

Invited Mini Review

Structure biology of selective autophagy receptors

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Autophagy is a process tightly regulated by various autophagy-related proteins. It is generally classified into non-selective and selective autophagy. Whereas non-selective autophagy is triggered when the cell is under starvation, selective autophagy is involved in eliminating dysfunctional organelles, misfolded and/or ubiquitylated proteins, and intracellular pathogens. These components are recognized by autophagy receptors and delivered to phagophores. Several selective autophagy receptors have been identified and characterized. They usually have some common domains, such as LC3-interacting-region (LIR) motif, a specific cargo interacting (ubiquitin-dependent or ubiquitin-independent) domain. Recently, structural data of these autophagy receptors has been described, which provides an insight of their function in the selective autophagic process. In this review, we summarize the most up-to-date findings about the structure-function of autophagy receptors that regulates selective autophagy. [BMB Reports 2016; 49(2): 73-80]

INTRODUCTION

The term 'autophagy' comes from the Greek words 'auto' and 'phagy' which means 'self' and 'eating', respectively. The name was coined by Dr. Christian de Duve for the first time in 1963 (1, 2). For several decades, there were few publications on autophagy. However, the identification of autophagy-related genes (*atg*) in yeast by Dr. Oshumi's group in the late 1990s invoked an interest in this field (3). The core machinery involved in the major steps of autophagy, such as autophagy initiation, autophagosome elongation and maturation, and lysosomal fusion, have been extensively studied for the last 10 years (2, 4-16). Initially, autophagy was thought to be the non-selective and bulk degradation process, which is a main pathway induced in response to starvation. However, it is obvious that the elimination of pathogens and the removal of

damaged organelles or proteins, are highly selective processes that require specific cargo recognition by the autophagy machinery (17-20). Therefore, autophagy can be divided into two groups: non-selective and selective autophagy. Some specific proteins, known as autophagy receptors, recognize such target cargos and move them to the autophagosomal membrane. The selective autophagy is further divided into several sub-groups depending on its cargos: degradation of aggregated proteins (aggrephagy) (21-24), mitochondria (mitophagy) (25-32), peroxisomes (pexophagy) (33-35), ribosomes (ribophagy) (36), endoplasmic reticulum (ERphagy) (37-39), lipid (lipophagy) (40), nuclear envelope (nucleophagy) (39), and pathogens (xenophagy) (41-44). Different autophagy receptors are involved in the recognition steps of particular cargos and thus, structural studies on autophagy receptors are necessary for understanding their specificities. In this review, we will introduce the domain architecture of several autophagy receptors and structural information of the domains. In addition, the structural basis for the interaction between these domains and its interacting partners will also be reviewed.

DOMAIN ARCHITECTURE OF AUTOPHAGY RECEPTORS

Autophagy receptors are identified by the yeast two-hybrid system and proteomic approaches in mammalian cells (22, 45). The five well-known autophagy receptors, SQSTM1, NBR1, NDP52, OPTN, and NIX, are introduced in this review (Fig. 1 and Table 1). Most of them harbor both LC3-interacting region (LIR) and ubiquitin-binding domain (UBD) (46, 47). The LIR motifs interact with ubiquitin-like proteins like LC3s or GABARAPs in autophagy, and UBD (diverse ubiquitin binding domains in each receptors) with the ubiquitin attached to cargos (19, 48).

The first identified selective autophagy receptor is SQSTM1 (sequestosome 1; also known as p62) (49). SQSTM1 is not only known to act as a scaffold protein in NF- κ B pathway, but also interacts to ubiquitin-containing proteins related to protein aggregation diseases (50-52). It has 5 domains: Phox and Bem1p domain (PB1), ZZ-type zinc finger domain (ZZ), LIR, Keap1-interacting region (KIR), and ubiquitin-associated (UBA) domain (53, 54).

NBR1 (Neighbor of BRCA1 gene 1) was cloned as a candidate gene for the ovarian cancer antigen originally (55). Quite similar to SQSTM1, it also contains PB1, ZZ, LIR, and UBA

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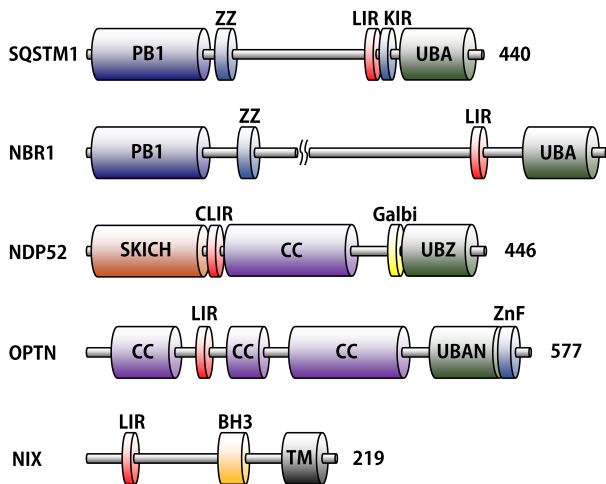


Fig. 1. Domain structures of autophagy receptor proteins. LC3-interacting motifs (LIR) and ubiquitin binding domains (UBA, UBZ, and UBAN) are distinguished by red and green color, respectively. The other unique domains represent different colors. The following abbreviations are used for each domain: PB1, Phox and Bem1p; ZZ, ZZ-type zinc finger; LIR, LC3-interacting region; KIR, Keap1-interacting region; UBA, ubiquitin-associated; SKICH, skeletal muscle and kidney-enriched inositol phosphatase carboxyl homology; CLIR, non-canonical LIR; CC, coiled-coil; Galbi, galectin-8 binding region; UBZ, ubiquitin-binding zinc finger; UBAN, ubiquitin-binding in ABIN and NEMO; ZnF, Zn-finger; BH3, Bcl-2 homology region 3; TM, transmembrane.

(Fig. 1). Due to a PB1 domain, NBR1 oligomerizes itself or with SQSTM1, and acts independently or cooperatively to degrade the cargos (56).

NDP52 (also known as CALCOCO2), expressed in all human tissues and cells, was originally found in nuclear promyelocytic leukemia bodies (57). It comprises an N-terminal skeletal muscle and kidney-enriched inositol phosphatase carboxyl homology (SKICH) domain, non-canonical LIR (CLIR) motif that can interact with the LC3C specifically, coiled-coil (CC) domain, galectin-8 binding region (Galbi), and ubiquitin-binding zinc finger (UBZ) domain (58-60). It is especially involved in xenophagy for clearing pathogens such as *Salmonella*, *Listeria*, *Shigella*, and *Mycobacterium* (42, 61, 62).

OPTN (optineurin) is the most recently identified autophagy receptor. It has LIR motif, MYO6 binding region, CC, ubiquitin-binding in ABIN and NEMO (UBAN) domain, and Zn-finger (ZnF) domain (23, 63-66). Interestingly, many other receptors have common LIR motif, but OPTN has a unique serine residue at the upstream LIR sequence, which is modified to phosphoserine by Ser/Thr TANK-binding kinase 1 (TBK1) (43).

NIX (also known as BNIP3L) protein, one of BH3-only members of the Bcl-2 family, has LIR motif, BH3 domain, and a transmembrane region (67, 68). As the sole mitophagy receptor, it has only an LIR motif, but no ubiquitin interacting domain (Fig. 1). Table 1 summarizes the function and structural information of each domain in different autophagy receptors.

Table 1. Summary of the determined structures of autophagy receptors

Name	Domain/motif	Functions	PDB IDs	References
SQSTM1	PB1	Oligomerization and binding to PKC ζ	2KKC, 4UF8, 4UF9, 2KTR, 4MJS,	(82-84, 96)
	ZZ	Interaction with RIPK1 and R-Bip	-	(86)
	LIR	LC3 binding	2K6Q, 2ZJD	(71)
	KIR	Keap1 binding	3ADE, 3WDZ	(53, 90)
	UBA	Ubiquitin binding	1Q02, 2RRU, 3B0F, 2K0B, 2KNV, 2JY7	(76, 78, 97, 98)
NBR1	PB1	Oligomerization	1WJ6, 2BKF, 2G4S	(85, 99)
	ZZ	Unknown	-	-
	LIR	LC3 binding	2L8J	(70)
NDP52	UBA	Ubiquitin binding	2CP8, 2MJ5, 2MGW	(79)
	SKICH	Unknown	3VVV	(58)
	CLIR	LC3C binding	3VWW	(58)
	Coiled-Coil	Homo-dimerization	-	(60)
	Galbi	Galectin-8 binding	4GXL, 4HAN	(60, 93)
OPTN	UBZ	Ubiquitin binding	2MXP, 4XKL	(59)
	Coiled-Coil	Oligomerization and binding to protein aggregates	-	(23)
	LIR	LC3 binding	2LUE, 3VTV, 3VTW	(73)
	UBAN	Ubiquitin binding	-	(66)
	ZnF	Unknown	2LO4	-
NIX	LIR	GABARAP binding	4WAA	(28)
	BH3	Cell death and autophagy pathway	-	(67)
	TM	Membrane insertion	-	-

STRUCTURAL BASIS FOR THE RECOGNITION OF LIR-MOTIF

There are 6 mammalian homologs of Atg8 comprising the microtubule-associated protein (MAP) light chain 3 (LC3) and γ -aminobutyric acid (GABA)-receptor-associated proteins (GABARAP). Therefore, mammalian LIR motif is similar to AIM (Atg8-interacting motif) in yeast (69). These proteins are involved in the elongation and maturation of autophagosome, respectively (70). All autophagy receptors possess the LIR motif for interacting with LC3 (Fig. 1 and Table 1). A general definition of the LIR sequence is Ω -x-x- Ψ , where Ω and Ψ are aromatic and hydrophobic residues respectively, and the two residues in between are any other amino acids. Preferentially, one or more acidic residues are needed before the LIR sequence (69).

Thus far, detailed structural information of several LC3-LIR complexes has been reported. The first complex structure in mammals is LC3B-SQSTM1 LIR peptide [PDB ID: 2ZJD] (Fig. 2A) (71). This complex shows how canonical LIR motif (W-x-x-L) binds to LC3. The N-terminal aromatic tryptophan residue (Ω) of LIR sequence plugs into the hydrophobic pockets and the downstream hydrophobic leucine residue (Ψ) binds to another hydrophobic pocket of LC3B. The acidic residues prior to LIR sequence bind to the basic side chains of LC3B (Fig. 2A) (21, 71). Structure of NIX LIR-fused LC3B [PDB ID: 4WAA] is quite similar to that of LC3B-SQSTM1 LIR (Fig. 2B). The LIR sequence of NBR1 is 'Y-I-I-I'. The structure of GABARAPL1-NBR1 LIR peptide [PDB ID: 2L8J] shows that all four residues in the peptide interact with GABARAPL1, and the tyrosine and isoleucine residues are in the same Ω -x-x- Ψ

manner as the LC3B-SQSTM1 LIR (Fig. 2C) (72).

Deviated LIR sequences have also been identified (43, 58). The NDP52 and OPTN of xenophagy receptors, especially those related to *Salmonella*, have unique and non-canonical LIR motif. The LIR sequence of NDP52 comprises the 'I-L-V-V' and lacks the aromatic residue. The crystal structure of LC3C-NDP52 complex [PDB ID: 3VVW] revealed that the non-canonical LIR (termed CLIR) binds to the β 2 strand of LC3C, but does not reach the hydrophobic pocket that normally binds the tryptophan in a canonical LIR (Fig 2D). The Asp132 residue of NDP52 outside CLIR interacts with Lys57 of LC3C (58).

Another xenophagy receptor protein, OPTN, is activated by phosphorylation. The LIR sequence of OPTN is 'F-V-E-I' which is a canonical LIR motif, but the binding affinity of LC3B-OPTN LIR is only 64.5-40.0 μ M, which is lower than that of LC3B-OPTN phospho-LIR (4 μ M). The NMR structure of LC3B-OPTN phospho-LIR [PDB ID: 2LUE] revealed that phospho-Ser177 upstream of the LIR motif in OPTN tightly binds to Arg11 and Lys51 of LC3B (Fig. 2E) (43, 73). These results clearly show that the acidic residue or phosphorylation site upstream of the LIR region also needs to be taken into account.

UBIQUITIN BINDING DOMAIN OF AUTOPHAGY RECEPTORS

According to recent papers, modification of targets with ubiquitin relates not only to proteasome degradation systems, but also degradation signals in selective autophagy (19, 74). When invaded pathogens in cytosol are decorated by a poly-ubiquitin, like K48- or K63-linked chain, they are recognized by

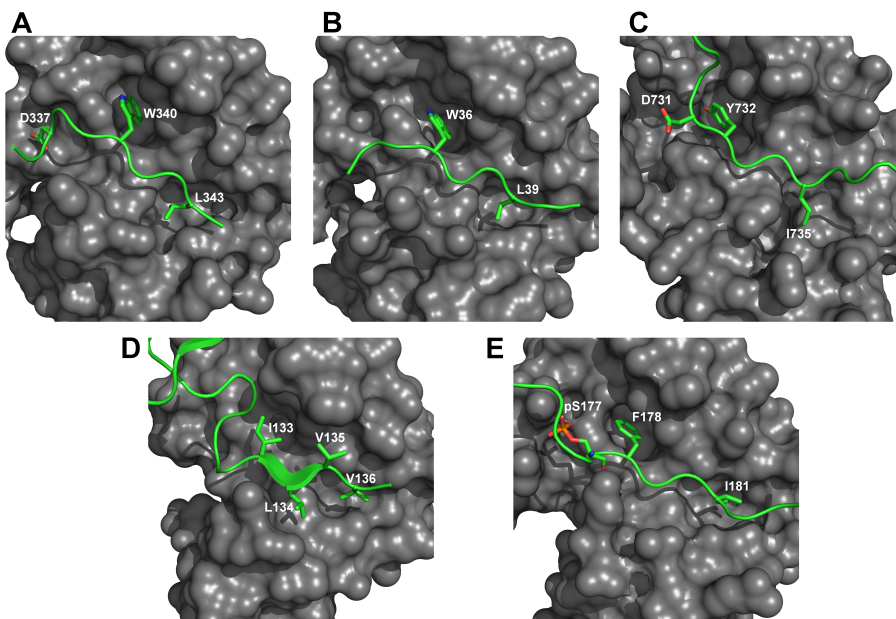


Fig. 2. Structures of LC3-LIR complex. (A) LC3B/SQSTM1-LIR peptide complex [2ZJD], (B) NIX-LIR fused LC3B [4WAA], (C) GABARAPL1/NBR1-LIR peptide complex [2L8J], (D) LC3C/NDP52 SKICH-LIR region complex [3VVW], and (E) LC3B/OPTN-phosphoLIR peptide complex [2LUE]. Surface of LC3 or GABARAP protein is shown in dark gray color, and the ribbon of LIR motifs in green. The side chain of LC3 interacting residues is also shown in stick model, colored green, red, blue, and orange for carbon, oxygen, nitrogen, and phosphorus, respectively.

the UBD of autophagy receptors and removed to phagophores (61, 75). SQSTM1/p62, NBR1, NDP52, and OPTN each have unique UBD (Table 1). Three UBD structures of SQSTM1, NBR1 and NDP52 are currently elucidated, and the details are as follows.

The solution and crystal structure of UBA domain of SQSTM1 were determined in 2003 and 2011, respectively (Fig. 3A) (76, 77). Although the complex structure between SQSTM1 UBA and ubiquitin is still unknown, conformational changes such as $\alpha 3$ helix extension and side chain flipping at Gln434 and Tyr435 upon ubiquitin binding are revealed through NMR titration (77). Interestingly, SQSTM1 UBA domain prefers to bind K48- or K63-linked di-ubiquitin rather than mono-ubiquitin (78). The structure of NBR1 UBA domain is very similar to that of UBA domain of SQSTM1 (Fig. 3B). Although NBR1 UBA domain also consists of three helices, the main difference is in the position of helix-3, which encloses a larger angle with helix-2 (16° difference) and is extended by approximately one turn (4 residues) in NBR1 as compared with SQSTM1 (79). The alanine mutation of Phe929 and Leu954 could completely abolish ubiquitin interaction. Substitution of Glu926 for alanine decreases the affinity to ubiquitin about 3-fold, thereby highlighting the contribution of the electrostatic interaction with ubiquitin. The overall structures of UBA domain of SQSTM1-Ub and NBR1-Ub complex resemble the well-known canonical UBA domain of DSK2 and ubiquitin complex, in which the UBA helices adopt a similar geometry, and interfacial residues are involved in forming similar hydrophobic contacts (80).

The structures of NDP52 UBZ domain with or without ubiquitin [PDB IDs: 2MXP and 4XKL] have recently been determined (Fig. 3C) (59). The UBZ domain has two zinc-finger domains, a dynamic unconventional zinc finger (ZF1) and a canonical C_2H_2 -type zinc finger (ZF2). Surprisingly, only one of two zinc-fingers, ZF2, can interact with 3 types of poly-ubiq-

uitin chains (K48- and K63-linked as well as M1 (methionine 1)-linked) (59).

UBIQUITIN-INDEPENDENT INTERACTION DOMAINS OF AUTOPHAGY RECEPTORS

PB1 domain is a scaffold module that interacts with each other in a front-to-back mode to arrange heterodimers or homo-oligomers. PB1-containing proteins display the topology of an ubiquitin-like β -grasp fold, including six-stranded β -sheets and two α -helices (Fig. 4A) (81). The folding pattern of PB1 domain of SQSTM1 is quite similar with that of other PB1-containing proteins. The p62 dimer or oligomers are formed by the electrostatic interaction of two opposite charged surface patches with conserved acidic and basic residues (Fig. 4A) (82). The PB1 (residues 1-122) domain of SQSTM1 assembles to form flexible helical polymers (83). On interacting with K63-linked octa-ubiquitin chain (but not with mono- and di-ubiquitin, or LC3), the SQSTM1 helical filament could dissociate by induced conformational changes (83, 84). The structure of the PB1 domain of NBR1 is also similar and reminiscent of those from previously characterized PB1 domains, including SQSTM1 (85).

ZZ-type zinc finger (ZZ domain, residue 122-167) is a mysterious domain in SQSTM1 (Fig. 1 and Table 1). The ZZ domain is related to NF- κ B signaling and glucose regulation. This domain could associate with RIPK1. Recently, some studies reveal that the ZZ domain of SQSTM1 could link autophagy to N-end rule pathway (86, 87). However, the mechanism between SQSTM1 and N-end rule is still unclear due to lack of structural information of ZZ domain.

TRAF6 binding (TB) domain of SQSTM1 interacts with TRAF6, an E3 ubiquitin ligase that regulates mTORC1 activity by K63-ubiquitination in a SQSTM1-interaction dependent manner. Through this TB domain, SQSTM1 could function as

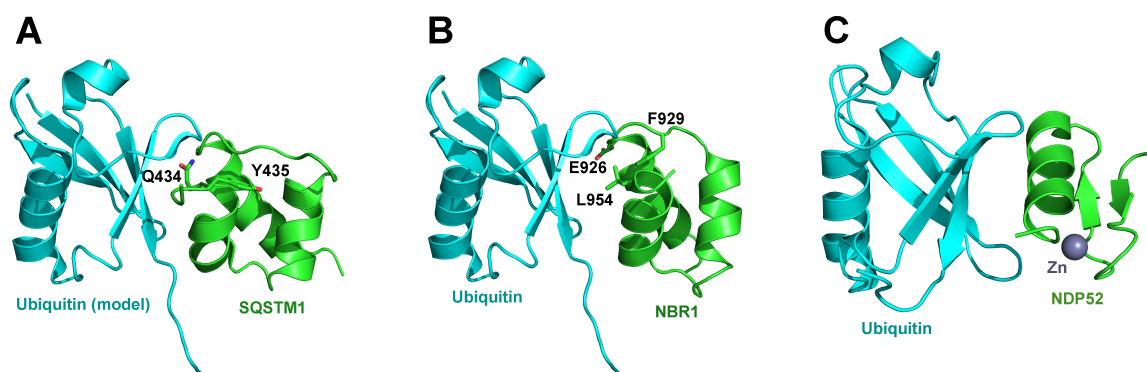


Fig. 3. Overall structures of ubiquitin binding domain (UBD) and ubiquitin complex. (A) Ubiquitin (model) with SQSTM1 UBA domain [1Q02] and (B) Ubiquitin with NBR1 UBA domain [2M]5 are shown in ribbon. The ubiquitin interacting Gln434 and Tyr435 of SQSTM1 UBA, and Glu926, Phe929, and Leu954 of NBR1 UBA are shown in stick model. (C) Ubiquitin with NDP52 ZF2 domain [4XKL]. A bound zinc atom is shown as a gray ball. UBD and ubiquitin are colored green and cyan, respectively.

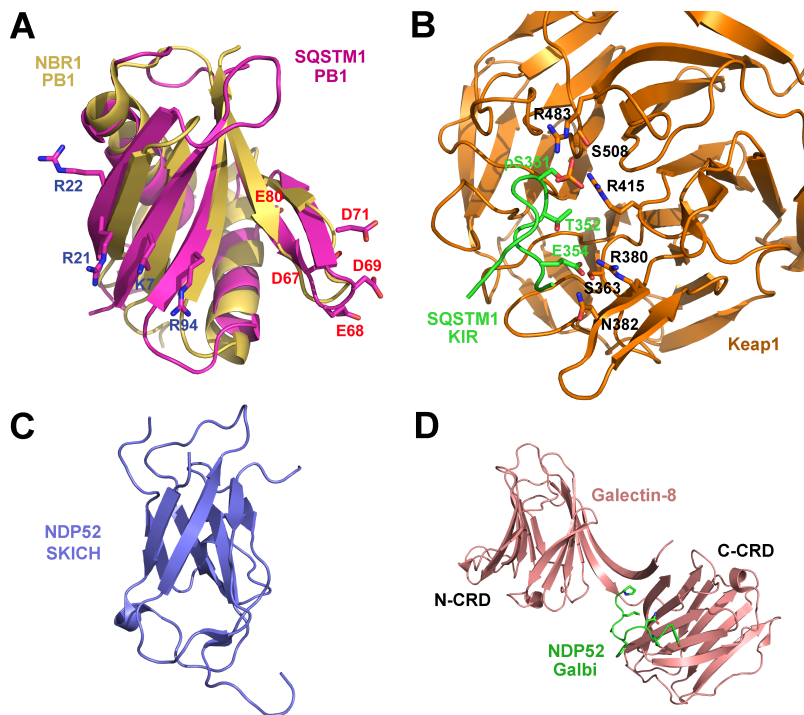


Fig. 4. Structures of ubiquitin-independent binding domains in autophagy receptors. (A) PB1 domains of SQSTM1 (magenta) and NBR1 (yellow) are superposed. Basic side chains of Lys7, Arg21, Arg22, and Arg94 residues and acidic side chains of Asp67, Glu68, Asp69, Asp71, and Glu80 residues involved in the oligomerization are shown in stick model. (B) Complex of Keap1 (orange) and SQSTM1-KIR peptide (green). The β -propeller structure of Keap1 recognizes KIR peptide containing phosphorylated Ser351. (C) NDP52 SKICH domain (slate) [3VVV], and (D) Complex between galectin-8 (salmon) and NDP52 Galbi peptide (green) [4HAN]. N-terminal carbohydrate binding domain (N-CRD) and C-terminal carbohydrate binding domain (C-CRD) are labeled. Ribbon diagram of overall structure and stick model of bound peptides are shown.

a crucial mediator for mTORC1 activity regulation (88).

Keap1 interacting region (KIR) is the most well studied domain of SQSTM1. KEAP1-NRF2 system is a response for oxidative stress (89). Under normal conditions, KEAP1 is dimerized and tightly interacts with NRF2. However, stress conditions induce the phosphorylation of S351 in KIR by mTORC1, and this causes the dissociation of KEAP1-NRF2 complex, leading to the formation of the KEAP1-SQSTM1 complex. The phosphorylated SQSTM1 KIR is recognized by β -propeller structure of Keap1 (Fig. 4B). In comparison to the non-phosphorylated state, two additional Keap1 residues (Arg483 and Ser508) were involved in the binding interaction with phosphorylated KIR (90). This phosphorylation of Ser351 in KIR is important for the antioxidant pathway as well as SQSTM1 mediated autophagy.

The SKICH domain of NDP52, which is responsible for the interaction with AZI2/NAP1 and its crystal structure, has been determined at 1.35 Å (Fig. 4C) (58). It shares the immunoglobulin-fold, and its closest structural homolog is the human C3 complement component (42, 91). Recently, the SKICH domain is reported to interact with phosphorylated tau and this suggests that NDP52 plays a role in autophagy-mediated degradation of phosphorylated tau (92).

NDP52 also interacts with galectin-8, a sugar receptor in the region between the CC and UBZ domain (Fig. 1) (44). The galectin-8 has two homologous carbohydrate-recognition domains (N- and C-CRD) linked by a single polypeptide chain. It serves as a danger receptor because it recruits NDP52 to the ruptured *Salmonella*-containing vesicles by recognizing carbo-

hydrate moiety. The complex structures of galectin-8 and NDP52 peptide (Galbi region, residues 372-380) were determined by two independent groups (60, 93). Only C-CRD of galectin-8, through an opposing site of sugar recognition, directly binds to the Galbi region of NDP52 (Fig. 4D).

CONCLUDING REMARKS

Selective autophagy has attracted a great interest for understanding the autophagic process and the subsequent cellular homeostasis. Different autophagy receptors play a critical role for cargo selection using particular domains or motifs (Table 1). For clear understanding of their selectivity, the structural data of the complex between these recognition domains and target molecules are inevitable. Although many structures of the domain of autophagy receptors are now available, structural information of some domains is still unknown (Table 1). More importantly, it is critical to have the structures of full-length protein but not a domain structure, because the cooperative action of individual domains occurs for efficient process of selective autophagy. So far, there is no structural information of full-length autophagy receptors at high resolution and it would be necessary to tackle this problem using various other techniques, especially advanced cryo-electron microscopy (94), or using combined methods such as small-angle X-ray scattering with a high resolution structure of each domain (95). These structural works in combination with cell biology, and *in vivo* data will provide the groundwork for understanding

the molecular mechanism of each autophagy receptors.

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