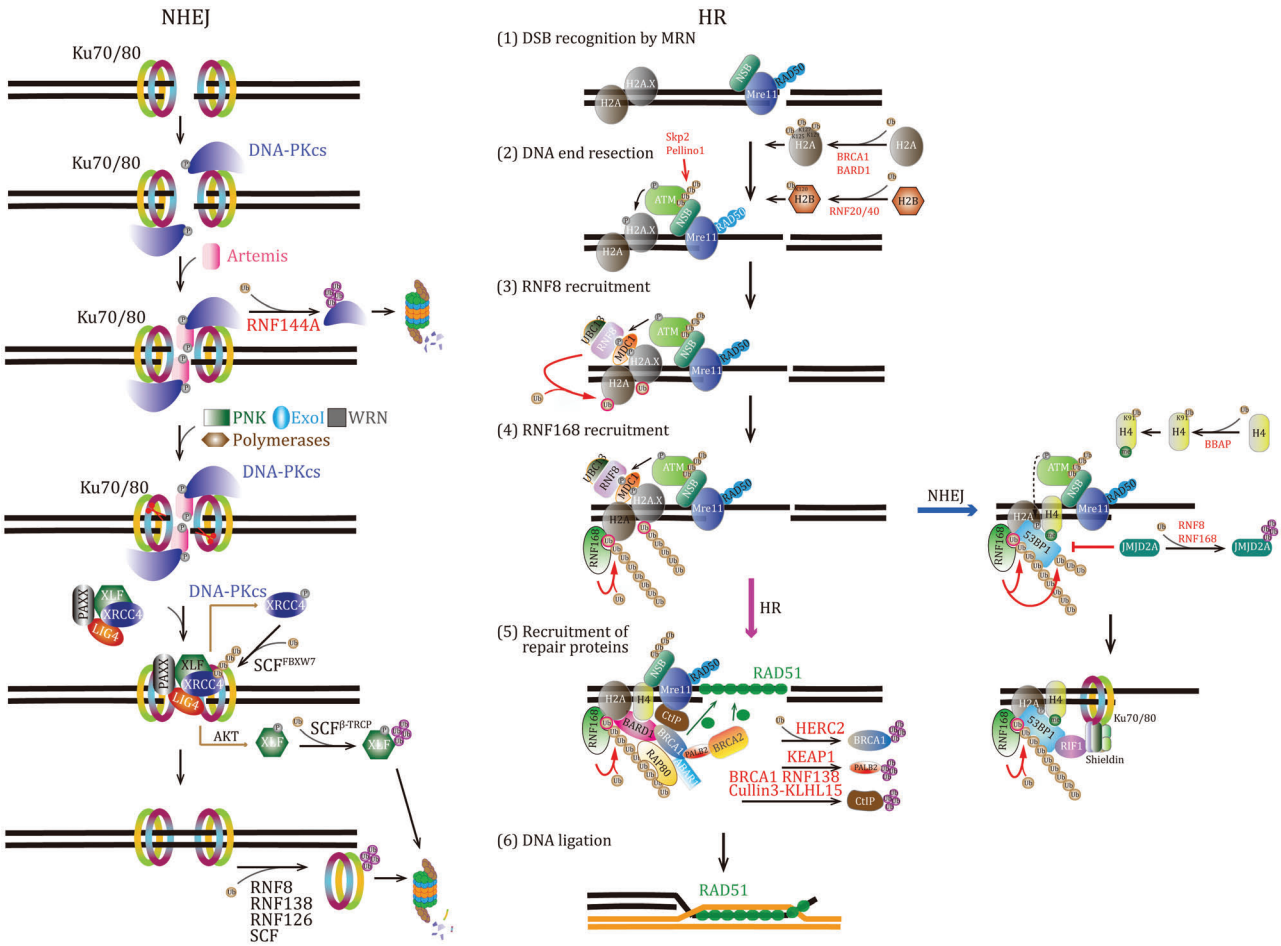


Figure 5. Ubiquitination controls DNA damage repair responses. Some E3 ligases have shown important functions in NHEJ pathway (left) or HR repair (right).

components of the ligation complex could be targeted by ubiquitination. XLF undergoes Akt-mediated phosphorylation at Thr181, which leads to its dissociation from the ligation complex and cytoplasmic retention; in the cytoplasm, an E3 ligase complex $SCF^{\beta\text{-}TRCP}$ ubiquitinates and degrades phosphorylated XLF, impairing NHEJ and promoting tumorigenesis (Liu et al. 2015). Another component XRCC4 was previously demonstrated to undergo monoubiquitination, which is increased in etoposide-induced DNA damage and may play roles in stabilizing DNA ligase IV (Foster et al. 2006). XRCC4 phosphorylation at S325/326 by $DNA\text{-}PK_{CS}$ can promote its interaction with FBXW7, which leads to its K63-linked ubiquitination at Lys296 catalyzed by SCF^{FBXW7} E3 complex (Zhang et al. 2016). XRCC4 polyubiquitination may work as an extended scaffold to recruit other NHEJ factors to facilitate NHEJ complex formation and promote NHEJ repair ability. Therefore, ubiquitination could be an efficient tool to modulate the NHEJ process.

HR repair response

HR primarily works in the S and G^2 phases of the cell cycle, and has high accuracy to repair DSBs by using a sister chromatid as the homologous template. HR repair owns a ubiquitination-dependent process that is more complicated than NHEJ repair.

DSB recognition by MRN complex

The MRN complex, comprising MRE11, RAD50, and NBS1 proteins, is an important DSB sensor, and has intrinsic DNA-binding activity and nucleolytic activities (Paull and Gellert 1998; de Jager et al. 2001). MRN searches and binds to broken DNA DSB ends, leading to Ku70/80 removal and DNA end resection that generates a long single-strand DNA (ssDNA) for searching homologous sequences. Next, an E3 ligase Skp2 is recruited by MRN and catalyzes NBS1 ubiquitination in a K63-linkage-dependent manner to attract kinase ATM to the DNA damage site, where ATM induces the phosphorylation on serine 139 of histone H2A.X and promotes γ-H2A.X foci formation (Falck et al. 2005; Lee and Paull 2005; Wu et al. 2012). In addition to Spk2, another E3 ligase Pellino-1 also ubiquitinate NBS1, controlling ATM recruitment to DSBs (Ha et al. 2019). Thus, K63-linked ubiquitination contributes to the initiation of HR repair.

RNF8 recruitment

MDC1 directly binds to and protects γ-H2A.X via the BRCT domain to amplify DNA damage signals, which leads to MDC1 phosphorylation that is required for the recruitment of RNF8, a very critical E3 Ub ligase (Stucki et al. 2005; Lou et al. 2006; Kolas et al. 2007). Upon binding phosphorylated MDC1 rapidly through the FHA domain, RNF8 can ubiquitylate histone H2A and H2A.X via cooperating with E2 enzyme UBC13 at damage sites, which is very critical for DDR foci formations of various proteins and promotes repair (Huen et al. 2007; Kolas et al. 2007; Mailand et al. 2007; Wang and Elledge 2007). Once the initial monoubiquitination is catalyzed by RNF8, another E3 ligase RNF168 will subsequently participate to transmit the repair signal (Doil et al. 2009).

The recruitment of RNF168

Mutations of *RNF168* lead to RIDDLE syndrome, an immunodeficiency and radiosensitivity disorder (Stewart et al. 2009; Bohgaki et al. 2011; Pietrucha et al. 2017). The RING domain of RNF168 is required for the accumulation of downstream 53BP1 and BRCA1, but not for RNF168 loading at DSBs, indicating RNF168-mediated ubiquitination is dispensable for RNF168 recruitment at DSBs. Instead, its two Ub-binding motifs MIU1 and MIU2 are responsible for RNF168 itself accumulation at damage sites through physical interaction with RNF8-mediated ubiquitylated H2A (Doil et al. 2009; Stewart et al. 2009). Interestingly, this widely accepted model is not supported by an *in vitro* assay showing that RNF8 is inactive toward nucleosomal H2A, but shows a high activity to ubiquitinate free H2A (Mattiroli et al. 2012). The study thus proposes a different model by which RNF8 mediates the ubiquitylation of other non-nucleosomal proteins, providing the docking sites for RNF168 recruitment. Recently, L3MBTL2 and KMT5A had been proposed to be such two non-nucleosomal proteins that are ubiquitinated by RNF8 and facilitate RNF168 recruitment to the DSB sites (Nowsheen et al. 2018; Lu et al. 2021). However, given that the biggest difference between nucleosomal H2A and isolated H2A is the presence or absence of the intact DNA, we here propose a novel hypothesis that RNF8 may directly recognize H2A molecules exposed at DNA damage sites, but cannot catalyze the H2A covered with intact DNA strands in the nucleosomes. Thus, our analysis supports the first model that RNF8-dependent H2A monoubiquitination is the first step and represents the docking sites for the following recruitment of RNF168 to the DNA damage sites. Further research is needed to verify the proposed selectivity of RNF8 toward free H2A molecule without DNA coverage. Altogether, RNF168 recruitment to DSBs is primarily mediated by RNF8-dependent ubiquitination of H2A.

Additionally, RNF8 and HUWE1 may also catalyze K63-linked polyubiquitination of histone H1 to recruit RNF168 to DSBs (Thorslund et al. 2015; Mandemaker et al. 2017). However, the removal of histone H1 from the entire γH2A.X domain has been observed in a genome-wide analysis of multiple chromatin features at DSBs using ChIP-seq (Clouaire et al. 2018). So, the hypothesized role for RNF168 docking of H1 ubiquitination remains controversial and needs further mechanistic analysis. Upon recruitment to the initial ubiquitinated H2A, RNF168 can ubiquitylate Lys13 or Lys15 of H2A/H2A.X to generate K63-linked polyubiquitin conjugates to amplify Ub signal, which serves as a binding platform that allows accumulation of proper repair factors at DNA damage sites (Mattiroli et al. 2012; Horn et al. 2019). Moreover, RNF168 also catalyzes noncanonical K27-linked H2As ubiquitination to signal DNA damage by assembling DDR foci at DSBs (Gatti et al. 2015). Although RNF168-dependent both K27- and K63-linked Ub chains on histone H2As are required to recruit proper repair factors to drive DDR, the interplay between them remains unclear.

DNA ligation

The effector proteins that are recruited to DDR foci determine the type of DNA repair pathway. The Ub-interacting motifs (UIM) of RAD80 protein binds RNF8/RNF168-induced Ub conjugates and facilitates RAD80/ABRA1/BRCA1/BARD1 complex assembly at the sites of DNA damage, activating HR repair (Kim et al. 2007; Sobhian et al. 2007; Wang et al. 2007). BARD1 (the partner protein of BRCA1) binds damage-containing nucleosomes bivalently by recognizing ubiquitinated Lys15 of H2A (H2AK15ub) via its BUDR motif, and unmethylated Lys20 of histone 4 (H4K20) by its ankyrin repeat domains (Becker et al. 2021). Moreover, BRCA1 binds to BRCA2 through PALB2 to assemble a complex that enhances recombinase RAD51 loading at DSBs and thus promotes HR repair (Sy et al. 2009; Zhang et al. 2009). Recently, BRCA1/BARD1 complex is also demonstrated to directly interact with RAD51 and increase the recombinase activity of RAD51 (Zhao et al. 2017). On the other side, by simultaneous recognition of ubiquitylated H2AK15 and methylated Lys20 of histone 4 (H4K20me) via its ubiquitylation-dependent recruitment (UDR) motif and the tandem Tudor domain, respectively, 53BP1 can be recruited to the damage sites, which will attract Ku70/80 complex and initiate NHEJ-mediated repair (Fradet-Turcotte et al. 2013; Wilson et al. 2016). These studies thus suggested that ubiquitination triggers the recruitment of repair proteins, and methylation determines which proteins could be recruited to initiate the corresponding repair pathway.

Ubiquitination modulates the switch between HR and NHEJ

The balance between HR and NHEJ can be elegantly maintained by several Ub-associated mechanisms. First, as mentioned above, RNF168-induced H2AK15ub is the

common base for recruiting BRCA1/BARD1 or 53BP1 to damage sites, but methylated H4K20 prefers 53BP1 oligomers while unmethylated H4K20 chooses BRCA1/BARD1, which switches DNA repair pathways (Fradet-Turcotte et al. 2013; Becker et al. 2021). Second, a novel phosphorylation of Ub molecule at Thr12 (pUbT12) regulates the function of H2AK15ub (Walser et al. 2020). The pUbT12 of RNF168-induced H2AK15ub accumulates in DDR foci upon DNA damage and specifically suppresses 53BP1 recruitment by impeding the recognition of 53BP1 UDR domain, but is still permissive to HR mediators BRCA1/BARD1, RAD51, and RNF169, inhibiting NHEJ but promoting HR repair. Although the pUbT12-positive foci are induced in a DDR kinases-dependent manner, the kinase that directly phosphorylates Ub is still a subject of investigation.

Moreover, many E3 ligases show critical impacts on the switch between HR and NHEJ. On one hand, some E3s inhibit HR repair. A HECT-type E3 HERC2 ubiquitinates BRCA1, which promotes the degradation of BARD1-uncoupled BRCA1 and prevents the HR pathway (Wu et al. 2010). Keap1 ubiquitinates BRCA1-interacted site on PALB2 and thus suppresses BRCA1–PALB2 interaction and HR (Orthwein et al. 2015). Moreover, BRCA1, RNF138, and Cullin3-KLHL15 can mediate the ubiquitination of CtIP, the binding partner of BRCA1, to regulate HR repair (Yu et al. 2006; Schmidt et al. 2015; Ferretti et al. 2016). On the other hand, some E3s target 53BP1 to control NHEJ repair. TIRR (Tudor interacting repair regulator) directly interacts with 53BP1 and impedes 53BP1 recruitment to H4K20me, restricting NHEJ reaction (Drané et al. 2017). Interestingly, RNF168 is reported to ubiquitylate 53BP1 and the ubiquitination of 53BP1 contributes its recruitment to DSBs (Bohgaki et al. 2013). Additionally, Tudor domain-containing JMJD2A can antagonize 53BP1 recruitment to DSB sites by binding to demethylated H4K20, but RNF8 and RNF168 trigger JMJD2A ubiquitination and degradation to facilitate 53BP1 foci formation (Mallette et al. 2012). BRCA1 prevents the accumulation of 53BP1/RIF1 complex and inhibits NHEJ by recruiting CtIP protein at DSB sites (Escribano-Díaz et al. 2013). Therefore, E3 ligases can work as effective switch regulators in DNA repair pathways.

Besides the ubiquitination discussed above, Ub conjugated to other sites of histone H2A or to other histone proteins beyond H2A further increases the complexity of DDR regulation. While E3 complex PRC1-catalyzed H2AK118/119ub is involved in transcriptional repression near the break site (Ui et al. 2015; Uckelmann and Sixma 2017), BRCA1/BARD1 complex can trigger ubiquitination at K125/127/129 of H2A, which facilitates DNA end resection in DNA repair (Kalb et al. 2014; Densham et al. 2016). E3 ligases RNF20/40 can promote monoubiquitination at Lys120 of histone H2B (H2BK120ub) to promote chromatin opening and accumulate DNA repair proteins at DSBs (Fierz et al. 2011; Moyal et al. 2011; Nakamura et al. 2011; So et al. 2019). Interestingly, H2BK120 could undergo a switch from ubiquitination to acetylation, and H2BK120ub displays a progressive loss, upon DSB induction (Clouaire et al. 2018). The different PTMs on H2BK120 may exhibit distinct functions at the different stages of DDR. Similar to H2BK120ub, H2B Lys34 ubiquitination (H2BK34ub) can also induce nucleosome distortion for Dot1L binding and activation, stimulating Dot1L-mediated H3K79 methylation (Ai et al. 2022). Additionally, histone H3 and H4 ubiquitination is mediated by E3 ligase complex CUL4-DDB-ROC1 and participates in cellular response to DNA damage (Wang et al. 2006). And, E3 ligase BBAP can monoubiquitylate H4K91, which promotes H4K20me generation and 53BP1 foci formation at the DNA damage sites (Yan et al. 2009). Based on these studies, it is reasonable to speculate that ubiquitination happening on histone proteins usually have impacts on DDR. Although these ubiquitination events cannot cause protein degradation, they effectively modify DDR.

Notably, some E3 ligases can modulate different steps of DDR via catalyzing proteolytic ubiquitination. Two SUMO E3 PIAS1 and PIAS4 mediate MDC1 SUMOylation, which further recruits a SUMO-targeted E3 ligase RNF4 to ubiquitinate and degrade SUMOylated MDC1 in a K48-linkage-dependent manner, promoting MDC foci turnover at damage sites and thus maintaining a proper DDR level (Shi et al. 2008; Galanty et al. 2012; Luo et al. 2012; Yin et al. 2012). TRIP12 and UBR5, two HECT E3s, can induce RNF168 polyubiquitination, preventing the massive accumulation of RNF168 and Ub conjugates at DSBs (Gudjonsson et al. 2012). Moreover, E3 ligase PRP19, RFWD3, or RNF4 had been found to regulate DNA damage-induced ubiquitination and the stability of the RPA complex, an essential regulator in genome maintenance (Galanty et al. 2012; Maréchal et al. 2014; Elia et al. 2015). Protein CLASPIN also plays an important role in DDR activation and undergoes the ubiquitination-dependent degradation mediated by APC/Cdh1 or βTrCP-SCF (Mailand et al. 2006; Peschiaroli et al. 2006; Bassermann et al. 2008). Recently, TRIM21 was also demonstrated to target CLASPIN for K63-linked ubiquitination (Zhu et al. 2022). Although it is non-proteolytic, it counteracts K6-linked ubiquitination and the chromatin loading of CLASPIN, proposing a regulatory role of TRIM21-mediated ubiquitination on DNA repair.

In summary, ubiquitination regulates the initiation, progression, and termination of DDR, and controls the switch between different types of cellular responses, which makes DDR an elegant and accurate machinery.

Ub stress

As one of the most abundant proteins in eukaryotic cells, Ub concentration has been estimated to be

~500 pmol/mg total cell protein or ~85 μmol/L per cell (Kaiser et al. 2011). In cells, Ub molecule undergoes a dynamic equilibrium between "conjugated" Ub covalently attached to substrates or "free" Ub available for ubiquitination reactions (Park and Ryu 2014). In various cell lines, ~23% of Ub is presented as free form, ~76% as conjugated status (65% for monoubiquitinated substrates and 11% for polyUb chains); In contrast, in mammalian brains, 60%–80% of Ub exists as free Ub monomer, which may facilitate the rapid response of neurons upon stresses (Kaiser et al. 2011). Maintenance of free Ub levels can balance a wide range of cellular processes, and cells develop different regulatory mechanisms to control cellular Ub homeostasis (Kimura and Tanaka 2010; Chen et al. 2011). Many environmental, genetic, or pathological factors could lead to the alteration of Ub homeostasis, which can be termed as Ub stress (Hanna et al. 2007; Peng et al. 2017). According to the changes in cellular Ub level, Ub stress can be classified as Ub^+ stress or Ub^- stress.

Heat shock, DNA damage reagents, Ub overexpression, or prolonged proteasomal inhibition could increase Ub concentrations and lead to Ub^+ stress (Bond and Schlesinger 1985; Fornace et al. 1989; Kaiser et al. 2011). Our group previously had demonstrated that Ub^+ stress can trigger autophagy in a p62-dependent manner (Peng et al. 2017). Specifically, Ub^+ stress promotes UBE2D2/3-mediated ubiquitylation on multiple sites (including K420 in the UBA domain) of p62, which disrupts UBA domain-mediated p62 dimerization and thus allows p62 oligomerization. This conformational change of p62 favors polyubiquitinated cargoes for selective autophagy (Liu et al. 2014; Peng et al. 2017; Pohl and Dikic 2019). Interestingly, UBE2D2/3-catalyzed p62 ubiquitylation under Ub^+ stress does not require a special E3 ligase. In another study, the K420 residue of p62 was also reported to be ubiquitinated, which was mediated by an E3 complex Keap1/Cullin3 (Lee et al. 2017). Keap1/Cullin3 enhances autophagic activity by targeting dimeric p62, which thus increases p62 oligomerization and its subsequent degradation. Although these studies consistently support the conclusion that ubiquitination of a conserved lysine K420 can lead to p62 oligomerization, they have different opinions on the requirement of E3 ligase for K420 ubiquitination. This discrepancy may be explained by different Ub stress states. Ub^+ stress may preferentially induce E3-independent K420 ubiquitination of p62. Recently, USP8 suppresses autophagy by directly deubiquitinating p62 principally at K420 (Peng et al. 2020). In addition, E3 ligase TRIM72 was found to ubiquitinate and degrade an alternative short form of p62, thereby modulating selective autophagy (Wang et al. 2022a). Further investigation is needed to explore whether Keap1/Cullin3 or TRIM72 may play roles in Ub stress response. Together, Ub^+ stress leads to autophagic events.

On the other side, Ub depletion by cycloheximide treatment or deletion of polyubiquitin genes (*UBI4* in yeast, *UBB/UBC* in mammals) could cause Ub^- stress, which leads to decreased cellular functions, reduced viability or impaired cellular resistance to stress conditions (Park and Ryu 2014). Moreover, DUBs show important roles in regulating Ub homeostasis. Deletion of some DUB genes in mice also leads to a decreased level of free Ub and triggers neurological abnormalities and severe symptoms (Walters et al. 2008). Therefore, Ub depletion can inhibit the overall function of the Ub system, which may endanger various biological processes and cell survival. For such kind of lethal stress, mammalian cells may have few adaptive responses. However, as mentioned above, Ub is encoded by four different genes in the human genome, which may be an evolutionarily acquired safe strategy for cells to ensure adequate Ub supply and avoid the situations of Ub deficiency in the best possible way.

ER stress

As an important intracellular organelle, ER predominantly mediates folding, translocation, and posttranslational processing of secretory and membrane proteins. Many genetic and environmental insults, such as hypoxia, heat shock, or glucose starvation, could perturb ER function, which results in accumulation of misfolded or unfolded proteins in the ER, called ER stress (Sano and Reed 2013). Once ER stress happens, ER-associated protein degradation (ERAD) could be activated to eliminate unfolded proteins, and the unfolded protein response (UPR), a highly conserved corrective mechanism, is also triggered to rapidly improve protein-folding capacity to restore ER homeostasis (Hwang and Qi 2018). However, if the ER stress cannot be alleviated, prolonged ER stress often causes cell death. Ubiquitination has shown critical roles in regulating both ERAD and UPR (Fig. 6), which facilitates ER homeostasis and cell survival.

ERAD has three different pathways, containing ERAD-L, ERAD-M, and ERAD-C, which degrades misfolded substrates in the ER lumen, within the ER membrane, and on the cytoplasm, respectively (Wu and Rapoport 2018). E3 Ub ligases are required for these ERAD pathways. In yeast, a RING E3 ligase Hrd1-containing protein complexes mainly mediate ERAD-L and ERAD-M pathways to remove misfolded proteins; another E3 ligase Doa10 is responsible for ERAD-C. In addition, a Ub ligase complex, consisting of Asi1, 2, and 3, can degrade misfolded proteins at the inner nuclear membrane (Foresti et al. 2014; Khmelinskii et al. 2014). Mammalian cells possess a highly consistent ERAD machinery, which is mediated by homologous E3 ligases SEL1L-HRD1, TEB4, and gp78/CHIP/RMA1 complex (Lopata et al. 2020). After being polyubiquitinated, ERAD-L and ERAD-M substrates can be pulled out from the membrane by p97 (Cdc48 in

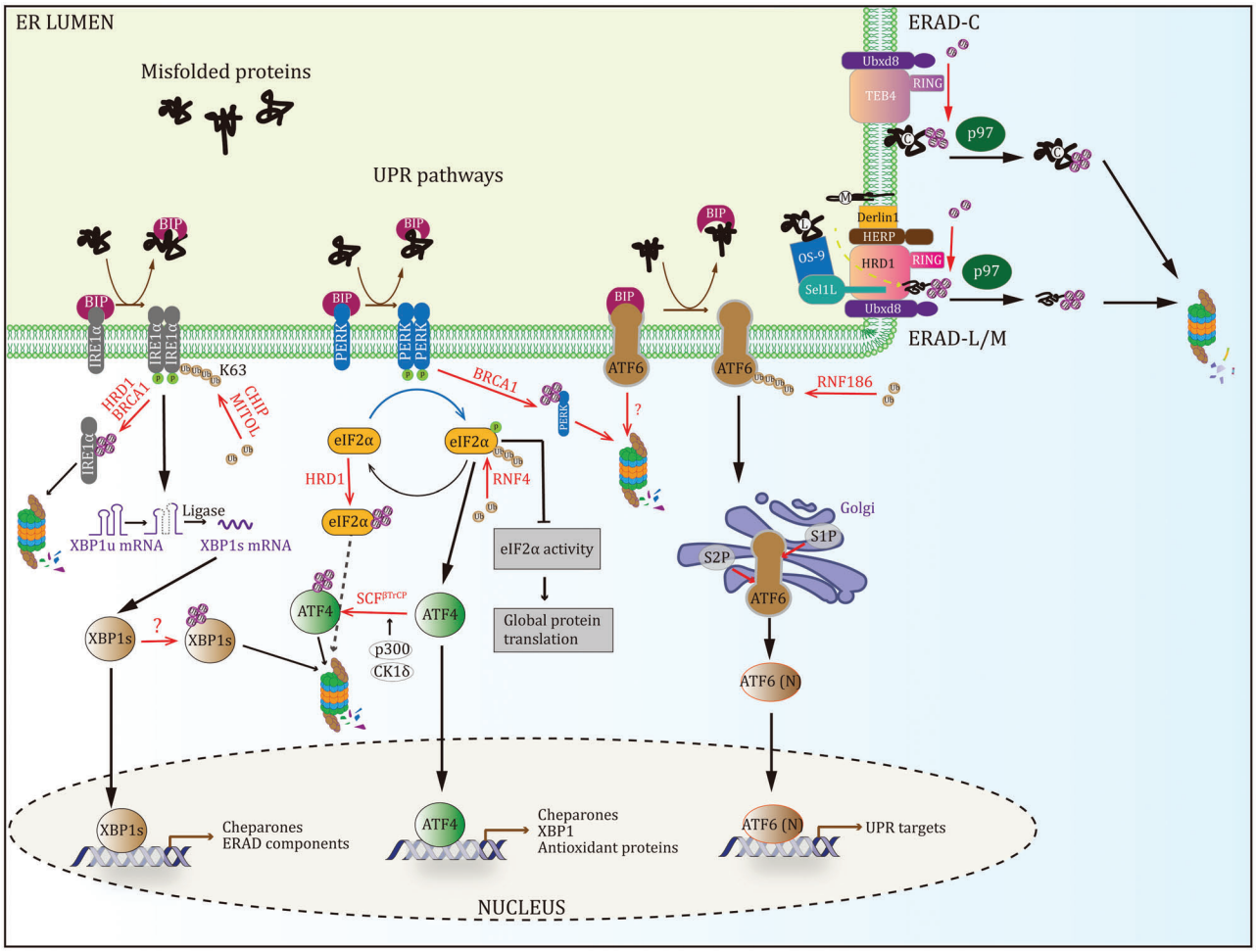


Figure 6. Ubiquitination regulates ER stress by modulating ERAD and UPR pathways. On one hand, misfolded proteins accumulated in the ER trigger UPR pathway. Ubiquitination can regulate protein-folding capacity of ER by targeting UPR sensors or related transcription factors. On the other hand, ERAD promotes the degradation of unfolded proteins to alleviate ER stress.

yeast) and undergo proteasome-dependent degradation in the cytoplasm. Thus, ubiquitination-mediated ERAD functions to rapidly clear misfolded proteins and alleviate ER stress.

At the same time, under ER stress, UPR increases protein-folding capacity of ER and decreases the generation of misfolded proteins. UPR is generally initiated by the activation of three ER transmembrane sensors: IRE1, PERK, and ATF6 (Oakes and Papa 2015). These sensors usually bind to an ER molecular chaperone BIP (GRP78 or HSPA5), and exist as inactive forms under the non-stressful conditions. When ER stress occurs, accumulated unfolded proteins recruit GRP78 and drive its dissociation from IRE1, PERK, and ATF6. Free IRE1, PERK, and ATF6 are activated, and trigger the activation of transcription factors XBP1s, ATF4, and ATF6(N) to selectively upregulate targets that can expand ER capacity to fold proteins, and simultaneously inhibits the activity of translation initiation factor eIF2α to slow down global protein synthesis and decrease the protein load on the ER (Clarke et al. 2014; Iurlaro and Muñoz-Pinedo 2016). Recently, increasing numbers of studies showed that ubiquitination regulates UPR by targeting ER sensors.

First, several E3 ligases regulate IRE1. An E3 ligase CHIP was found to catalyze K63-linked ubiquitination on K545 and K828 of IRE1, which promotes IRE1 phosphorylation and IRE1–TRAF2 interaction, and resists to ER-stressed-mediated senescence (Zhu et al. 2014). Another E3 ligase MITOL can also promote K63-linked IRE1 ubiquitination, albeit at a different lysine residue (K481), and protect cells from stress-induced apoptosis (Takeda et al. 2019). Notably, E3 HRD1-contained ERAD complex can target IRE1 for ubiquitination and degradation, which proposes a self-regulatory mechanism of the ER quality control system (Sun et al. 2015). Second, BRCA1 controls ER homeostasis by targeting PERK and IRE1 for ubiquitination and proteasomal degradation (Hromas et al. 2022). BRCA1 recruits both PERK and IRE1, which suggests that BRCA1, PERK, and IRE1 could form a complex under ER stress; BRCA1 deficiency leads to the accumulation of PERK and IRE1, which constitutively activates UPR and promotes survival of BRCA1-deficient

cancer cells. Interestingly, under ER stress, PERK phosphorylates several E3 Ub ligases (MARCH5, MULAN, and Parkin), which increases the Ub ligases activities of these E3s (Toyofuku et al. 2020). LRRK2 can regulate PERK-mediated phosphorylation of these E3 ligases and determine ER-mitochondrial tethering. Third, ATF6 had been shown to be a direct target of the UPS (Hong et al. 2004). However, the E3 ligase that is responsible for ATF6 degradation remains unknown. A recent study demonstrated that an ER-localized E3 ligase RNF186 can catalyze non-proteolytic ubiquitination of K152 on ATF6 and improve UPR (Ranjan et al. 2021). Thus, three critical UPR sensors can be modified by ubiquitination under ER stress.

Ubiquitination also manipulates UPR by targeting transcription factors downstream of UPR pathways. $SCF^{\beta TrCP}$ complex can bind to phosphorylated ATF4, which results in ATF4 ubiquitination and degradation (Lassot et al. 2001). Histone acetyltransferase p300 and casein kinase 1 delta (CK1δ) show roles on $SCF^{\beta TrCP}$-mediated ATF4 ubiquitination (Lassot et al. 2005; Feng et al. 2021). Also, Ub conjugated on K60 and K77 of XBP1s had been reported to be required for its degradation, with unknown E3 ligases (Sun et al. 2020). Finally, translation initiation factor eIF2α is also modulated by some E3 ligases. HRD1 interacts with, ubiquitinates, and degrades eIF2α; however, another E3 RNF4 ubiquitinates and stabilizes phosphorylated eIF2α (Huang et al. 2017; Avitan-Hersh et al. 2020). Via targeting transcription factors of UPR, E3 ligases could directly guide UPR and control ER stress response. How these E3s cooperate with each other deserves further investigation.

In summary, to alleviate ER stress and maintain cell survival, ubiquitination-dependent ERAD and ubiquitin-regulated UPR are rapidly activated to degrade accumulated unfolded substrates or suppress the generation of unfold proteins. Moreover, a self-regulatory mechanism for ER recovery can be executed by ERAD-mediated ubiquitination of some components of UPR pathways.

Oxidative stress

As one kind of extremely harmful products in cells, reactive oxidants can cause damages to various biological macromolecules, such as proteins, carbohydrates, nucleic acids, and lipids (Sies et al. 2017). A relative balance between oxidants and antioxidants is required for normal cellular functions. Reactive oxidants usually contain ROS, including superoxide anion radical ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($HO^{\bullet}$), and reactive nitrogen species (RNS), i.e., nitric oxide ($NO^{\bullet}$) and peroxynitrite anion ($ONOO^-$) (Hayes et al. 2020). A relative excess of ROS or RNS when compared with antioxidants is defined as oxidative stress, which could be triggered by most environmental or intercellular stressors, including hypoxia, heat stress, DNA damage stress, ER stress, and other stresses that are not reviewed here. Cells possess multiple mechanisms to overcome oxidative stress and provide effective prevention of damages, which can enable cell survival. However, sustained oxidative stress may result in cell death (ferroptosis) and human diseases.

ROS can be produced in cytosol, but predominantly from the mitochondria, which requires multiple enzymes. NADPH oxidases (NOX enzymes), mitochondrial complexes I/II/III, superoxide dismutases (SOD1/2/3), cytochromes P-450, NADH oxidases, and many other oxidases are usually responsible for ROS generation; however, catalase, thioredoxin, glutathione peroxidase (GPX1-8), Peroxiredoxins (Prx1-6), glutathione (GSH), and other antioxidant factors can counteract ROS or ROS-induced damages (Sies et al. 2017). Ubiquitination or other PTMs of these enzymes that generate ROS or antioxidants definitely can modulate cellular responses to oxidative stress.

Many E3 Ub ligases have shown regulatory roles on ROS production by targeting these critical enzymes. First, several E3s can modulate the functions of NADPH oxidases. A HECT ligase HACE1 controls ROS generation by targeting Rac1-dependent NADPH oxidase complexes (Daugaard et al. 2013). Loss of HACE1 in human cell lines led to NADPH-oxidase-dependent ROS elevation, which further causes DNA damage. Notably, the study showed that Hace2 directly targeted Rac1, but not NADPH oxidase. Another E3 ligase Fbxw7 can directly bind to and degrade NADPH oxidase 1 (Nox1) (Wang et al. 2021). RNF34 ablation was also reported to enhance the generation of NADPH oxidase-dependent ROS (Fang et al. 2021). Although their target substrates are different, Hac1, Fbxw7, and RNF34 have similar inhibitory roles on NADPH oxidase-mediated ROS production. Besides NADPH oxidases, superoxide dismutase SOD1 can also be targeted by ubiquitination. Mitochondrial E3 ligase MITOL can ubiquitinate and degrade mutant SOD1, but not wild-type SOD1, which suppresses mutant SOD1-induced ROS generation (Yonashiro et al. 2009). Moreover, antioxidant proteins could be regulated by some E3 ligases. For example, antioxidant peroxidases 1 (Prx1) had been found to undergo E6AP-mediated ubiquitination and degradation. E6AP deficiency leads to the elevation of Prx1 and an enhanced cell capacity to tolerate an oxidative stress situation (Nasu et al. 2010; Wolyniec et al. 2013). In addition, other E3 ligases, including TRIM32 and TRAF6, have shown regulatory roles on mitochondrial ROS production (Prajapati et al. 2020; Shen et al. 2020; Wang et al. 2022b). There, targeting ROS-associated enzymes by ubiquitination could be a powerful strategy cells use to manipulate oxidative stress responses.

Furthermore, ROS sensor Keap1 is involved in controlling ubiquitination and stability of Nrf2, a transcription factor that plays a key role in oxidative stress

resistance. Under oxidative stress, Nrf2 can trigger the expression of diverse cytoprotective proteins, including antioxidant, detoxification, and anti-inflammatory proteins (Kaspar et al. 2009). Keap1 possesses a BTB domain, a C-terminal Kelch domain, and an intervening region (IVR), and functions as the substrate recognition module of a Cullin3-based E3 ligase complex (Keap1/Cullin3/Rbx1) (Dinkova-Kostova et al. 2017). Under non-stressful conditions, dimeric Keap1 recruits an Nrf2 molecule, which facilitates Nrf2 polyubiquitination and subsequent proteasome-mediated degradation (Furukawa and Xiong 2005). Interestingly, human Keap1 is a cysteine-rich protein containing 27 highly conserved cysteines. These reactive cysteine residues can act as electrophilic sensors for cellular ROS. Once oxidative stress occurs, these cysteines of Keap1, especially Cys151 in the BTB domain and Cys273 and Cys288 in the IVR domain, can undergo oxidative modification, leading to Keap1 conformational change and Nrf2 dissociation from Keap1 CRL complex (Kobayashi et al. 2006; Rachakonda et al. 2008). The Nrf2 dissociation from Keap1 blocks the E3 activity of the Keap1 complex, stabilizes Nrf2, and promotes its nuclear accumulation, which initiates anti-ROS gene expression and contributes to cell survival in the stress. Collectively, ubiquitination is a critical executor in the Keap1-Nrf2 system to regulate Nrf2 stability and anti-ROS cellular responses.

Additionally, the N-end rule pathway acts as a nitric oxide sensor to control E3 ligases-mediated degradation of regulatory proteins bearing N-terminal cysteine (Hu et al. 2005, 2008). The N-end rule pathway is a proteolytic pathway that can destabilize N-terminal residues of target proteins (Varshavsky 2011). N-terminal cysteine of target substrates, such as RGS proteins, can be oxidated by nitric oxide, which is required for substrate arginylation by R-transferase ATE1. Arginylated cysteine of target proteins is further recognized, ubiquitinated, and degraded by E3 Ub ligases (including UBR1 and UBR2) of the N-end rule pathway. Therefore, by triggering ubiquitination-dependent substrate degradation, the N-end rule pathway regulates cellular response after sensing RNS/ROS levels.

Interestingly, ER stress response can also regulate oxidative stress response. On one hand, protein folding events on the ER produce a large volume of ROS as a byproduct, which directly has impacts on cellular oxidative stress. On the other hand, ROS accumulation within ER can cause excess oxidation of ER-located proteins or disrupt disulfide bond formation, which results in protein conformational changes or misfolded or unfold protein generation on the ER, thereby leading to ER stress and UPR (Cao and Kaufman 2014). UPR has shown critical roles in oxidative stress response via multiple molecular mechanisms, which has been summarized in a recent review (Ong and Logue 2023). Thus, it could be concluded that ubiquitination could regulate oxidative stress by controlling UPR.

Mitophagy

Mitophagy is an evolutionarily conserved mechanism that eliminates ROS-producing mitochondria via autophagy, which has a complex relationship with oxidative stress (Schofield and Schafer 2021). As the major source of intracellular ROS, mitochondria are constantly challenged and damaged by ROS accumulation. Mitophagy plays a fundamental role in the mitochondrial quality and quantity control via selective degradation of damaged mitochondria. There are two types of mitophagy in mammals: receptor-mediated mitophagy and Ub-mediated mitophagy (Onishi et al. 2021).

Ubiquitination can modulate receptor-mediated mitophagy. Mitophagy receptors on the OMM, such as BNIP3, BNIP3L (NIX), and FUNDC1, have conserved LC3-interacting regions (LIRs) and can recruit the autophagic machinery to degrade mitochondria (Youle and Narendra 2011). Interestingly, RBR E3 ligase parkin mediates ubiquitination of NIX, which in turn recruits autophagy adaptor NBR1 and promotes autophagosome formation surrounding mitochondria (Gao et al. 2015). Moreover, E3 ligase MARCH5 also regulates receptor-mediated mitophagy by controlling FUNDC1 protein levels (Chen et al. 2017). MARCH5 directly binds to FUNDC1 and catalyze proteolytic ubiquitination at Lys119 of FUNDC1, which impairs hypoxia-induced mitophagy. Thus, ubiquitination shows a regulatory role in receptor-mediated mitophagy.

On the other hand, the mitochondrially localized serine-threonine kinase PINK1 and the E3 ligase Parkin exhibit central roles in ubiquitin-mediated mitophagy. Once mitochondria are damaged and mitochondrial membrane potential is changed, PINK1 forms a dimer and its Ser228 is phosphorylated via autophosphorylation (Okatsu et al. 2012). This autophosphorylation recruits, phosphorylates, and activates Parkin on Ser65 in its Ubl domain. Moreover, PINK1 autophosphorylation can also stabilize its Insert 3 regions to an appropriate position, which recruits Ub and promotes Ser65 phosphorylation of Ub; Importantly, Ub phosphorylation by PINK1 accelerates Parkin E3 ligase activity (Kane et al. 2014; Kazlauskaite et al. 2014; Koyano et al. 2014). This PINK1-Parkin axis results in poly-p-Ub accumulation on damaged mitochondria, which recruits a series of autophagy adaptors (p62/SQSTM1, NDP52/CALCOCO2, NBR1, OPTN, TAX1BP1) and LC3, finally facilitating autophagosome closure and autophagosome-lysosome fusion (Ordureau et al. 2014; Onishi et al. 2021). Collectively, mitochondria-generated excessive ROS harms mitochondria, which triggers ubiquitin-regulated or mediated mitophagy to remove damaged mitochondria and alleviate oxidative stress to protect cells.

Ferroptosis

Ferroptosis, one type of cell death that can be triggered by excessive ROS stress, is modulated by the ubiquitination system. Ferroptosis is an iron-dependent and non-apoptotic form of regulated cell death, which is characterized by the accumulation of ROS and lipid peroxidation products (Li et al. 2020c). By regulating lipid peroxidation and iron metabolism, some proteins show very important effects on the ferroptosis process, including p53, GPX4, SLC7A11, FSP1, HSPB1, VDAC2/3, and NRF2 (Xie et al. 2016). Ub covalently conjugated to these molecules usually have important impacts on ferroptosis.

SLC7A11, a crucial subunit of the cystine/glutamate antiporter to block ferroptosis, had been found to be ubiquitinated by E3 ligase TRIM26 and deubiquitinated by OTUB1 (Liu et al. 2019b; Zhu et al. 2021b). While OTUB1 stabilizes SLC7A11 to prevent ferroptosis, TRIM26-mediated ubiquitination degrades SLC7A11 and enhances ferroptosis. Two recent studies had also reported that a SOCS2-containing E3 complex or HRD1 can interact with SLC7A11 and catalyze K48-linked proteolytic ubiquitination of SLC7A11, thus promoting ferroptosis (Chen et al. 2023; Wang et al. 2023). Moreover, another key ferroptosis regulator GPX4 is modulated by ubiquitination. A novel small molecule inhibitor (Bufotalin) of GPX4 was recently reported to induce GPX4 ubiquitination and degradation, lipid peroxidation, and ferroptosis, and thus could serve as potential anti-tumor agents (Zhang et al. 2022). Although the E3 ligase that mediates Bufotalin-induced GPX4 degradation remains unknown, several E3 ligases target GPX4. Recent studies have demonstrated that TRIM21, TRIM59, or NEDD4L promotes ferroptosis by inducing K48-linked ubiquitination and degradation of GPX4 (Sun et al. 2023; Tang et al. 2023; Zhang et al. 2023). However, LUBAC E3 complex recruits GPX4 and catalyzes M1-linked linear ubiquitination of GPX4, which can stabilize GPX4 and improve cellular defenses against ferroptosis (Dong et al. 2022). Interestingly, preexisting K48- or K63-linked Ub chains on GPX4 could enable LUBAC recruitment to GPX4. Based on these studies, a complicated Ub-associated mechanism for ferroptosis regulation could be proposed. K48-linked ubiquitination can lead to GPX4 degradation, which either promotes ferroptosis or recruits LUBAC to product linear Ub chains on GPX4 and thus block the ferroptosis process. Additionally, voltage-dependent anion channel proteins VDAC2/3 bind to the ferroptosis activator (erastin) and undergo ubiquitination and degradation, which is mediated by a special E3 ligase Nedd4 (Yang et al. 2020). Nedd4 expression is induced by erastin, which exacerbates VDAC2/3 degradation and thus enhances cell tolerance to erastin treatment. FBXW7 also targets VDAC3 for ubiquitination and degradation (Zhu et al. 2021a). Finally, some other E3 ligases can modulate other regulatory proteins of ferroptosis. For example, TRIM69 E3 ligase ubiquitinates and degrades FSP1 (ferroptosis suppressor protein 1) to promote ferroptosis (Yuan et al. 2022). However, two E3 ligases MARCHF6 and HUWE1 can target p53, ACSL4, and transferrin receptor for ubiquitination and proteosome-mediated degradation, which suppresses ferroptosis (Nguyen et al. 2022; Wu et al. 2022). Given that ferroptosis is currently a research focus that attracts much attention, roles of more E3 ligases in regulating ferroptosis will be explored in the recent futures.

Collectively, via targeting some crucial ROS enzymes, the Keap1-Nrf2 axis, the UPR process, mitophagy, and ferroptosis, the ubiquitination system exhibits critical functions to regulate cellular adaptive responses to oxidative stress.

Conclusions and future perspectives

In summary, as one fundamental and omnipresent PTM in mammals, ubiquitination shows important regulatory functions in controlling distinct cellular responses to different stressors. Multiple elements of cellular response pathways, such as receptors, adaptors, signal transducers, or transcription factors, are modified and controlled by the ubiquitination system, which thus manages stresses, repairs damages, and restores homeostasis. Although ubiquitination targets different substrates in different cellular stress responses, it renders cells the capacity to resist stresses, showing housekeeping roles in sustaining cellular homeostasis.

As described above, under some stresses, roles of ubiquitination in cellular responses remain poorly investigated, such as extreme cold stress. Some mammals can tolerate extreme cold stress (close to 0°C) during hibernation (Mohr et al. 2020), however, whether ubiquitination or other PTMs, modulates the tolerance to extreme cold is unclear. Moreover, human skin (in frostbite) and tissues (in organ preservation) also undergo extreme cold stress (Petrone et al. 2014; Jing et al. 2018). Further investigations are still needed to explore whether ubiquitination is involved in cellular protection against cold-induced damage.

In some cellular stress response pathways, one critical component can be targeted by multiple E3s-mediated ubiquitination. Some E3s promote the activity of the substrate, while other E3s inactivate or destroy the substrate. How cell coordinates these E3 ligases to act, and how they regulate each other in stress responses deserve more integrated investigations.

Significant advances have been addressed in understanding classical ubiquitination system in the last decades, but emerging evidence highlights the importance of atypical ubiquitination. On one hand, more atypical E3 ligases may exist. The discovery of an E3 ligase MYCB1 having esterification activity and targeting threonine

residues suggests that a group of human E3 ligases may be selectively responsible for non-lysine ubiquitination. Studies of bacterial E1/E2-independent E3 ligases also lead to a possibility that mammalian cells may express similar E3 Ub ligases. On the other hand, non-proteinaceous substrates are needed to be further explored. To date, only bacterial LPS and eukaryotic phospholipids PE were reported to be ubiquitinated by E3 ligases RNF213 and Tul1, respectively, proposing a novel class of ubiquitination substrates beyond protein (Otten et al. 2021; Sakamaki et al. 2022). However, it is unknown if other non-proteinaceous materials, carbohydrates, or nucleic acids, could undergo ubiquitination. It is expected that exploring atypical E3 ligases and identifying non-proteinaceous targets that regulate cellular stress responses will greatly extend our knowledge of the ubiquitination system.

Currently, there is high interest in academia and industry in exploring tissue- and cell-type-specific expression profiles of human E3 ligases under normal or diseased/stressed conditions, especially for the development of targeted protein degradation (TPD) (Békés et al. 2022). TPD is an attractive therapeutic modality to degrade disease-causing proteins via designed strategies, of which proteolysis-targeting chimeras (PROTACs) is the best-known technology showing great therapeutic potentials in oncology, inflammation, autoimmune diseases, virus infection, and neurodegeneration. A PROTAC molecule is a small heterobifunctional compound that contains two linked ligands, which can bridge a choice E3 ligase and a protein of interest (POI), leading to POI ubiquitination and proteasomal degradation (Sakamoto et al. 2001; Toure and Crews 2016). In the last two decades, the PROTAC field grows very exponentially and several PROTAC degraders have been entered in preclinical and early clinical development. Although a systematic assessment of protein targets that are suitable to be degraded by PROTAC molecules had been performed (Schneider et al. 2021), only a few validated E3 ligases are utilized in PROTAC, such as CRBN, MDM2, VHL, DCAFs, IAPs, KLHLs, RNF4, RNF114, KEAP1, TRIM9, TRIM71, and ZNRF3 (Békés et al. 2022; Marei et al. 2022). Although PROTAC degraders that use these validated E3 ligases work very well, there is still a need to explore now E3 ligases for PROTAC. Based on some recent studies, it could be proposed that, for a specified target, one E3 ligase in particular may work better than others (Bondeson et al. 2018; Bond et al. 2020; Zeng et al. 2020). Moreover, in oncology, resistance mechanisms to PROTAC agents could be developed rapidly by cancer cells to inhibit the abilities of PROTAC E3 ligases and therefore evade degraders, which may require alternative ligases for developing next-generation degraders. Therefore, for development of PROTAC, it is very important to identify new E3 ligases to pair with any target of choice. Several factors can determine the degradation profiles of E3 ligase: shape complementarity of the ligase and the target, degradation-competent ternary complex formation, subcellular localization of ligase and target, and cell-type- or tissue-specific expression profiles of E3 ligases (Békés et al. 2022). Using PROTAC molecules based on cell-type- or tissue-specific E3 ligases can degrade target proteins in the special cancer cells or tissues/organs, which could avoid systemic off-targets and side effects. Several studies had analyzed expression profile of E3 ligases in healthy and diseased states according to the publicly available datasets (He et al. 2020; Khan et al. 2020; Shirasaki et al. 2021). However, analysis of expression levels of a single subunit of multi-subunit E3 complexes in these studies cannot represent the accurate expression profiles of the E3 complexes. Collectively, to systematically investigate tissue- and cell-type-specific expression profiles of E3 Ub ligases, under normal or diseased/stressed states, will be an attracting topic in the next decade either to comprehensively understand the biology of E3 ligases in different diseases/stresses or to extend PROTAC applications.

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Conflict of interest

The authors declare that they have no conflict of interest.

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