Similar but Different: RBR E3 Ligases and their Domains that are Crucial for Function

Abstract

Background: The E3 ubiquitin ligases can be subdivided into four distinct types (RING, HECT, U-box, and RBR type) based on their domain architecture and ubiquitin transfer mechanism. Recent structures of different RBR E3 ligases have been solved showing enzymes in their autoinhibited state. The only exception is HOIP/HOIL-1L which was recently solved in its “active” conformation. This review discusses the structural and functional characteristics of three different members of the RBR E3 ubiquitin ligase family: Parkin, HOIP/HOIL-1L, and HHARI.

Methods: Searches were performed using PubMed. Search term includes “RBR E3 Ligase”, “Parkin”, “HOIP/HOIL-1L”, “HHARI”, “Ubch7”, and “E2”. In the end, 25 journal articles were selected as the foundation of this review. The structural coordinates of Parkin, HOIP, and HHARI were accessed from the PDB (www.rcsb.org) with the PDB IDs 4ZYV, 5EDV, and 4KBL, respectively.

Summary: Currently, most solved RBR E3 ligase structures are only in their inactive forms, except for HOIP/HOIL-1L and these inactive forms provide valuable information on how these proteins are regulated in vivo. All the RBR E3 ligases have common domains, but their structures and functions are heavily dependent on their accessory domains, which serve as regulators that orchestrate certain ubiquitin chain syntheses and play a role in the autoinhibition of RBR E3 ligases. Although these domains are structurally different, they use distinct molecular interactions to achieve the same goal. While the regulation of most RBR E3 ligases has been extensively studied, more structural studies are required to further characterize the mechanism that these enzymes use to build different ubiquitin chains. Understanding the mechanisms underlying the formation of each type of ubiquitin chain could help elucidate their functions and related pathways.

Introduction

Protein turnover is an essential cellular process that allows non-functional or nonessential proteins to be degraded and new ones to be made. (1) One of the most prevalent degradation pathways is the protein ubiquitination pathway. (1,2) Protein ubiquitination is a key post-translational modification involved in the marking of cellular proteins for 26S proteasomal degradation. (1) The ubiquitin degradation pathway occurs via three steps: ubiquitin activation, conjugation, and the actual transfer of ubiquitin to the substrate. (1) The ubiquitin is activated by an E1 activation enzyme (E1) in an ATP-dependent reaction. (3) Ubiquitin is then transferred to an E2 conjugating enzyme (E2). (3) The E2 enzyme catalyzes the transfer of ubiquitin to either the E3 ligase (E3) or the substrate, depending on the type of E3 ligases used in the reaction. (4) These enzymes, particularly the RBR E3 ligases, accomplish the transfer of ubiquitin through the formation of thioester bonds. During the last step of the ubiquitination pathway, a stable isopeptide bond is formed between the substrate and the C-terminus of ubiquitin, as shown in Fig. 1. (3)

Ubiquitination can also occur multiple times on the same substrate, generating multiple ubiquitination sites. (1) Ubiquitination usually occurs on a lysine residue of the substrate, but can also occur on the N-terminus or more rarely on serine and threonine residues. Ubiquitination can also happen to ubiquitin to generate ubiquitin chains. (1) Different types of polyubiquitination chains lead to different signalling pathways, but often lead to protein degradation (Fig. 2). (1) For example, linear chains, which are generated by the HOIP/HOIL-1L complex or Linear Ubiquitin Assembly Complex (LUBAC), signal for the NF-kB pathway during an immune response. (1) In addition, K63 polyubiquitination often signals for DNA repair and lysosomal degradation, (1) whereas the K48 polyubiquitination signals for proteasomal or lysosomal degradation. (1, 5)

Since the human proteome is quite large, it is reasonable for there to be many kinds of E3 ligases to accommodate the different substrates. Four different categories of E3s have been characterized based on their domains and ubiquitin transfer mechanism. (7) The first type is the RING type ligase, which allows the direct transfer of ubiquitin from the E2 enzyme to the substrate. (7) The second type of E3 is the HECT type ligase, in which the ubiquitin is first transferred to a cysteine in the E3 ligase and then to the substrate. (7) The third type of ligase is the U-box ligases, in which the transfer mechanism resembles RING type ligases using their U-Box domain.
Structural Characterisitics of the RBR E3 Ligase Family

The RBR E3 ubiquitin ligases have three characteristic Zn2+ binding domains known as the RING1, in-between RING (IBR), and RING2 domains (Fig 3). In all of these domains, the coordination with Zn2+ ions occurs through seven cysteines and one histidine. (10) The RING1 domain is structurally similar and exhibits the same functional role for binding an E2 enzyme as typically observed for the RING domains of RING-type E3 ligases. (10) The RING1 domain, which is highly conserved among the RBR E3 ligases in sequence and structure, is important for the binding of an E2 enzyme. (10) For example, the RING1 domain displays the characteristic cross-brace motif structure observed in RING ligases (Fig 4). (10) The RING2 domain in the RBR ligases, despite bearing the same name, is not a true RING domain. Indeed, it is not able to bind to E2 conjugating enzymes and has a different structure. (10) In the RBR E3 ligases, the transfer of ubiquitin is done between the E2 enzyme and the RING2 domain through trans-thiolation (Fig 1) (3) The role of the IBR domain remains unclear, but the IBR domain is required for function and displays flexibility in known structures. (10, 12)

What sets these RBR E3 ligases apart?

In addition to their three core domains, each RBR E3 ligase has other different domains that have similar functions. Parkin activity is modulated by its Ubl (ubiquitin-like) domain, a unique RING0 domain, and the Repressed Element of Parkin (REP) linker. Indeed, the Ubl domain and the REP linker of Parkin affect its binding to E2. (10) The Ubl domain is crucial for Parkin as it contains a site for phosphorylation by PINK1, a kinase localized at the mitochondria, during the mitophagy pathway. (17) PINK1 phosphorylation of Parkin is required for the activation of Parkin ubiquitination activity when mitochondria are depolarized. (17, 18) Parkin will then ubiquitinate mitochondrial protein to promote mitophagy. (17) In addition, Parkin’s unique RING0 domain contributes to the maintenance of the Parkin autoinhibited conformation in the context of healthy mitochondria. (19)

HOIP and HOIL-1L are other types of RBR E3 ligases. Unlike other RBR E3 ligases, they both ligases form a complex to carry out polyubiquitini-
How do cells regulate the ubiquitination activity of Parkin, A?

Although all the RBR E3 ligases have the three core domains, they are regulated very differently. Generally, the E3 ligases are autoinhibited due to the other domains within the ligase. (23) Different RBR E3 ligases have been found to inhibit themselves via various mechanisms involving different accessory domains. While these accessory domains may have similar functions, they have different structures as in the case with the of Parkin and the Ariadne Family.

The autoinhibition of Parkin

Parkin has two structural features, the Ubl domain and REP linker, which regulate its activity by modulating the access of its E2 binding site. It has been shown that the Ubl domain interacts with the RING1 domain, acting as a switch between inactive and active Parkin. (26, 27) Lysine 48 (K48) is required for the Ubl to interact with Parkin, with any mutations to K48 resulting in the loss of Ubl-dependent autoinhibition. (10). Furthermore, binding of phospho-ubiquitin and the phosphorylated Ubl domain induce a structural change in Parkin which increases Parkin activity. (12, 27-29) The phosphorylation of Parkin on serine 65 (S65) is performed by PINK1. (17) Phosphorylation of the Ubl domain decreases its affinity to RING1 which results in the activation of the protein. (10, 17)

In addition to the Ubl domain, the REP linker has been shown to occlude the E2 binding site. However, after the phosphorylation of the Ubl domain, a change in its conformation makes the E2 binding site accessible. (26) In the REP linker, tryptophan 403 (W403) is involved in the binding of RING1 domain. Mutations on W403 have been associated with higher Parkin activity because the hydrophobic residue fits in the RING1 domain. (10) Despite the crucial tryptophan residue, this hydrophobic residue, is crucial for the interaction between the REP linker and the RING1 domain, not all hydrophobic residues have the same effect. (10) A mutation to alanine, also a hydrophobic residue, has been shown to increase Parkin’s activity, possibly because alanine is not long enough to bind to the groove that would otherwise be occupied by tryptophan. (10) Aside from the E2 binding site, Parkin has another regulation site located at the RING2 -RING0 interface. (10) The active cysteine (C431) required for the thiol-transfer is buried between the hydrophobic interfaces of the RING2 and RING0 domain. (10) Mutations in the hydrophobic residues involved in the interaction between RING2 and RING0, such as phenylalanine 146, have been shown to increase Parkin’s activity. (10) Without this hydrophobic interaction, the RING2 domain can easily dissociate from the RING0 domain, thus exposing the active cysteine to the surrounding environment.

HOIP/HOIL-1L regulation mechanism

Similar to Parkin, an interaction between the third ubiquitin binding region (UBR3) and ubiquitin is important for HOIP’s activity. (9) However, the difference between Parkin and HOIP is that Parkin is activated upon the binding by phosphorylated ubiquitin, whereas HOIP is activated by non-phosphorylated ubiquitin. (9) In HOIP, the autoinhibitory function is carried out by its UBA domain, however, upon interaction with HOIL-1L, HOIP becomes activated. (9) Furthermore, linear di-ubiquitin could also remove HOIP-UBA autoinhibition. (9) It has been shown that residues such as ile807 and Glu809 in the Ubiquitin-Binding Region 3 (UBR3) of HOIP are important in order to bind to ubiquitin or ubiquitin chains. (9) These interactions between the UBR3 and ubiquitin allows HOIP/HOIL-1L to form linear ubiquitin chains. (9)

Ariadne family is also autoinhibited like Parkin

Similar to Parkin, the Ariadne family also has accessory domains that aid in its autoinhibition. These domains are structurally different as Ariadne domains exist as four alpha helices in HHARI, while Parkin’s RING0 contains two beta sheets and one alpha helix, as shown in Fig 5. (10, 11) The Ariadne domain behaves functionally like the RING0 domain in Parkin; however, the way that these interactions occur is completely different. The Ariadne domain interacts with its RING2 domain via hydrogen bonds. (11) Consequently, the active cysteine in HHARI (C357) is occluded from interacting with the E2-bound ubiquitin. Not only is the Ariadne domain occluding the RING2 active site, but it also intercalates between the IBR and RING0 domain. (11) The intercalation between IBR and the RING2 domain separates the active site from the RING1-bound E2. (11)

The RING2 domain of HHARI contains 14% aromatic residues. (30) Trends between HHARI and other RBR E3 ligases were compared, and a sequence alignment suggests that the tryptophan and histidine observed in both HHARI and Parkin might play a role in maintaining their protein fold. (30, 31) In Parkin, these aromatics, such as His 433, are involved in the transfer of ubiquitin to its target. (10) Therefore, it is possible that these aromatics on HHARI also play an important role in the transfer of ubiquitin.

Types of ubiquitin chains formed by RBR E3 ligases

The ubiquitination signaling pathway is vital in the elimination of misfolded or unwanted proteins. (1) The only way for these different chains to form is through E3 ligases. These chains could be linear, K48, K63, or branched polyubiquitin. (1) Ubiquitin has seven lysine residues and the amino group of its N-terminus can be used to form isopeptide bonds. It is suggested that the type of E2 utilized will affect the types of chains that are made. However, in some cases, the rules might not be as rigid.

It has been previously suggested that Parkin is able to form branched ubiquitin chains such as the formation of Lys 63, Lys 48, and Lys 27 branched polyubiquitin chains. (32) Recently, it was discovered that Parkin has a preference to form Lys 6 chains, although its signalling pathway is poorly understood. (32) Structurally, the crucial residue that has been implicated to aid the transfer of ubiquitin is the histidine residue that is two residues away from the catalytic cysteine. (10) Although the preference for a certain type of chain has been discovered, the mechanisms in which they are transferred are still unclear. It should be expected that the structural difference between these RBR E3 ligases will contribute more to the formation of different ubiquitin chains. Since these E3s use different E2s, it is most likely that the structural difference between these E2s and the structural difference between the E3s contributes to the different chain formations.

In contrast to other RBR ligases, HOIP/HOIL-1L are complexed in the LUBAC (linear ubiquitin chain assembly complex) pathway. (15) It has been shown that HOIL-1L contains a UBL domain, however, in contrast to Parkin, this UBL domain does not participate in the autoinhibitory mechanism, but is used to activate the HOIP once in complex with HOIL-1L and SHARPIN. Furthermore, both HOIL-1L and SHARPIN have the
NZF domain that coordinates one zinc ion with four conserved cysteine residues. (21) These NZF domains facilitate the binding to ubiquitin via a conserved TF/Ω motif. (21) Furthermore, HOIL-1L has been shown to bind linear chains via hydrophobic interaction between the NZF domains and certain ubiquitin residues. (21) It has been shown that Phe4 and Ile44 have been shown to interact with the NZF core and its tail. (21) It is possible that the NZF domain of HOIL-1L or SHARPIN is used to stabilize the di-ubiquitin that could facilitate the linear conjugation of further linear polymers. (21) Although both proteins have an NZF domain, the NZF domain of SHARPIN is also involved in programmed cell death. (21) Although the mechanism for the formation of different ubiquitin chains is not clear, using the model of the HOIP/OHIL-1L system might shed some light onto the structural variance that can give rise to different types of chains made by the LUBAC complex.

Conclusion

Due to the recentness of its discovery, a lot of ambiguity still surrounds the Hect-type mechanism used by RBR ED ligases. Although all RBR E3 ligases have common domains, their functions are heavily dependent on the accessory domains. In the case of HOIP/OHIL-1L, the NZF domain from HOIL-1L serves as an aid to form linear ubiquitin chains. For the Parkin and Ariadne family, there are accessory domains dedicated to autoinhibition. Their structural differences provide different interactions between these accessory domains and the RING2 domain. In contrast to the Ariadne family and Parkin, the HOIP/OHIL-1L system activation requires another RBR E3 ligase (HOIL-1L). Since the regulation of most RBR E3 ligases has been extensively studied, more structural studies are required to characterize the mechanism in which different ubiquitin chains are formed. Understanding the mechanisms underlying the formation of each type of ubiquitin chain could help to elucidate their functions and related signaling pathways.

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References
