Functional Roles of Ubiquitin-Like Domain (ULD) and Ubiquitin-Binding Domain (UBD) Containing Proteins

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Contents

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1. Introduction

Post-translational modifications used in the form of small proteinaceous moieties provide a system much more versatile and flexible than small molecule modifications such as phosphorylation and acetylation. Since its discovery in the mid-1970s, the pioneering ubiquitin (Ub) protein has been accompanied by a number of ubiquitin-like (UBL) modifiers, all taking advantage of the globular β-grasp ubiquitin superfold. Together they form a rather large and divergent superfamily of UBL molecules, which are involved in the regulation of cellular activities extending into almost every corner of eukaryotic life. Accurate interpretation of the signals mediated via small protein modifiers is dependent on the correct employment of a multitude of ubiquitin- and UBL-binding domains (UBDs), which have coevolved alongside their interaction partners.

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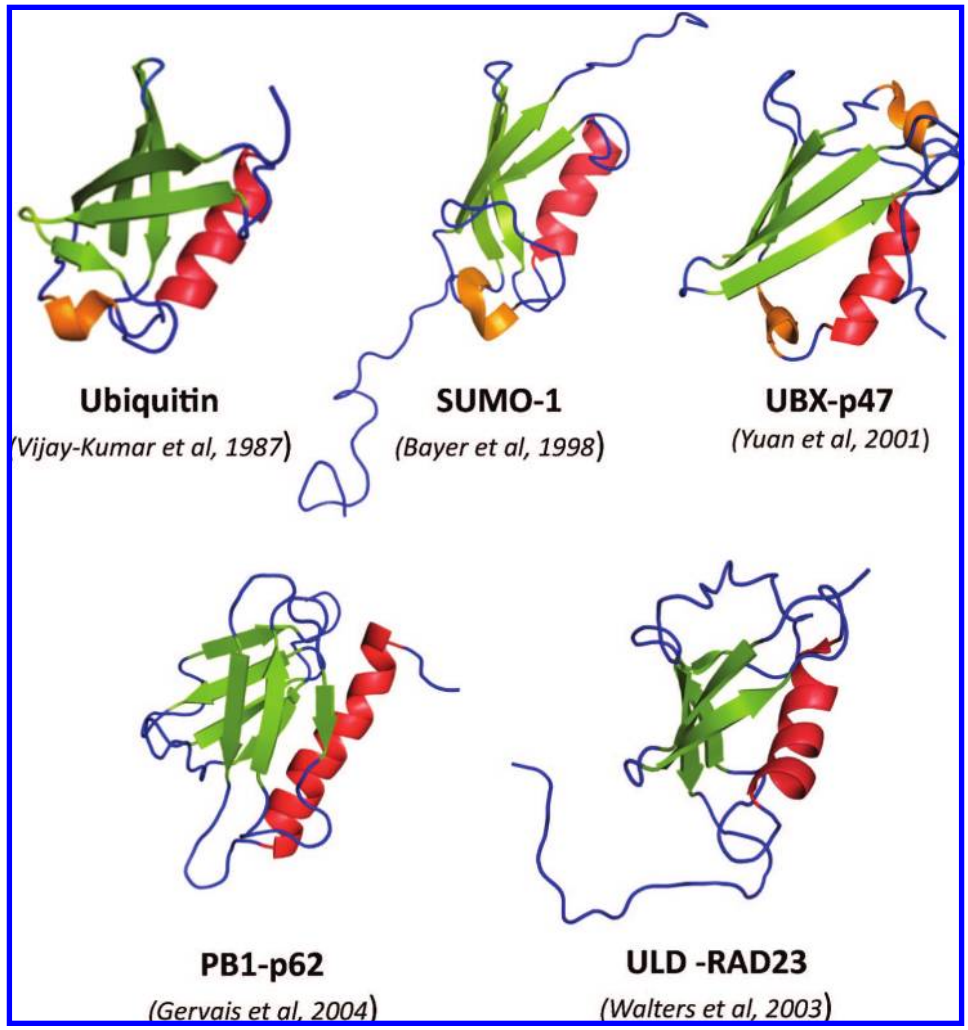

Figure 1. Variations of the ubiquitin β-grasp superfold in protein domains. Structural comparison of the ubiquitin-like folds characterized to date, based on published structural information provided by NMR- and crystallography-based studies as follows: (1) ubiquitin[1] (PDB code 1UBQ), (2) SUMO-1[146] (PDB code 1A5R), (3) the UBX domain in p47[147] (PDB code 1I42), (4) the PB1-domain in p62[148] (PDB code 1PFJ), and (5) the UBL in Rad23[149] (PDB code 1OQY). The figures were prepared using the program Pymol (http://pymol.sourceforge.net/).

In parallel to the single entities known as UBLs, ubiquitin-like folds are now also recognized as important integral elements of proteins, forming so-called ubiquitin-like domains (ULDs), which are present in a large variety of protein families. A subgroup of such proteins has combined a ULD with a UBD within the same polypeptide chain, endowing the resulting protein with unique properties, such as intramolecular interaction and autoregulation. The most studied of the ULD/UBD proteins are the ubiquitin shuttle receptor families (RAD23, the Dsk2/ubiquilin proteins and DDI1), which are involved in the targeting of polyubiquitinated proteins for proteasomal degradation. Alongside the ULD/UBA-containing proteasomal shuttle factors, new proteins with alternative ULD/UBD combinations are being identified, including the UBX/UBA proteins primarily functioning in the ERAD pathway as well as SLD/SIM proteins, which contain modules resembling the small ubiquitin-like modifier (SUMO), SUMO-like domain (SLD), together with SUMO-interacting motifs (SIMs). In this review, we focus on proteins displaying a ULD/UBD architecture and discuss their roles in the regulation of various cellular activities as well as in the etiology of human diseases such as neurodegenerative disorders, muscle atrophy, and tumorigenesis.

2. Ubiquitin Superfold Domains

2.1. Ubiquitin: An Overview

The importance of ubiquitin is convincingly illustrated by the evolutionary conservation of its 76 amino acids, which between mammals, yeast, and plants only differs at three positions. Structurally, ubiquitin adopts a compact globular fold, known as the "ubiquitin fold" or "ubiquitin superfold", characterized by a five-stranded β-sheet with a single helix on top and an exposed C-terminal tail which extends to participate in the covalent linkage to target proteins[1] (Figure 1). This conjugation, relying on the sequential activity of the ubiquitin activating (E1), conjugating (E2), and ligating (E3) enzymes, results in the addition of a ubiquitin moiety to either the ε-amino group of a lysine residue or the extreme N-terminus of a polypeptide, which further has the potential to be extended by the action of E4 elongation enzymes to form polyubiquitin chains.[2,3] Traditionally, ubiquitin conjugation has been believed to invariably serve as the final station in the destiny of a protein, serving to target its substrates for degradation by the proteasome. Today, however, we know that ubiquitination influences a broad repertoire of cellular processes and that the fate of the target

protein depends on the number of ubiquitin moieties conjugated as well as the type of lysine linkage used for the ubiquitin−ubiquitin conjugation.[4] Whereas the addition of a single ubiquitin to a target protein (monoubiquitination) may alter protein activity and localization (regulating endocytosis, lysosomal targeting, meiosis, and chromatin remodelling), the formation of a diverse array of ubiquitin chains (polyubiquitination) is implicated in events such as proteasomal targeting, immune signaling pathways (e.g., the NFκB cascade), and DNA repair.[5] Importantly, in order to enable a dynamic regulation of signaling events, ubiquitination is, similar to phosphorylation, a reversible process which is specifically counteracted by the deubiquitination (DUB) family of proteases.[6]

2.2. Ubiquitin-Like Modifiers

Together with an average 10^7-10^8 copies of ubiquitin per cell,[7−9] there are a large number of small proteins resembling ubiquitin in their primary or higher order structures, which can be conjugated to and, consequently, alter the fate of their target proteins. Common to most of these so-called ubiquitin-like modifiers (UBLs) is the utilization of an E1/E2/E3-like conjugation machinery as well as a requirement of proteolytic processing prior to protein activation. The UBL modifiers characterized to date include the SUMOs (small ubiquitin-like modifiers) -1, -2, and -3[10] (Figure 1), which like ubiquitin also have the capacity to participate in chain formation, Nedd8 (aka Rub1 or related to Ub-1), the ATG8 (Autophagy-8) family, UFM-1 (ubiquitin-fold modifier-1), HUB-1 (homology to Ub-1), the diubiquitin molecules ISG15 (Interferon-stimulated gene 15) and FAT10 (human leukocyte antigen F associated),[11] as well as MUB (membrane-anchored UBL-fold). The discovery of the most recent UBL family member, the MUB, has revealed yet a unique feature in the ubiquitin system, providing a UBL which is elegantly targeted to cellular membranes by prenylation.[12]

Besides performing overlapping as well as unique roles, there appears to be considerable cross-talk and sometimes also competition between ubiquitin and the various UBLs.[10] For instance, a subgroup of ubiquitin ligases, including the E3 TOPORS, seems to display dual activities and has the capacity to conjugate both ubiquitin and SUMO-1 to target proteins.[13,14] Other ubiquitin E3 ligases are instead dependent on a preceding sumoylation event, as shown for RNF4, which specifically mediates K48-linked polyubiquitination and proteasomal degradation of sumoylated PML-RARα oncoprotein in acute promyelocytic leukemia (APL) patients treated with arsenic trioxide.[15,16] In other cases, target proteins have been found to be modified, in a competitive manner, by either ubiquitin or SUMO-1 at the same residue, providing a powerful switch mechanism by which the fate of a protein can rapidly be altered.[10] Moreover, a direct connection between different UBLs has been made evident by the discovery of polyubiquitin chains in which UBLs, such as SUMO and Nedd8, are integrated, forming so-called heterologous ubiquitin chains. However, the mechanism behind the generation of such chains, as well as their physiological relevance, is currently poorly understood.[4]

2.3. Ubiquitin-Binding Domains

In comparison to small molecule modifiers, protein modifications generated by the addition of an entire protein moiety provide a larger and chemically more varied surface area with the enclosed potency of multifaceted cellular interpretations. This feature has promoted the coevolution of UBLs, on one hand, and a multitude of UBL-binding domains (UBDs), on the other. The $\geq$16 thus far characterized UBDs are in general rather small (20−150 amino acids) and diverge in both structure and patterns of ubiquitin recognition. A majority of the UBDs fold into α-helical-based structures, including the UBA (ubiquitin-associated domain), UIM (ubiquitin-interacting motif), DIUM (double-sided ubiquitin-interacting motif), MIU (motif interacting with ubiquitin), CUE (coupling of ubiquitin conjugation to ER degradation), GAT [GGA (Golgi-localized, gamma-ear-containing, ADP-ribosylation-factor-binding protein), and TOM (target of Myb) domains. Nonhelical UBDs are also frequent and can be exemplified by the different ubiquitin-binding zinc fingers (ZnF) such as NZF (Npl4 zinc finger) and PAZ (polyubiquitin-associated zinc finger), the Ubc domain present in E2 enzymes, as well as the UEV (ubiquitin-conjugating enzyme variant), GLUE (GRAM-like ubiquitin-binding in Eap45), Jab1/MPN, and PFU (PLAA family ubiquitin binding) domains. Besides their structural similarities, helical UBDs also share a common attraction to the same binding surface on the ubiquitin moiety, formed by the hydrophobic patch including and surrounding isoleucine 44 (Ile44). In contrast, ZnF-based UBDs, such as the A20-ZnF and the ZnF-UBP, display highly variable modes of ubiquitin recognition, which is in keeping with their highly divergent biological roles (for review see ref 17). Furthermore, while some UBDs appear to be strictly connected to a certain protein function, such as the exclusive presence of UBZ and UBM domains in Y-family DNA polymerases,[18] others fail to follow any general rules in correlation to functionality.

Although in most cases the binding between the to date described UBDs and ubiquitin is of low or moderate affinity, characterizing ubiquitin-mediated protein interactions as flexible and highly dynamic, there are examples where the interaction is much stronger.[19−21] In fact, K_d values between the various UBDs and ubiquitin range between 2 and 500 μM, and in many cases the affinity of binding is strongly enhanced (10−100 times) by the recruitment of multiple ubiquitin moieties in the form of multiple consecutive monoubiquitins or polyubiquitin chains. Interestingly, the UBD that displays the strongest binding affinity to ubiquitin is the ZnF-UBP module present in proteins such as HDAC6, BRAP2/IMP, IsoT, and Usp5. The ZnF-UBPs in these proteins fold into a compact globular structure with a deep binding pocket that accommodates the C-terminal diglycine motif of ubiquitin with K_d values in the low micromolar range.[20,21]

On the basis of the apparent correlation between linkage specificity and cellular functionality, it has been proposed that different ubiquitin chain conformations ought to be recognized by distinct UBDs. However, so far in vitro experiments have been unable to identify any selectivity of the majority of UBDs toward any type of linkage.[22] Instead, specificity appears to be conveyed by surrounding domains within the same protein or another protein within a larger protein complex. Then again, when evaluating and comparing affinities and specificities in regard to UBDs, it is important to keep in mind that most of the to date published interaction studies have been performed utilizing methods which measure the binding properties of recombinantly expressed isolated protein domains. Moreover, it is still unclear whether

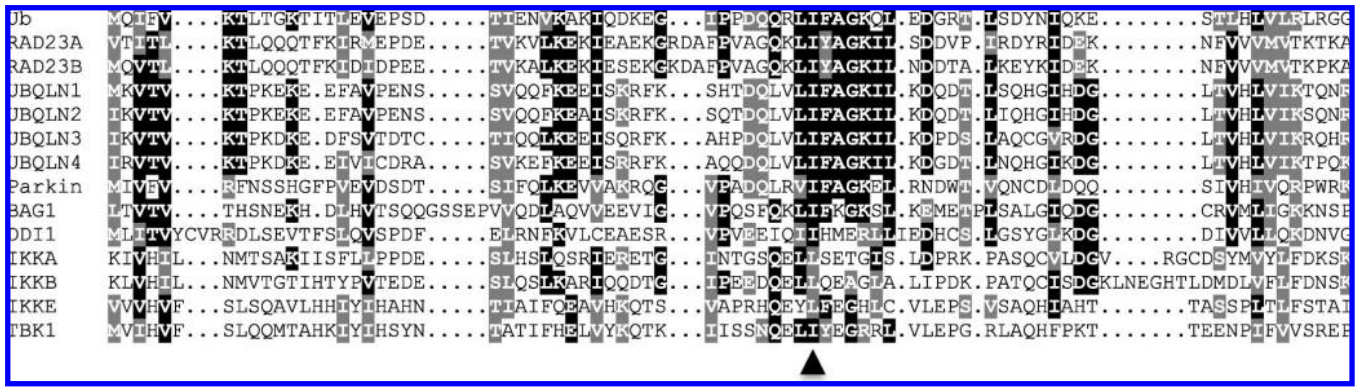

Figure 2. Sequence alignment of ULDs displayed by proteins involved in UPS-mediated degradation and immune signaling pathways. Sequence comparison of the ULDs in RAD23A, RAD23B, UBQLN1, UBQLN2, UBQLN3, UBQLN4, Parkin, Bag1, DDI1, IKKA, IKKB, IKKE, and TBK1. Domain boundaries were chosen by aligning the corresponding protein sequences with ubiquitin, where after the resulting ULDs were aligned using the MAFFT program.[150] Conserved residues are shown in black or gray background. Whereas ULDs derived from proteins within the same protein family or from proteins with similar functions share quite high sequence homologies, ULDs in unrelated proteins can be rather divergent. In most cases, however, the hydrophobic core centered around Ile44 (arrowhead) in ubiquitin is preserved also in integral ULD domains.

local concentrations of the binding partners can create environments where the effective affinity is significantly amplified in the context of a living cell.

Alongside the discovery of new protein domain variants resembling the ubiquitin superfold, new types of UBDs have also been described. Importantly, one such domain was recently characterized in the Rpn13 subunit of the proteasomal regulatory particle, which was found to contain a novel ubiquitin-binding motif in the N-terminal part of the protein. Given its structural similarity to phosopholipid-binding PH domains, this new motif was named pleckstrin-like receptor for ubiquitin or in short Pru. The Pru domain folds into two continuous, antiparallel β-sheets that are connected by loop regions, where the loop regions unexpectedly extend to contact ubiquitin with high affinity ($K_d \approx 30$ and 90 nM for mono- and diubiquitin, respectively).[23,24] In addition to Pru and the conventional ubiquitin-binding domains discussed above, the discovery of a ubiquitin-binding function in a subclass of proline-recognizing SH3 domains[25,26] further underscores a complexity of ubiquitin-mediated regulation that is yet to be explored.

2.4. UBL-Binding Domains

In contrast to the well-studied UBDs, protein domains that recognize the related UBL modifiers are still quite poorly characterized. However, multiple studies have recently described motifs specifically recognizing SUMOs, brought together under the term SUMO-interacting motif (SIM) (previously also known as SUMO-binding domain (SBD)), which rather than forming a modular structure, interacts with SUMO via merely a few amino acids. The SIMs commonly consist of a hydrophobic consensus sequence including clusters of Val, Ile, and Leu, which is flanked by a stretch of acidic and/or phosphorylated residues.[27−30] Interestingly, the interaction between a SUMO and the corresponding SIM is in general of significantly higher affinity than most ubiquitin/UBD interactions, displaying K_d values of 2−3 μM. This feature may explain the observation that a single SUMO/SIM binding event is in general sufficient to generate a biological readout,[31] while ubiquitin-mediated signaling in many cases requires chain formation or multiple or tandem interactions. Moreover, analogous to ubiquitin itself, a SUMO/SIM interaction event can be reinforced by SUMO polymerization as well as the presence of multiple SIMs in the target protein.[32]

In the context of UBL-binding domains, it is important to mention that in addition to ubiquitin, many of the classical UBDs also recognize a variety of integral ULDs as well as the UBLs Nedd8 and FAT10, most likely due to their high sequence and structural homologies. A few examples of crucial importance are the recruitment of proteasomal shuttle factors (e.g., RAD23/Dsk2/DDI1) to the UIM domains in the proteasomal subunit Rpn10/S5a,[33−36] the intramolecular interaction between the ULD and UBA2 domains in RAD23,[37] as well as the UBA domain-mediated recruitment of FAT10 by Nub1L.[38] Furthermore, novel Nedd8-interacting motifs are also in the process of being identified, for instance, in Nub1/Nub1L, which contains conserved stretches of leucine-rich sequences that appear to recognize Nedd8.[39] In conclusion, the relationship between the various UBL/ULDs and their corresponding UBDs is highly complex. It should without a doubt be expected that the current paradigms will be subject to modification when challenged in vivo.

2.5. Integral Ubiquitin-Like Domains (ULDs)

In conjunction with the evolution of UBLs, larger cellular proteins have also taken advantage of the valuable properties displayed by ubiquitin and genetically integrated ubiquitin-like folds within their coding region. Such integral ubiquitin-like domains come in several flavors, among which the most frequently occurring is the ubiquitin-like domain, the ULD. The ULD (also known as UBQ, ubiquitin homologues) is defined as a region of 45−80 amino acids which strongly resembles ubiquitin in primary sequence as well as 3D structure[40] (Figures 1 and 2). ULD motifs are widely spread in eukaryotic proteins and appear in proteasomal shuttle factors such as RAD23 (Figure 1) and Dsk2/ubiquilin, E3 ligases including Parkin and Elongin B (a component of the multisubunit VHL E3 ubiquitin complex), the chaperone cofactors Bag1 and Scythe, as well as the DUB enzyme USP14 (for review, see ref 41). Besides these ULDs found in proteins linked to the cellular machinery coping with protein folding and degradation, the presence of integral ULDs extends also into proteins involved in the regulation of signal transduction and enzymatic activity. For instance, IKKα and IKKβ (IκB kinase α and β), two related serine/

threonine kinases required for phosphorylation of IκB and subsequent NFκB activation, both comprise regions that resemble ubiquitin. However, whereas IKKβ displays a distinct ULD, which is essential for its kinase activity, the actual presence of a ULD in IKKα is still an area of controversy.[42] More recently, ULD domains were also identified in two additional immune-response inducible kinases resembling the IKKs, namely, TBK1 and IKK-*i*, both proteins in which the ULD was shown to mediate functions essential for substrate binding as well as kinase activity per se.[43] It is interesting to note that while substitution of the RAD23 ULD with the authentic sequence of ubiquitin can fully restore the UV-protective functions of the protein,[44] the catalytic activity of IKKβ is abolished when replacing the ULD with bona fide ubiquitin,[42] suggesting that the intrinsic differences between integral ULDs are important for protein function.

Another variant of an integral protein domain that resembles ubiquitin is the ubiquitin-regulatory X domain (UBX), a protein module which despite sharing only low sequence homology with ubiquitin nevertheless folds into a structure highly similar to the ubiquitin superfold, differing from ubiquitin itself only by one expanded surface loop between the third and fourth β-strands of the domain[45] (Figure 1). UBX domains are commonly placed in the absolute C-terminal region of the host protein, which in most cases belong to one of several evolutionary conserved families, including FAF-1, p47, SAKS1, TUG, UBXD1, UBXD3, and Rep8.[40,46]

A third group of integral ULDs is formed by the PB1 (Phox and Bem1) domains, which is present in proteins such as p62 (Figure 1), MEK5, and PKC (ζ and ι/λ) and represents a functionally incoherent group of ubiquitin-resembling folds that within a higher order structure have shown importance for signal transmission.[47,48]

The family of ULD-containing proteins is continuously growing, and it should be mentioned that many proteins appear to contain borderline ULD domains, in which the ULDs are rather divergent and may or may not be connected with the ubiquitin-proteasome pathway. In addition to the above-mentioned ULDs, there are additional integral protein domains that resemble other ubiquitin-like modifiers. One example of such domains is the SUMO-like domain (SLD), which will be further discussed below.

2.6. Further Applications of the Ubiquitin Superfold in Protein Design

In light of the poor sequence similarities between many of the identified proteins utilizing the ubiquitin superfold, Kiel and Serrano recently presented a new approach to classify the proteins, known to date, that display the structural features of a β-grasp ubiquitin superfold within their coding region.[49] By making manual estimations based on structural data they thereby formulated a consensus fingerprint sequence that can be used in order to classify the established ULDs and in addition simplify identification of novel ULDs. Deviating from the above-described classical ULDs, domains exploiting the ubiquitin superfold also include the RA (RalGDS/AF6 Ras-association domain), RBD (Raf-like Ras-binding domain), PI3_rbd (Ras-binding domain of PI3Kinase-like proteins), as well as P1 subdomain of the Band 4.1/FERM domain, referred to as the B41/ERM domain.[49] These domains all fold into a ubiquitin-like α/β roll, which by using small structural alterations display unique surfaces that expose different binding epitopes, thus enabling the recruitment of specific interaction partners and regulation of a broad array of cellular activities.[49]

3. ULD/UBDs in Combination

In eukaryotic proteins, the different types of ULD and UBD domains are used in combination with an extensive assortment of other protein modular domains and are thereby involved in a wide spectrum of cellular processes. A common feature of UBD-containing proteins is their predilection to become ubiquitinated themselves, an event mediated via a process known as "coupled monoubiquitination", a unique type of ubiquitination which is dependent on the presence of a functional UBD and in many cases independent of an E3 ligase.[50−53] Coupled monoubiquitination of UBD-containing proteins importantly provides a means of intramolecular interaction and consequently intramolecular regulation of protein activity (e.g., autoinhibition). One group of proteins has taken this attribute one step further by combining a ULD and a UBD within a single open reading frame, giving rise to the ULD/UBD family of proteins (Figure 3), among which the best characterized group is formed by the ULD/UBA ubiquitin shuttle receptors/factors.

3.1. ULD/UBA Proteins: Shuttle Buses to the Final Destination

The main site of protein degradation in the cell is the proteasome, a multisubunit protease that recognizes and degrades proteins tagged by ubiquitin chains. Traditionally, ULD/UBA proteins are commonly entitled "proteasomal shuttle factors", based on their mutual ability of simultaneously binding ubiquitin chains[54] and directly interacting with the 26S proteasome,[34,55] thus targeting ubiquitinated proteins for proteasomal degradation (for reviews, see refs 56−58). Members of the ULD/UBA family were first discovered in yeast and include RAD23 (radiation-sensitive mutant 23) and Dsk2 (Dominant suppressor of Kar1)/ubiquilin proteins, together with DDI1 (DNA damage-inducible protein), proteins which all display an N-terminal ULD, combined with one or two C-terminal UBA domains (Figure 3). Given the essential nature of protein degradation, evolution has provided the ULD/UBA ubiquitin shuttle factors with multiple docking sites on the proteasome, ensuring the proper delivery of its cargo (Figure 4). Utilizing their ULD domain RAD23, Dsk2/ubiquilin, and DDI1 all bind directly to both Rpn10/S5a and Rpn1,[33,55,59] two subunits of the 19S proteasomal regulatory particle, a targeting mechanism which is further backed up by a direct recognition of ubiquitin chains by the proteasome itself. Traditionally, Rpn10/S5a has been considered the major proteasomal receptor for ubiquitin, but recently Rpn13, another subunit of the regulatory particle, has also been found to function as a direct binding site for K48-linked ubiquitin chains.[23,24] The functional relevance of Rpn13 as a ubiquitin receptor is strongly reinforced by in vivo data from yeast experiments, in which the loss of Rpn13, together with Rpn10-deficiency, results in a reduced ability to handle stress induced by increased levels of misfolded proteins.[23,24] Interestingly, the ubiquitin-binding Pru domain of Rpn13 was found to interact potently with the proteasomal shuttle factors RAD23 and Dsk2/ubiquilin, further ensuring the targeting of proteins destined for degradation, to the proteasome.[23,24] The relevance of ULD/UBA proteins for this targeting process is

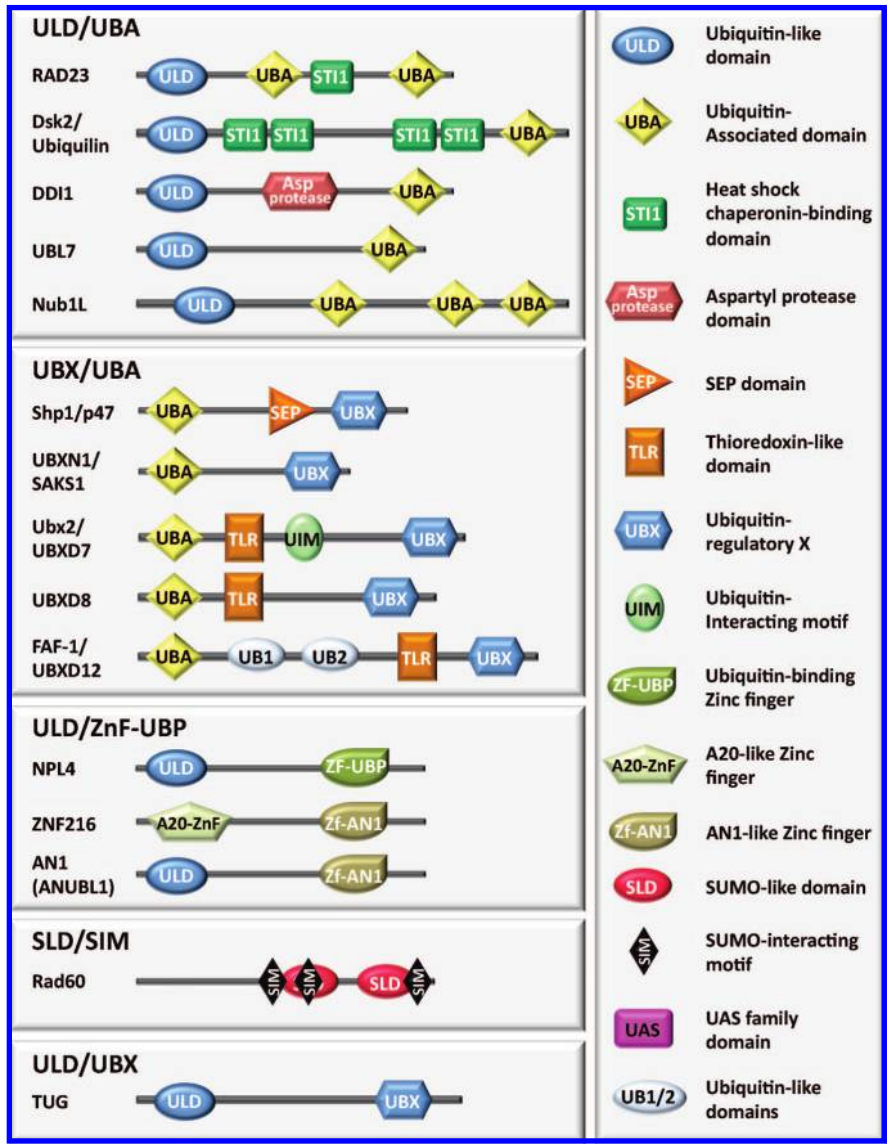

Figure 3. Domain organization of a selection of the so far characterized ULD/UBD proteins. A selection of proteins containing different combinations of ULD and UBD domains, arranged in the following families: ULD/UBA, UBX/UBA, ULD/ZnF-UBP, and SLD/SIM. In addition, the ULD/UBX protein TUG is shown in the bottom panel. Abbreviations are indicated in the right panel.

evident, given that alteration of yeast Dsk2 and/or RAD23 expression levels strongly affects the efficiency of protein degradation. Individual overexpression of either Dsk2 or RAD23 gives rise to accumulation of polyubiquitinated proteins and cell toxicity,[34,60,61] while deletion of Dsk2 and/or RAD23 likewise results in impaired protein degradation.[62]

ULD/UBA proteins have been evolutionarily conserved from yeast to man, expanding in number and functionality with the complexity of the organism. The lack of lethality observed in single mutant yeast strains suggests a partially overlapping functional relationship between the ULD/UBA proteins. Indeed, the ubiquitin shuttle factors have been reported to both physically interact with as well as in some cases have the capacity to substitute for one another. However, the fact that these proteins have acquired unique and specific functions later in evolution is evident from the analysis of mice deficient for the proteasomal shuttle factor RAD23. Specifically, despite the presence of two murine RAD23 homologues (RAD23A and RAD23B), mice lacking RAD23B barely survive into adulthood, displaying severe developmental impairment and intrauterine or neonatal lethality in 90% of the cases.[63] These observations in higher mammals, together with studies in yeast, imply several separate functions of the RAD23 proteins, mediated via different regions of the protein. In addition to its ULD and duplicate of UBAs, RAD23 also contains an STI domain (overlapping with the domain formerly known as the XPC-binding domain or R4BD) which mediates an interaction with XPC (Xeroderma Pigmentosum group C protein), a protein essential for recognition of DNA damage and initiation of nucleotide excision repair (NER).[64] In fact, RAD23 was initially identified as a modulator of NER given the reduced NER activity observed in RAD23-deficient yeast strains.[65] Even though the exact function of RAD23 in this process is not yet fully understood, multiple studies point toward a role in the stabilization of the XPC protein (providing protection from the ubiquitin/proteasome system) and/or a scaffolding function during the recruitment of XPC to NER lesions.[66,67]

The proteasomal shuttle factor Dsk2 has even further multiplied throughout evolution and in mammals given rise to four family members, known as the ubiquilins or PLICs (ubiquitin-like proteins or proteins linking integrin-associated protein (IAP) to the cytoskeleton, respectively), distinguished by numbers 1−4. Similar to RAD23, mammalian ubiquilins

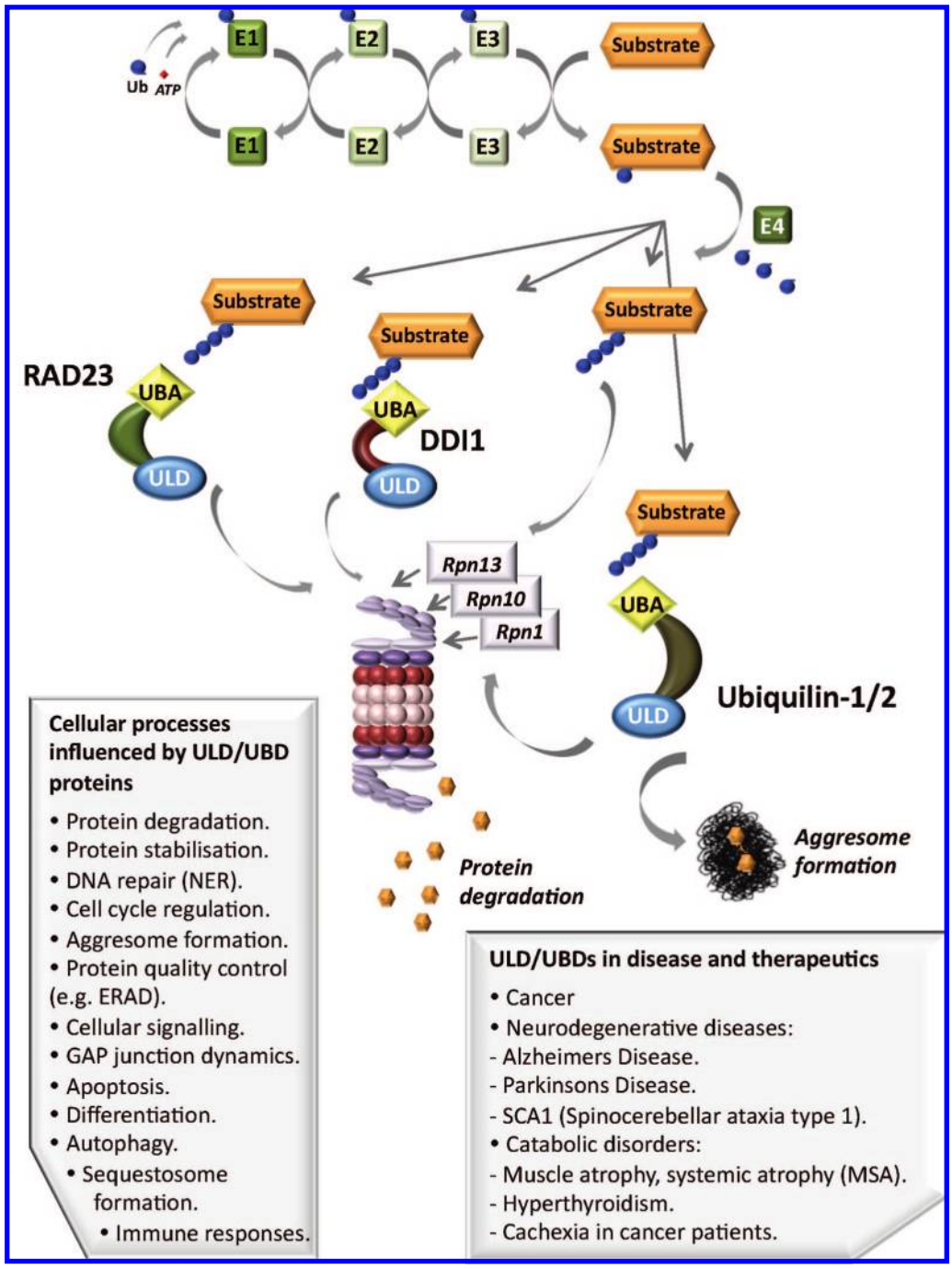

Figure 4. Role of ULD/UBA proteins in proteasomal degradation. After the sequential activity of ubiquitin E1 activating, E2 conjugating, E3 ligating, and E4 elongating enzymes, polyubiquitinated substrates are recognized by the UBA domain in the proteasomal shuttle factors, RAD23, ubiquilin family proteins, and DDI1. Subsequently, utilizing their integral ULD domains, the shuttle factors directly interacts with multiple sites on the proteasome (Rpn1, Rpn10, and Rpn13), thus serving to target their ubiquitinated cargo for degradation. Given the requirement of protein degradation for removal of damaged, abnormally folded, or simply undesired proteins, as well as the proper regulation of most cellular activities, the entire UPS system offers an important source of human pathogenesis.

have retained their shuttling activity and recently been suggested to be involved in formation of aggresomes, given the reported augmentation of ubiquilin-1 transcription in response to misfolded protein stress,[68] together with the recently observed recruitment of ubiquilin-2 protein to sites of aggresome formation[69] (Figure 4). A role of ubiquilins in this process is entirely in line with their implied involvement in the etiology of neurodegenerative diseases caused by protein misfolding and aggregation. More specifically, based on the observed accumulation of ubiquilin proteins in pathogenic Lewy bodies, their interaction with presenilins,[69,70] and the reported involvement of ubiquilin-1 in the trafficking of the amyloid precursor protein APP,[71] the ubiquilins have been linked to Alzheimer's Disease (AD) as well as spinocerebellar ataxia type I (SCA1).[72,73] An important role of ubiquilin proteins in AD has been further supported by in vivo studies in *Drosophila*, where loss of the sole ubiquilin family member, ubiquilin-1, has been linked to age-dependent neurodegeneration and reduced lifespan, processes during which it genetically interacts with the presenilins.[74,75] Interestingly, ubiquilins have been found to interact with Eps15, a binding event that by extension suggests a putative model in which ubiquilin-1/2 plays crucial roles during the trafficking of protein aggregates to the aggresome.[68,76]

The least studied of the ULD/UBA proteins is DDI1. DDI1 contains a retroviral protease-like domain, in contrast to the STI1-like domains of RAD23 and Dsk2/ubiquilin, and has been proposed, although not proven, to participate in the preparation (e.g., deubiquitination) of substrates for the proteasome.[77] A recent study of DDI1 defined this ULD/UBA family member as a multifunctional protein and concluded that the different domains displayed by the DDI1 open reading frame are individually responsible for the involvement of DDI1 in regulated protein turnover, in Pds1-dependent S phase checkpoint control, and in exocytosis (mediated via a direct interaction with SNARE proteins (t/v)), respectively.[78]

3.2. Crosstalk Makes Perfection

There is an obvious, however not fully understood, crosstalk between the three main families of proteasomal shuttle factors. Clearly, they are all involved in homo- as well as heterodimerization, forming complexes that can be interrupted in the presence of ubiquitin since UBA/ubiquitin (or ULD/Rpn10) binding is preferred over a ULD/UBA interaction.[79] In agreement, it has been shown that disruption of intramolecular ULD−UBA domain interactions in RAD23 potentiates its binding to ubiquitin. Interestingly, structural investigations suggest that RAD23 activity is regulated in a competitive manner, given that the integral UBA domain, the proteasome itself as well as the ubiquitin ligase Ufd2, all recognize the same binding surface of the RAD23 ULD domain.[80] The clustering of ULD/UBA proteins indeed exhibits a regulatory role but may also be important for protein functionality given the current belief that multiple ubiquitin receptor molecules have the capacity to interact with a single polyubiquitin chain, thereby joining forces to ensure a safe delivery to the proteasome and protect the ubiquitin chain from premature deubiquitination in the cytoplasm.[79] This model is backed up by recent data showing that ubiquitin chains form rather flexible and accessible structures in solution.[81,82] This is true for K63-linked ubiquitin chains in particular, which under physiological conditions form elongated structures without any stable intersubunit interfaces.[82,83] In contrast, at neutral pH K48-linked chains fold into a relatively closed conformation, consequently hiding important residues such as L8, I44, and V70 at the interdomain interface.[84] Despite this sequestration of key interaction surfaces, studies have indicated that the interdomain interfaces nevertheless retain a high flexibility, thereby allowing these residues to interact with their cognate UBDs.[84] This finding is further endorsed by the observation that the recruitment of K48 diubiquitin to Rpn10 induces a conformational transition in the ubiquitin chain, thus allowing a direct contact between the hydrophobic patch of ubiquitin and the two UIM motifs of Rpn10.[85]

In most cases the binding of a ubiquitinated substrate to a proteasomal shuttle factor is synonymous with destruction. However, there are several layers of complexity in this system that ought to be remembered. First, in some situations the binding to a ULD/UBA protein has been found to stabilize the interaction partner. This has been observed for the ubiquilin proteins, which stabilize p53 and IκBα, as well as for RAD23 that protects the XPC protein from proteasomal degradation.[34,61] Second, whereas shuttle proteins display rather promiscuous binding to substrates in assays performed in vitro, in vivo situations appear to provide a higher level of specificity, where non-UBA sequences together with other, external, factors are likely to contribute to a meticulous fine tuning of the system.[22] This has, for instance, shown to be the case for Sic1, which in contrast to in vitro results in the living cell appears to be solely governed by RAD23.[58] Third, the intimate relationship between shuttle factors and ubiquitin ligases are also likely to contribute to the specificity.

In light of the high structural homology shared by the multitude of UBL modifiers and integral ULDs, it is interesting to note that while ubiquitin as well as most ULDs are directly recruited to the proteasome, others fail to interact with any of the proteasomal subunits. To understand the underlying mechanism behind this, the so far characterized ULDs have been compared in extensive bioinformatic analyses. Interestingly, these studies have identified a proteasomal-targeting motif (PIM) present in all domains known to interact with the proteasome, including ubiquitin itself, as well as Nedd8 and the ULDs in RAD23, ubiquilin proteins, and Parkin. In agreement, the SUMO proteins, which are not to recruited to the proteasome, are all lacking such a motif.[86]

Given the close proximity of the proteasomal shuttle factors to the proteasome itself, constantly delivering ubiquitinated proteins for degradation, it is worth noting that they themselves are highly stable within the cell. The burning question of how RAD23, Dsk2/ubiquilin, and DDI1 are protected from proteasomal degradation has been addressed by Dantuma and co-workers, who by a combinatorial approach based on biochemistry and yeast genetics could elegantly establish that the C-terminally located UBA domains of these proteins display an evolutionary conserved, cis-acting, protective function which is essential for protein stability as well as functionality. In their experiments they observed that in the absence of an intact UBA domain, proteasomal shuttling factors themselves become short-lived proteasomal substrates that are directly recruited to the proteasome via their ULDs.[37]

3.3. UBX/UBA: Bridging the Proteasome with ERAD

There are areas in the cell in which proteasomal degradation is not sufficient but requires reinforcement by other AAA-type ATPase complexes with more specialized activities. One such is the p97/Cdc48/VCP (valosin-containing protein) complex, which complements proteasomes in the obliteration of misfolded and unassembled proteins and protein complexes in the endoplasmatic reticulum (ER), in the process known as ERAD (ER-associated protein degradation)[87] (Figure 5). Residing in close proximity to the ER, where it functions to dislocate substrate proteins into the cytoplasm through a retro-translocation pore, p97 forms a ring-shaped complex consisting of six identical subunits (homohexamer), each composed of two ATPase domains and an N-terminal domain responsible for substrate binding. Although p97 itself has the ability to interact with ubiquitin, efficient targeting of ubiquitinated substrates to the ERAD pathway is dependent on ubiquitin-binding adaptor proteins. Similar to the UPS system, ERAD shuttle factors are characterized by a stereotypic combination of one UBD together with a ubiquitin-like module. However, in contrast to the proteasome, p97 specifically interacts with UBX-domain-containing proteins rather than the classical ULDs (Figure 5). One of the best-studied p97 adaptors is the UBX/UBA-containing protein p47 (Shp1 in yeast), which utilizes its UBX domain to directly bind the p97 N-terminus in order

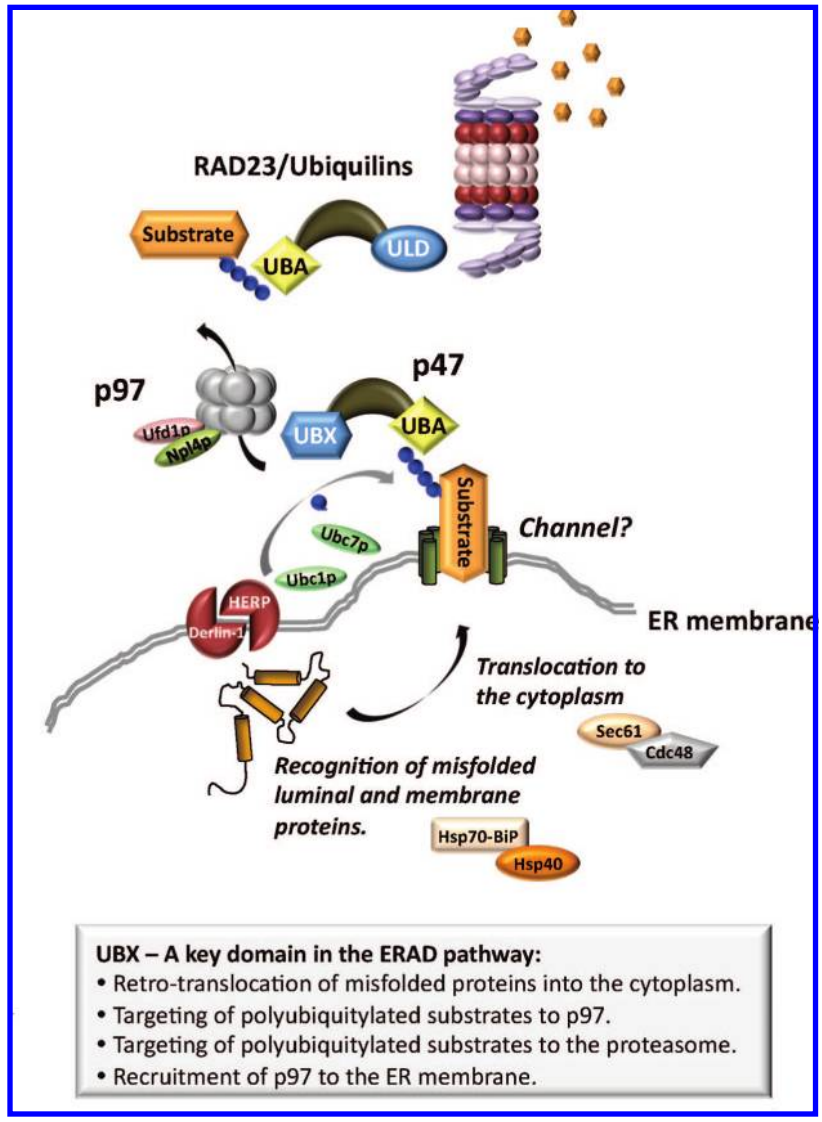

Figure 5. UBX proteins: Major regulators of ERAD. The ERAD pathway is essential for removal of misfolded and improperly assembled proteins and protein complexes in the ER. Following the recognition of an ERAD-destined substrate, target proteins are ubiquitinated and retro-translocated into the cytoplasm by the combined action of among others, the AAA-type ATPase p97, the transmembrane proteins Derlin-1 and HERP, as well as multiple UBX-containing proteins. Importantly, UBX/UBD-containing proteins such as p47 and the NPL4/UFD1 complex functions as essential cofactors of p97, exhibiting essential roles as ERAD-substrate shuttle factors, anchors for p97 at the ER membrane, and in addition display auxiliary roles in the final delivery of ERAD substrates to the proteasome.

to deliver its cargo for disassembly. In addition to the founder p47, vertebrate genomes contain at least two additional homologous family members, p37 and UBXD4. Importantly, p37 does not contain a UBA domain and is not implicated in the ERAD pathway but rather in a novel membrane fusion pathway essential for the biogenesis and maintenance of the ER and Golgi compartments.[88] Also essential to the ERAD pathway is the highly conserved NPL4-UFD1 complex, which in yeast comes together to form a ULD/UBD-containing protein complex, where the ULD in NPL4 is complemented by discrete ubiquitin-binding domains in UFD1. In higher species, NPL4 has acquired an additional C-terminal ubiquitin-binding zinc finger, thereby in itself fulfilling the criteria for an ERAD shuttle factor.[89,90]

Besides the classical set of p47 homologues, there are other proteins in which a UBX is combined with a UBA domain or an alternative UBD. A few such proteins, all implicated in the ERAD pathway (as well as other functions), include SAKS1 (aka 2B28 or Y33K)[91] and FAF-1 (Fas-associated factor 1), both showing a UBA/UBX topology (which in the latter case is complemented by two supplementary ubiquitin-like motifs of unknown function),[92] as well as the related UBXD7 protein, which in addition to UBA/UBX domains also contains a UIM motif. It is clear that the duplication of UBDs in UBXD7 mediates a stronger binding to ubiquitin chains, but the functional relevance of this event remains to be established.[93] What is clear, however, is that UBXD7 is crucial for the recruitment of p97 to the ER membrane.[94] Besides these examples of UBX/UBD-containing proteins there is one, so far, identified protein in which a UBX domain is found in combination with a classical ULD. This recently described protein, TUG (tether-containing UBX domain for GLUT4)/ASPL/UBXD9, does not appear to be involved in protein degradation but rather in protein redistribution within the cell, more specifically in insulin-induced GLUT4 mobilization to the plasma membrane and by extension glucose uptake.[95,96]

Also recruited to p97, together with the UBX/UBD shuttle factors and their ubiquitinated cargo, are the deubiquitinating enzymes VCIP135[97,98] and Otu1[99] as well as Ufd2.[99] Ufd2

is an E4 enzyme, which in addition to extending ubiquitin chains by a few moieties, also has the capacity to recruit RAD23 and Dsk2/ubiquilin proteins, an event important for the subsequent delivery and final destruction of ERAD substrates by the proteasome.[100] Interestingly, Ufd2 has been shown to compete with Rpn1 for a common binding site within the RAD23 ULD, an observation that has provided an elegant model for how ubiquitin conjugation per se and the process of proteasomal delivery may be linked.[101] In short, these three proteins are proposed to form a molecular platform where the proteasomal and ERAD degradative pathways may converge.[100]

The identification of TUG as a p97-interacting protein is only one example in a large group of ERAD-unrelated proteins, which in recent years have shown to be recruited to p97 and thereby expanded our view of p97 functionality and importance. In fact, all 13 UBX-containing proteins encoded in the human genome are targeted to p97, including the classical UBX/UBA shuttle factors, as well as UBX-only proteins, deficient in ubiquitin binding and consequently involved in activities uncoupled from protein degradation.[102] Indeed, p97 is known to play an essential role during the proteolytic activation of a subset of transcription factors,[103,104] in the regulation of ER-residing enzymes such as HMGR,[105,106] and in the targeted degradation of specific substrates.[102,107,108] Furthermore, a large number of ubiquitin ligases, deriving from all known families of E3s, were recently found to directly interact with the UBX/UBA shuttle proteins and/or p97 itself, consequently implicating a broader utilization of p97 for protein degradation than previously anticipated, extending far beyond ERAD.[102] These findings are entirely in keeping with the high abundance of p97 in mammalian cells. A comprehensive list of UBX- as well as UBX/UBD-containing proteins is still far from complete, and the characterization of new proteins as well as an increased knowledge regarding UBX/UBD protein function will be important subjects in the near future.

New levels of understanding, as well as complexity, of the proteolytic pathways are continuously reported, not only by identification of novel players and new shuttle factors but also in the discovery of new functions for old players. For instance, the ubiquilin family member ubiquilin-4/UBIN[109] was recently rediscovered (and confusingly named CIP75 for "Connexin43-interacting protein of 75 kDa") as a novel regulator of gap junction communication between cells. In this report, ubiquilin-4 was described as a protein mainly residing in the ER, where it was proposed to control the turnover of Connexin43, a major component of gap junctions. More specifically, ubiquilin-4 was shown to function both to facilitate the dislocation of Connexin43 from the ER as well as the subsequent targeting of the protein for ERAD-mediated degradation.[110]

3.4. Ubiquitin-Binding Zn Fingers: Providing Variations to the ULD/UBD Paradigm

In the past few years, several additional classes of proteins, in which alternative ULD/UBD combinations are employed, have accompanied the established ULD/UBA and UBX/UBA families. One such protein is the above-described ERAD-linked adaptor protein NPL4. Another example is the ZNF216 protein, in which a ubiquitin-binding A20-type of zinc finger is combined with an AN1-type zinc finger. Despite lacking a classical ULD, ZNF216 nevertheless shows the typical characteristics of a proteasomal shuttling factor (e.g., displays the dual capacity to interact simultaneously with both polyubiquitin chains and the proteasome) but additionally appears to function as a negative regulator of NFκB signaling.[111] A recent study has moreover implicated ZNF216 as well as the entire UPS system in the etiology of muscle dystrophy diseases, given the reported up-regulation of ZNF216 in response to induced muscle atrophy, together with the finding that mice deficient of ZNF216 display a resistance to atrophy.[112] Interestingly, the founder member of the AN-1 family of proteins, first discovered in *Xenopus*,[113] contains a ULD together with an AN1-type zinc finger. It is not clear whether the AN1-type zinc finger has the ability to bind ubiquitin, but if this is the case, the evolutionary conserved AN1 family of proteins may constitute a novel group of ULD/UBD proteins.

Another ULD/UBD-containing protein is p62/Sequestosome-1, a multifunctional protein, which in conjunction with a PB1 and UBA also contains a ZnF-ZZ domain.[114,115] p62 is implicated in the regulation of bone remodelling, inflammation, neurotrophin biology, and obesity[115] but is also recognized for its involvement in the formation of sequestosomes, cytoplasmic compartments where ubiquitinated proteins are stored while waiting for removal by the autophagic machinery.[114,116] Whereas the PB1 and ZnF-ZZ domain mediates interactions with atypical PKCs and the TNFα signaling adaptor RIP, respectively,[115] the UBA domain of p62, analogous to the ULD/UBA proteins, recognizes polyubiquitinated substrates and promotes their delivery to the proteasome (as demonstrated for ubiquitinated tau protein[117]). In fact, in the absence of p62 the targeting of tau to the proteasome is abolished[117] and has in the brain of p62 $-/-$ mice been observed to cause aggregation of K63-polyubiquitinated proteins (including tau),[118,119] resulting in phenotypes including anxiety, depression, and loss of working memory.[118] Formation of insoluble inclusion bodies as well as the behavioral abnormalities displayed by p62$-/-$ mice in many ways resemble the symptoms displayed by AD patients, which is in agreement with the suggested involvement of p62 in neurodegenerative disorders.

NBR1 (next to breast cancer 1/neighbor of BRCA1 gene 1) was recently discovered as a ULD/UBD structural homologue of p62, which together with p62 is recruited to ubiquitin-positive protein aggregates.[120] Both NBR1 and p62 have been reported to bind to the ATG8 family of autophagy-specific UBL proteins (in mammals represented by the LC3 and GABARAP subfamilies), a binding that is mediated via consensus motifs referred to as LIR (LC3-interacting region) or LRS (LC3-recognition sequence) domains.[120−122] Anchored in this dual capacity of simultaneously binding ubiquitin and ATG8 proteins, p62 and NBR1 have recently been suggested to promote the targeting of polyubiquitinated substrates to autophagosomes for lysosomal degradation.[120,122] Indeed, in parallel with the proteasomal and ERAD-linked shuttle factors, it is reasonable to also speculate the existence of autophagy-specific shuttle factors (e.g., p62 and NBR1), particularly in view of the fact that p62 and NBR1 have been shown to deliver ubiquitinated cargo into autophagosomes and are themselves also substrates for autophagy.

3.5. ULD/UBDs: Important Regulators of the Cell Cycle

A dynamic and finely tuned control of cell cycle regulatory proteins by the proteasome is essential for cell proliferation.[123] Indeed, the three yeast ULD/UBA protein families

display partially redundant roles required for progression through mitosis given that the main defect in yeast strains deficient of Rad23, Dsk2, and Ddi1 is a delay in G2/M transition as well as anaphase,[124] a phenotype that when combined with loss of Rpn10 (e.g., *Rad23/Dsk2/Rpn10* LOF) generates a mitotic arrest.[54] Besides these conventional players, other ULD/UBD proteins appear to display more dynamic and specialized functions in the regulation of cell cycle progression. One of these is KPC2 (Kip1 ubiquitination-promoting complex 2), a ULD/UBA protein which in complex with KPC1 forms the ubiquitin ligase KPC,[125] responsible for ubiquitination of the CDK inhibitor $p27^{Kip1}$ at the G0 → G1 transition. The close proximity of the two KPC subunits enables newly ubiquitinated $p27^{Kip1}$ to immediately be recognized by KPC2 and delivered to the proteasome, thereby providing a dynamic and rapid route by which a cell is permitted to leave the G0 resting status and initiate proliferation.[126] The role of ULD/UBA proteins in cell cycle control has been further emphasized by studies in *Xenopus laevis*, where the ubiquilin-1 homologue XDRP1 has been found to be important for the degradation of mitotic cyclins in egg extracts.[127]

3.6. ULD/UBA Proteins: Also Targeting Nedd8 and FAT10

Besides the above-discussed proteins, the ULD/UBD family has one additional member, somewhat hidden behind the scenes. In contrast to the other shuttle factors, Nub1 (Nedd8 ultimate buster-1/negative regulator of ubiquitin-like proteins 1) as well as its splice variant Nub1L does not bind ubiquitin itself but instead interacts with the UBL modifiers Nedd8 and Fat10, thus targeting other types of substrates for proteasomal degradation.[38,128,129] Similar to other ULD/UBAs, Nub-1L utilizes its three UBA domains to recruit Nedd8/Fat10 and its N-terminal ULD to deliver its cargo to the proteasome.[38] However, in contrast to RAD23 and Dsk2/ubiquilin proteins, Nub1 and Nub1L are not protected from proteasomal degradation but are themselves degraded together with their substrates. Interestingly, Nub1L was recently shown to interact with synphilin-1-interacting protein and, similar to other ULD/UBAs, also accumulate in Lewy bodies in patients with Parkinson's disease (PD) and dementia as well as glial cytoplasmic inclusions in multiple system atrophy (MSA).[130]

4. Recycling of the Dogma: SLD/SIM Proteins

The rationale of combining a protein homology domain with the corresponding interacting domain is not unique to ULD/UBD-containing proteins but has been utilized in multiple other configurations, including the intramolecular interactions between poly proline-rich regions and the SH3 domain as well as phoshotyrosine residues and the SH2 domain in the cytoplasmic Src family kinases (SFKs).[131] Within the family of ubiquitin-like modifiers this phenomenon has expanded from ULD/UBDs to also include SUMO-like domains in combination with SUMO-interacting motifs. The discovery of integral SUMO-like domain proteins (SLDs) is relatively novel. As recently as 2006, the first examples of SLDs were reported and classified, forming a protein family denoted as the RENi family (based on the identity of the three first characterized family members, namely, Rad60, Esc2, and NIP45).[132] In parallel to the identification of Rad60 as a SLD protein, another report characterized Rad60 to additionally contain several SUMO-interacting motifs. Together these studies thereby exposed Rad60 as the first SLD/SIM-containing protein. The coexistence of these domains was moreover shown to be important for Rad60 self-association as well as Rad60 activation during the replication stress-response DNA repair,[133] thus indicating a functional significance of an SLD/SIM architecture in Rad60.

5. ULD/UBDs in Disease and Therapeutics

Given the requirement of a properly functioning proteasomal system for removal of abnormally folded and damaged proteins as well as regulatory proteins controlling proliferation, differentiation, and apoptosis, defects in such an important system are an indisputable source of human pathogenesis. As already discussed, ULD/UBA proteins, such as RAD23 and the ubiquilins, are strongly implicated in the etiology of neurodegenerative diseases and accumulate in pathogenic protein aggregates. In particular, ubiquilin-1 and -2 display multiple links to Alzheimer's disease (AD). First, ubiquilin-1 interacts directly with the presenilin proteins, essential subunits of the γ-secretase complex which is responsible for cleavage of amyloid precursor proteins, accumulation of Aβ peptides, and consequently formation of amyloid plaques.[72] Second, ubiquilin-1 has been shown, independently of the γ-secretase complex, to influence the intracellular trafficking and maturation of Aβ;[71] Third, genetic variations in the ubiquilin-1 loci have been proposed to be directly linked to AD.[134] However, this is still an area of controversy, and no specific mutations have yet been characterized.[135] Furthermore, other ubiquilin family members have been associated with different types of neurodegenerative disorders, including the reported involvement of ubiquilin-4 in SCA1 (spinocerebellar ataxia type 1), a disorder caused by a polyglutamine repeat expansion in the ataxin-1 protein.[73]

As there are always two sides to a coin, excessive protein degradation, similar to insufficient degradation, can also trigger human pathogenesis. Indeed, the degradative functions of the entire UPS system are accordingly implicated in the etiology of catabolic syndromes such as muscle atrophy, hyperthyroidism, sepsis, and cachexia in cancer patients.[136] Moreover, in light of the regulatory roles displayed by ULD/UBA proteins during cell-cycle control, these proteins may provide yet another source of carcinogenesis.[116]

Besides the proteasomal shuttle factors, proteins in the ERAD pathway have also been linked to disease. For instance, a single point mutation in p97, R155H, has been identified as an underlying cause of hereditary inclusion body myopathy with Paget disease of bone and frontolobal dementia (IBMPFD). Interestingly, the R155H mutation does not affect the ATPase activity or the typical hexameric configuration of p97. Nevertheless, p97 R155H mutant protein gives rise to impaired protein degradation, causing an abnormal accumulation of ubiquitinated proteins in IBMPFD patients.[137] Structural investigations of p97 in complex with NPL4-UFD1 have mapped R155 to the contact surface between p97 and the NPL4-UFD1 complex, and shown that an R→H mutation at this position completely abolishes binding, thus providing a molecular explanation for the disease.[138] It is interesting to remark that the same binding surface on p97 has been found to mediate the interaction with p47,[139] further emphasizing the importance

Table 1. Nomenclature for Proteins Involved in the UPS/ERAD Pathways (*S. cerevisiae* versus *H. sapiens*)

yeast nomenclature	mammalian nomenclature	function/physiological relevance
UPS system		
DDI1	DDI1	UBL/UBA ubiquitin shuttle receptors, UPS
	DDI2	S-phase checkpoint control (cell cycle) exocytosis
Dsk2	ubiquilin-1 (PLIC-1)	UBL/UBA ubiquitin shuttle receptor, UPS
	ubiquilin-2 (PLIC-2)	aggresome formation
	ubiquilin-3	neurodegenerative diseases
	ubiquilin-4 (UBIN)	regulation of gap junctions
RAD23	RAD23A (HHR23A)	UBL/UBA ubiquitin shuttle receptor, UPS
	RAD23B (HHR23B)	DNA repair (NER)
Rpn10	S5a (PSMD4)	19S proteasomal subunit, regulatory particle, ubiquitin receptor
Rpn13	Rpn13 (ADRM1)	19S proteasomal subunit, regulatory particle, ubiquitin receptor
Ubp6	USP14	deubiquitinating enzyme (DUB)
ERAD pathway		
Cdc48	p97/PSMD2/VPC	AAA-type ATPase, homohexameric, ERAD
Shp-1	p47	UBX/UBA protein, ubiquitin shuttle receptor in ERAD
	UBXN2A	
	UBXN2B/p37	
NPL4	NPL4	ULD/ZF-UBP containing ERAD shuttle factor
UFD1	UFD1	complements NPL4 to form an ERAD-specific ubiquitin shuttle receptor
UBX2	UBXD7	UBX/UBA protein with an additional UIM, ubiquitin shuttle receptor in ERAD
Faf1p	FAF-1/UBXD12	UBX/UBA protein involved in apoptosis
	SAKS1/UBXN1/UBXD10	UBX/UBA protein, involved in protein degradation
other functions		
	TUG (UBXD9, ASPL)	ULD/UBX protein, involved in insulin-induced GLUT4 mobilization to the plasma membrane and in extension glucose uptake
Ufd2	UBE4B	E4 ubiquitin elongation enzyme

of UBX/UBD shuttle factors for the proper targeting of ERAD substrates to the p97 complex.

Since the activity of ULD/UBA family members are important for the regulation of proteins including cell-cycle regulators, oncogenes. and tumor suppressors, targeting these proteins as well as the entire UPS system has become a promising strategy in the development of new cancer therapeutics.[140] One such approach is the application of the proteasomal inhibitor Bortezomib (Velcade and PS-341). Bortezomib is a dipeptide boronic acid analogue which has shown to limit cell proliferation, target the NFκB pathway, trigger ER stress, as well as induce caspase-dependent apoptosis and decrease angiogenic cytokine production in tumor cells.[141] It is already frequently used in relapsing multiple myeloma with good effects,[142] and its utilization in the treatment of other hematologic cancers as well as solid tumors is currently under investigation with promising results.[141] Besides its apparent application in the fight against cancer, Bortezomib and other proteasomal inhibitors have shown positive effects in therapies targeting disorders such as AL amyloidosis,[143] autoimmune and inflammatory diseases, and myocardial infarction.[144] Even though targeting nonenzymatic proteins such as ULD/UBDs is not a trivial assignment, they still comprise a group of highly interesting therapeutic targets. Identification of the small molecules ubistatins, specifically blocking the binding of ubiquitinated proteins to substrate shuttle factors,[145] may give a hint of what is achievable in the future.

6. Conclusions and Future Perspectives

Investigation of ULD/UBD proteins is an intense area of research that in the close future surely will expand and offer many surprises and opportunities. New classes of ULD domains and new combinations of ULD/UBDs are continuously being reported, revealing novel information not only in regard to functionality but also to the mechanisms of intramolecular regulation. In addition there are a multitude of questions concerning the already described ULD/UBDs and, in particular, the extensive cross-talk between them that remain to be addressed. Furthermore, even though ULD/UBA proteins have not been directly linked to cancer they are highly interesting as putative targets for cancer therapy. Targeting these proteins could provide higher flexibility and specificity as well as less unwanted side effects as compared with the currently available UPS-targeting substances that in most cases target the proteasome itself. Identification of the so-called ubistatins, small molecules specifically blocking the binding of ubiquitinated proteins to substrate shuttle factors,[145] is a good starting point on the road toward new strategies in the clinics.

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