5-5-2012

Selectivity of the Ubiquitin-Binding Modules

Simin Rahighi
Chapman University, rahighi@chapman.edu

Ivan Dikic
Goethe University

Recommended Citation
Simin Rahighi, Ivan Dikic (2012). Selectivity of the ubiquitin-binding modules. FEBS Lett. 586, 2705-2710. doi: 10.1016/j.febslet.2012.04.053

Selectivity of the Ubiquitin-Binding Modules

FEBS Letters 586 (2012) 2705–2710

journal homepage: www.FEBSLetters.org

Review

Selectivity of the ubiquitin-binding modules

Simin Rahighi, Ivan Dikic *

Buchmann Institute for Molecular Life Sciences and Institute of Biochemistry II, Goethe University School of Medicine, Theodor-Stern-Kai 7, 60590 Frankfurt am Main, Germany

ARTICLE INFO

Article history:
Received 29 March 2012
Revised 26 April 2012
Accepted 26 April 2012
Available online 5 May 2012

Edited by Marius Sudol, Gianni Cesareni, Giulio Superti-Furga and Wilhelm Just

Keywords:
Ubiquitin-binding module
Ubiquitin signaling
Ubiquitylation

ABSTRACT

Ubiquitin-binding modules are constituents of cellular proteins that mediate the effects of ubiquitylation by making transient, non-covalent interactions with ubiquitin molecules. While some ubiquitin-binding modules bind single ubiquitin moieties, others are selective for specific ubiquitin chains of different linkage types and lengths. In recent years, functions of ubiquitin chains that are polymerized through their Lys or N-terminal Met (i.e. linear chains) residues have been linked to a variety of cellular processes. Selectivity of ubiquitin-binding modules for different ubiquitin chain types appears as a key to the distinct regulatory consequences during protein quality control pathways, receptor endocytosis, gene transcription, signaling via the NF-κB pathway, and autophagy.

1. Introduction

Although, ubiquitin was initially identified in 1978 to be required for the breakdown of proteins in the proteasome, it is now widely accepted that this 76-amino-acid residue protein is implicated in a myriad of biological pathways [1]. Ubiquitin serves as a post-translational modifier and is involved in the regulation of a vast array of cellular processes such as cell cycle, endocytosis, and DNA repair [2–4]. Ubiquitin signals are generated through covalent attachment of ubiquitin molecules to the target proteins in a process known as ubiquitylation. The signals are then transmitted by means of the ubiquitin-binding modules that specifically recognize and transiently bind the ubiquitylated proteins [5]. Versatility of biological functions of ubiquitin is rendered by its ability to interact non-covalently with a variety of proteins not only as a single moiety but also as polyubiquitin chains with different types of linkages and lengths [5,6]. How ubiquitin-binding modules achieve their specificity toward different ubiquitin species and result in distinct signaling outcomes are the focuses of this review.

2. Ubiquitylation as a post-translational modification

Ubiquitin is covalently attached to other ubiquitin molecules or substrate proteins through a sequential enzymatic process known as ubiquitylation [1]. In this process, ubiquitin is first activated at the expense of ATP and transferred to the active site Cys residue of an ubiquitin-activating enzyme (E1) [1]. The activated ubiquitin is then further transferred to the active site of a family of ubiquitin-carrier or ubiquitin-conjugating enzymes (E2s) [1]. The final step is catalyzed by ubiquitin-ligases (E3s) in a way that carboxyl group of the C-terminal Gly residue of ubiquitin is ligated to the ε-amino group of an internal Lys or α-amino group of the N-terminal Met residue in the target protein [1,7]. Containing seven Lys residues, ubiquitin can form different chains that are linked via its Lys6, 11, 27, 29, 33, 48, and 63 [8]. Alternatively, linear or Met1-linked chains are polymerized through the N-terminal Met residue of ubiquitin [9,10].

In the same manner as other post-translational modifications such as phosphorylation and acetylation, ubiquitylation is a reversible process. Ubiquitylation is counter-regulated by the activity of a superfamily of isopeptidases known as deubiquitylating enzymes (DUBs) [11]. Different DUBs are classified into five groups among which are ubiquitin C-terminal hydrolases (UCHs), ubiquitin-specific proteases (USPs), ovarian tumor proteases (OTUs), Josephin and JAB1/MPN/MOV34 metalloenzymes (JAMMs) [11]. Coordinated activity of the E3 ubiquitin ligases and DUBs enables tight regulation of the ubiquitin signaling events.

3. Ubiquitin-binding modules

Ubiquitin-binding modules are specialized domains in larger proteins in the ubiquitin-signaling network that recognize ubiquitin molecules. These domains are structurally and functionally diverse reflecting the variety of ubiquitin signals. To date, almost 20 different families of ubiquitin-binding domains (UBDs) are characterized and the number is expected to grow as greater

* Corresponding author.
E-mail address: Ivan.dikic@biochem2.de (I. Dikic).

0014-5793 © 2012 Federation of European Biochemical Societies. Published by Elsevier B.V. Open access under CC BY-NC-ND license.
http://dx.doi.org/10.1016/j.febslet.2012.04.053

aspects of ubiquitin signaling are discovered. Characteristics of the UBDs are described in detail elsewhere [5,6].

Based on their structural folds, UBDs are categorized into four main groups. The largest group includes UBDs that fold into α-helical structures including UBA (ubiquitin-associated), UIM (ubiquitin-interacting motif), GAT (GGA and TOM), VHS (Vps27/Hrs/STAM), CUE (coupling ubiquitin conjugation to ER degradation), UBM (ubiquitin-binding motif) and UBAN (ubiquitin-binding domain in ABINs and NEMO). The second largest group of UBDs is composed of the ones that contain zinc fingers (ZnFs) in their structures such as NZF (Npl4 ZnF), A20 ZnF domains, ZnF UBP (ubiquitin-specific processing protease), and UBZ (ubiquitin-binding ZnF). The other two groups include PH (Plekstrin homology), and Ubc (ubiquitin-conjugating)-like domains. It has to be noted that some members of the above mentioned UBD families appear not to bind ubiquitin [12,13]. This emphasizes the importance of experimental verification of the modules that are being identified by motif searching.

4. Specificity of ubiquitin-binding modules for ubiquitin

An important challenge in the field of ubiquitin signaling is to unravel the mechanisms underlying the specificity of ubiquitin-binding modules for different ubiquitin species. Thus, great efforts have been put into understanding the determinants of such specificity provided by either the ubiquitin-binding modules or ubiquitin species.

Among various polyubiquitin chains, Lys48- and Lys63-linked types have been the focuses of initial ubiquitin-signaling investigations [14,15]. However, extensive functional and structural studies in recent years have manifested larger variety of ubiquitin chains such as Lys11- and Met1-linked polyubiquitins to be synthesized in vivo and participate in the biological processes [10,16–19].

So far, there are several factors proposed to determine the specificity of ubiquitin-binding modules for different ubiquitin species. From ubiquitin side, overall conformation of the chains including relative orientation of the ubiquitin moieties in the chain and extension of the linker regions, as well as the length of ubiquitin chains are proposed to contribute to their specific recognition by ubiquitin-binding modules. From UBDs side, multiplicity of the binding sites, their structural folds and interactions with the residues forming the linker regions in the ubiquitin chains are defined as the key determinants for their ubiquitin-binding specificity.

5. Specificity determinants provided by the ubiquitin species

5.1. Structural variability of the ubiquitin chains

Structural studies of ubiquitin chains in the free form or in complex with UBDs have greatly contributed to our understanding of the specificity of ubiquitin signaling. Solution structure of some of the ubiquitin chains including Lys63-, Lys48-, Lys11-, Lys6-, and Met1-linked ubiquitin chains indicate that while diubiquitin chains that are linked via residues Lys48, Lys11, and Lys6 adopt compact or closed conformations, Lys63-, and Met1-linked chains form relatively extended structures [20–24]. It has been also shown by molecular modeling that overall structure of Lys27-linked chains is likely to be compact compared to rather elongated Lys29- or Lys33-linked ubiquitin chains [25]. However, it has to be taken into account that in spite of the overall rigidity of the ubiquitin structure its C-terminal tail is quite flexible, resulting in the conformational variability of ubiquitin chains. This feature might be more pronounced in the case of polyubiquitins, which form less compact structures such as Met1- or Lys63-linked chains. Solution structure of Met1-linked diubiquitins indicate both elongated and compact conformations suggesting that the available crystal structures are only snapshots of many conformations that this type of chain may adopt in the free form in solution [21,23,24,26].

Importantly, structural malleability of ubiquitin chains allows a specific chain type to form distinct conformations upon binding to ubiquitin-binding modules. For instance, structural study of Lys63-linked diubiquitins in complex with different UBDs indicates marked divergence in the overall conformation of this type of chains (reviewed in [6], Fig. 1). Therefore, it is expected for the ubiquitin chains with any linkage type to exert some extent of flexibility and adaptation to their binding partners, thus providing specificity for each individual ubiquitin chain–UBD interaction.

5.2. Length of the ubiquitin chains

To date, the actual length of ubiquitin chains that are involved in the biological processes is not known and the use of diubiquitins as the shortest ubiquitin chains with less complexity in production and interpretation of the results has been more popular for the purpose of the structural studies. Diubiquitins appear to be the fundamental units recognized by ubiquitin-binding modules [27] and they can be purified directly from biological samples [28] suggesting their activity in vivo. In spite of that, it is likely that the extracted diubiquitins would be the building blocks of longer chains or generated by trimming of the chains in cells [28]. Moreover, there are several examples where stronger binding of ubiquitin-binding modules to longer ubiquitin chains is detected [29–32]. It is in general speculated that longer chain length increases the binding avidity, which in turn compensates for the low affinity ubiquitin–UBD interactions and might also give rise to the non-specific UBD–ubiquitin interactions [27]. Nonetheless, higher affinity for specific chain length could indicate optimal number of ubiquitins in a chain required for efficient signaling outcomes. Lys63-linked chains with minimum of three ubiquitins are shown

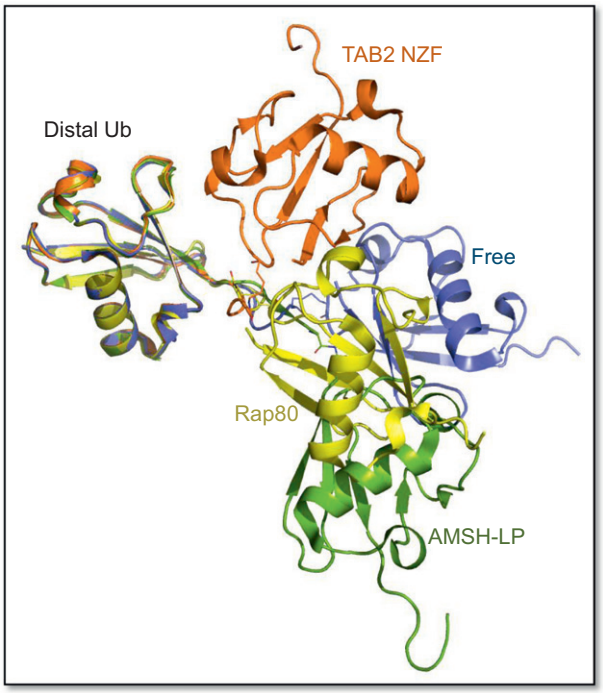

Fig. 1. Structural variability of ubiquitin chains. Superposition of the distal ubiquitin of Lys63-linked diubiquitin chains in the free form (PDB 2JF5) and in complex with the TAB2-NZF (PDB 3A9J), Rap80-UIMs (PDB 3A1Q), and AMSH-LP DUB (PDB 2ZNV). (Distal ubiquitin is indicated as a molecule in a diubiquitin chain with its C-terminal Gly residue being attached to the next ubiquitin moiety, as opposed to the proximal ubiquitin with an exposed C-terminal tail.)

to be potent activators of RIG-I (retinoic-acid-inducible gene-I) [33]. It is also reported that Lys48-linked tetraubiquitins are required to achieve efficient proteasomal targeting [14]. Although, more recent studies reveal that the ubiquitin-based signals for proteasomal degradation are more diverse ranging from monoubiquitins to different ubiquitin chain types, in particular Lys11-linked chains (reviewed in [18]).

6. Specificity determinants provided by ubiquitin-binding modules

6.1. Cooperative ubiquitin-binding of multiple UBDs

An important feature of ubiquitin binding modules is that they often harbor two or more ubiquitin-binding domains. There can be

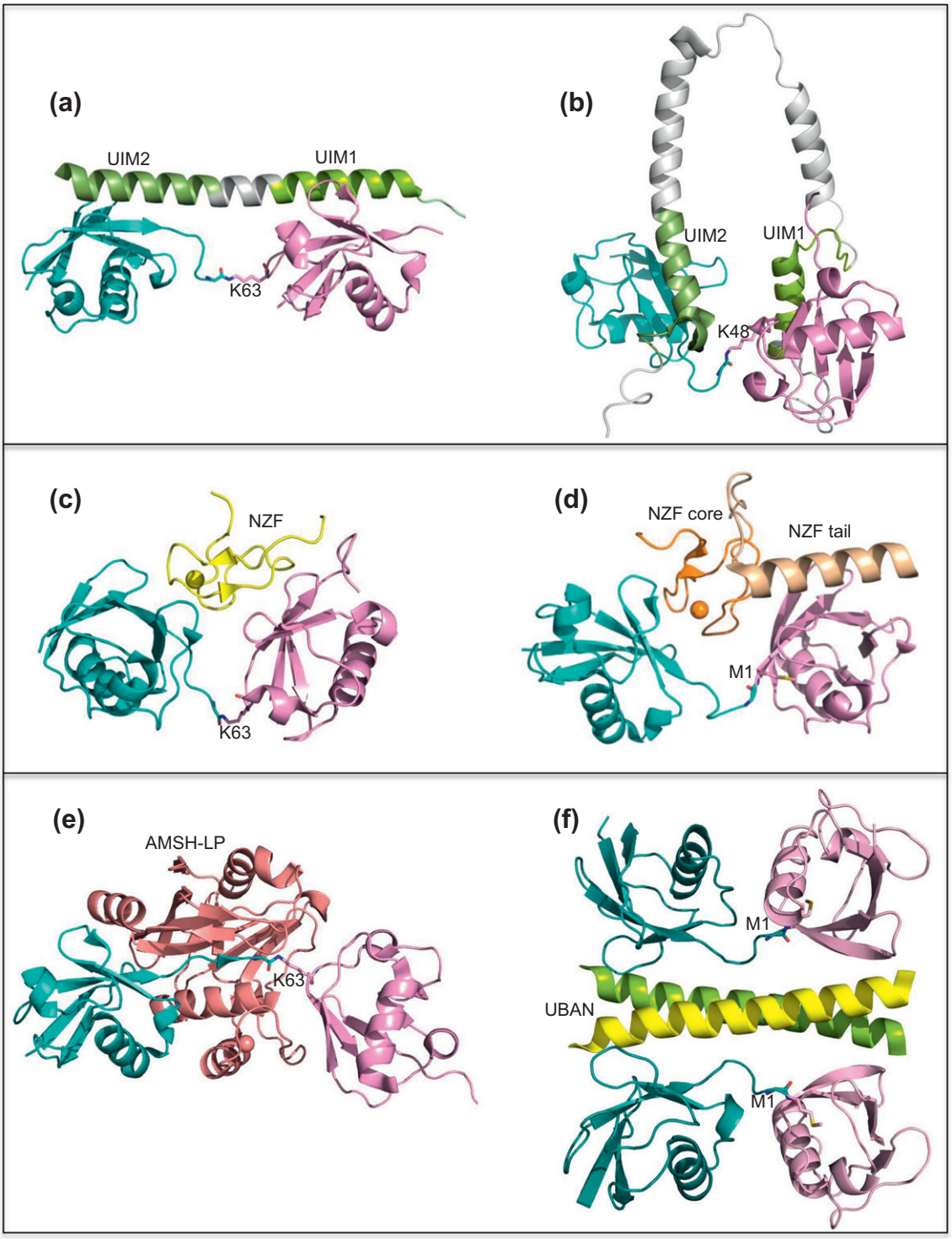

Fig. 2. Specificity determinants of the UBD–ubiquitin chain interactions. (a and b) Cooperative ubiquitin-binding by the (a) UIMs of Rap80 to Lys63-linked (PDB 3A1Q) and (b) UIMs of S5a to Lys48-linked diubiquitin chains (PDB 2KDE). (c and d) Multiple ubiquitin-binding by the (c) NZF domain of TAB2 to Lys63-linked (PDB 3A9J) and (d) NZF domain of HOIL-1L to Met1-linked (PDB 3B08) diubiquitins. (e and f) Recognition of the linker between the two ubiquitins in a (e) Lys63-linked diubiquitins by the AMSH-LP DUB (PDB 2ZNV) and (f) Met1-linked ubiquitin chains by the UBAN motif of NEMO (PDB 2ZVO). Distal and proximal ubiquitins are shown in blue and pink, respectively.

included UBDs of the same type such as a few copies of UIM [34,35], UBA [29,36], or A20 ZF domains [37], or combination of different domains like UBM-UBZ [38], UIM-VHS [31,39] or UBAN-ZF domains [32]. Indeed, these UBDs are capable of recognizing ubiquitin, individually. However, cooperative ubiquitin-binding of these domains can render selectivity of the adaptor proteins for specific ubiquitin species. Thus, ubiquitin-binding specificity of an individual, isolated ubiquitin-binding module may not be representative of the entire intact binding protein and results of such investigations should be tested in the context of the full-length protein. For instance, the tandem UIMs of the Rap80 protein that is involved in the DNA repair mechanisms are shown to obtain specificity toward Lys63-linked diubiquitin [34] (Fig. 2a). The two UIMs of S5a/Rpn10, a proteasomal ubiquitin receptor, bind specifically to Lys48-linked ubiquitin chains [40] (Fig. 2b). Ataxin-3 protein can recognize both Lys63- and Lys48-linked ubiquitin chains triggered by the cooperative activity of its two UIMs that are connected by a short and flexible linker [41]. Cooperative binding to ubiquitin is not confined to the UBDs of a same protein; rather UBDs from different proteins are shown to simultaneously interact with distinct surfaces on an ubiquitin molecule. A20-like ZnF (A20 ZnF) of the ubiquitin receptor ZNF216 and UBA domain of the p62 protein, which function in a same biological pathway bind to two different faces on a monoubiquitin [42]. Although, ubiquitin appears to have multiple potential binding surfaces, the most prevalently used surface for interaction with UBDs is a hydrophobic patch centered on Ile44 residue [6]. In this example, A20 ZnF binding site on ubiquitin is centered on Asp58, leaving the Ile44 patch free to interact with p62 UBA domain, thereby making the system compatible for cooperative binding to a single ubiquitin without causing steric hindrance [42].

It is likely that in some cases cooperation of UBDs increases affinity of proteins for more variety of ubiquitin chains rather than providing selectivity toward a specific ubiquitin chain type. All of these interactions might be relevant in vivo but vary in the sense that they occur in distinct cell types and are driven by different source of stimuli. One example would be NEMO protein that is the key regulator of the canonical NF-κB signaling pathway and is proposed to act as the high affinity receptor for Met1-linked ubiquitin chains [43]. In addition, the full size NEMO protein including the C-terminal zinc finger domain can also bind Lys11- or Lys63-linked ubiquitin chains [44–46]. These could indicate the contribution of these ubiquitin chains in the NEMO-related activation of NF-κB signaling pathway following stimulation in different cell types [43].

6.2. Multiple ubiquitin-binding by a single UBD

Recognition of multiple ubiquitin moieties by an individual module has also been shown to work as a specificity determinant for some ubiquitin–UBD interactions. Structural fold and binding surfaces on these UBDs require unique orientation of the ubiquitins resulting in selectivity toward a specific ubiquitin chain type. For instance, the NZF domains of both TAB2 and TAB3 (proteins that are implicated in activation of the NF-κB pathway) bind preferentially to Lys63-linked ubiquitin chains [47,48]. Although, from both ubiquitins the Ile44-centered hydrophobic patch is involved in the interactions with NZF domains, it is shown that such conformation is uniquely possible only for the Lys63-linked chains [47] (Fig. 2c). As shown by a recent study, methylation of a Cys residue in NZF domain of either TAB2 or TAB3 disrupts polyubiquitin recognition and blocks NF-κB signaling pathway [49]. Another example is HOIL-1L protein, which is a component of the linear ubiquitin chain assembly complex (LUBAC) [9]. HOIL-1L recognizes Met1-linked ubiquitin chains through its NZF domain. Crystal structure of the NZF domain in complex with Met1-linked diubiquitins reveals that a helical region that follows NZF domain (indicated as NZF tail) assists binding of HOIL-1L to the ubiquitin chains [26] (Fig. 2d). TRABID, the human ovarian tumor (OTU) domain deubiquitinase (DUB), also acquires selectivity for both Lys29- and Lys33-linkd diubiquitins through an ankyrin repeat domain that precedes its A20-like ZF domain [50].

6.3. Recognition of the linker region of ubiquitin chains

As discussed above, ubiquitin chain linkage type determines the overall conformation of chains and contributes to their specific recognition by UBDs. Direct interaction of ubiquitin-binding modules with the linker itself can be the most convincing observation to explain the ubiquitin chain linkage specificity [21,51]. Structure of the Zn-dependent AMSH-LP deubiquitinating enzyme (DUB) in complex with Lys63-linked diubiquitins provided the first evidence for recognition of an isopeptide bond-linked ubiquitin chain [51] (Fig. 2e). UBAN motif of NEMO (see above) also tightly interacts with the linker region of Met1-linked diubiquitins in a way that mutation of a single residue from UBAN that contacts the diubiquitin linker can abolish its binding to the ubiquitin chains [21] (Fig. 2f).

7. Affinity of ubiquitin-binding modules for ubiquitin

Affinity of ubiquitin-binding modules for ubiquitin is generally low [5]. In fact, weak and transient interaction of ubiquitin with proteins is the basis for rapid and timely regulation of cellular pathways by the ubiquitin signaling. Nonetheless, in order to produce specific signals biological system employs several strategies to amplify the ubiquitin-binding effects including increased avidity, contribution of UBD-independent sequences, posttranslational modifications like phosphorylation and conformational changes following the UBDs–ubiquitin interactions. These factors create environments in which the effective affinity and selectivity of ubiquitin–UBD interactions are functionally relevant in the context of a living cell.

Regardless of interacting with ubiquitin as a single molecule or chains, some UBDs bind more than one copy of these ubiquitin species. The binding mode for such multiple interactions is usually identical since UBDs employ essentially similar surfaces for binding to ubiquitin. The double-sided ubiquitin-binding of Hrs UIM [52] and dimerization of NEMO UBAN [21] are examples of this mechanism. Hrs protein is implicated in the endocytic sorting of the monoubiquitinated membrane proteins [53]. It binds two mono-ubiquitins on either sides of the α-helix and the key interacting residues are repeated along the UIM but shifted by two residues [52]. Dimerization of UBAN motif also allows NEMO (see above) to accommodate two Met1-linked diubiquitins on either side of the coiled-coil structure [21]. In both examples, the two-sided ubiquitin-binding is required for efficient activity of the proteins [21,52].

Modification of ubiquitin-binding modules by phosphorylation has been recently identified to increase their affinity for ubiquitin. For instance, phosphorylation of Ser403 in the UBA domain of p62/SQSTM1 that regulates selective autophagy of ubiquitylated proteins results in a significant increase in its affinity for polyubiquitin chains [54]. Conversely, binding to ubiquitin by Syntenin-1 that is involved in the trafficking of trans-membrane proteins is inhibited by phosphorylation [55]. Altogether, since phosphorylation as a reversible post-translational modification takes part in the regulation of various biological pathways; it is also expected to be involved in regulation of the wider range of the UBD–ubiquitin interactions.

8. Conclusions and future perspectives

Major challenges in ubiquitin-signaling field deal with the understanding of multimeric interactions between ubiquitin signals and their binding partners under physiological conditions. There are clearly many factors that determine specificity of interactions between ubiquitin-binding modules and ubiquitin species in the cells and their so-called "in vivo affinity" remains elusive. Often we extrapolate on more complex interactions by relying on the knowledge based on in vitro biophysical affinity measurements, with the use of isolated ubiquitin-binding domains and ligands. These parameters, even though very valuable, do not always reflect the biological conditions. Therefore, the major input in the field will come from development of methods that help to monitor dynamics and interactions between ubiquitinated substrates and effectors mediating signaling pathways, in vivo. Recent advances in mass spectroscopy technique [56], use of ubiquitin chain-specific antibodies, and development of ubiquitin sensors [57] provide promising avenues for future research in the complex networks of ubiquitin signaling pathways [20].

Acknowledgements

We apologize to scientists whose important contributions were not referenced in this review due to space limitations. We would like to thank Mateusz Putyrski and Ligia Carinha Gomes for critical reading of the manuscript. Research in the I.D. laboratory is supported by the Deutsche Forschungsgemeinschaft, the Cluster of Excellence "Macromolecular Complexes" of the Goethe University Frankfurt (EXC115), LOEWE OSF and a European Research Council Advanced Grant and in the C.B. laboratory through a LOEWE Cell and Gene Therapy (CGT) grant, SFB834 funding, LOEWE OSF and Frankfurt Autophagy Network (FAN) funding. S.R. is the recipient of the EMBO long term fellowship.

References

[1] Hershko, A. and Ciechanover, A. (1998) The ubiquitin system. Annu. Rev. Biochem. 67, 425–479.
[2] Mocciaro, A. and Rape, M. (2012) Emerging regulatory mechanisms in ubiquitin-dependent cell cycle control. J. Cell Sci. 125, 255–263.
[3] Haglund, K. and Dikic, I. (2012) The role of ubiquitylation in receptor endocytosis and endosomal sorting. J. Cell Sci. 125, 265–275.
[4] Ulrich, H.D. and Walden, H. (2010) Ubiquitin signaling in DNA replication and repair. Nat. Rev. Mol. Cell Biol. 11, 479–489.
[5] Husnjak, K. and Dikic, I. (2012) Ubiquitin-binding proteins: decoders of ubiquitin-mediated cellular functions. Annu. Rev. Biochem. 81, 30.1–30.32.
[6] Dikic, I., Wakatsuki, S. and Walters, K.J. (2009) Ubiquitin-binding domains from structures to functions. Nat. Mol. Cell Biol. 10, 659–671.
[7] Deshaies, R.J. and Joazeiro, C.A. (2009) RING domain E3 ubiquitin ligases. Annu. Rev. Biochem. 78, 399–434.
[8] Ikeda, F. and Dikic, I. (2008) Atypical ubiquitin chains: new molecular signals. EMBO Rep. 6, 536–542.
[9] Kirisako, T., Kamei, K., Murata, S., Kato, M., Fukumoto, H., Kanie, M., Sano, S., Tokunaga, F., Tanaka, K. and Iwai, K. (2006) A ubiquitin ligase complex assembles linear polyubiquitin chains. EMBO J. 25, 4877–4887.
[10] Walczak, H., Iwai, K. and Dikic, I. (2012) Generation and physiological roles of linear ubiquitin chains. BMC Biol. 10, 23–28.
[11] Clague, M.J., Coulson, J.M. and Urbé, S. (2012) Cellular functions of the DUBs. J. Cell Sci. 125, 277–286.
[12] Alam, S.L., Sun, J., Payne, M., Welch, B.D., Blake, B.K., Davis, D.R., Meyer, H.H., Emr, S.D. and Sundquist, W.I. (2004) Ubiquitin interactions of NZF zinc fingers. EMBO J. 23, 1411–1421.
[13] Hicke, L., Schubert, H.L. and Hill, C.P. (2005) Ubiquitin-binding domains. Nat. Mol. Cell Biol. 6, 610–621.
[14] Thrower, J.S., Hoffman, L., Rechsteiner, M. and Pickart, C.M. (2000) Recognition of polyubiquitin proteolytic signal. EMBO J. 19, 94–102.
[15] Deng, L., Wang, C., Spencer, E., Yang, L., Braun, A., You, J., Slaughter, C., Pickart, C. and Chen, Z.J. (2000) Activation of the IκB kinase complex by TRAF6 requires a dimeric ubiquitin-conjugating enzyme complex and a unique polyubiquitin chain. Cell 103, 351–361.
[16] Matsumoto, M.L., Wickliffe, K.E., Dong, K.C., Yu, C., Bosanac, I., Bustos, D., Phu, L., Kirkpatrick, D.S., Hymowitz, S.G., Rape, M., Kelley, R.F. and Dixit, V.M. (2010) K11-linked polyubiquitination in cell cycle control revealed by a K11 linkage-specific antibody. Mol. Cell 39, 477–484.
[17] Bedford, L., Layfield, R., Mayer, R.J., Peng, J. and Xu, P. (2011) Diverse polyubiquitin chains accumulate following 26S proteasomal dysfunction in mammalian neurones. Neurosci. Lett. 491, 44–47.
[18] Kravtsova-Ivantsiv, Y. and Ciechanover, A. (2012) Non-canonical ubiquitin-based signals for proteasomal degradation. J. Cell Sci. 125, 539–548.
[19] Emmerich, C.H., Schmukle, A.C. and Walczak, H. (2011) The emerging role of linear ubiquitination in cell signaling. Sci. Signal. 4, re5.
[20] Bremm, A., Freund, S.M.V. and Komander, D. (2010) Lys11-linked ubiquitin chains adopt compact conformations and are preferentially hydrolyzed by the deubiquitinase Cezanne. Nat. Struct. Mol. Biol. 17, 939–947.
[21] Rahighi, S., Ikeda, F., Kawasaki, M., Akutsu, M., Suzuki, N., Kato, R., Kensche, T., Uejima, T., Bloor, S., Komander, D., Randow, F., Wakatsuki, S. and Dikic, I. (2009) Specific recognition of linear ubiquitin chains by NEMO is important for NF-κB activation. Cell 136, 1098–1109.
[22] Virdee, S., Ye, Y., Nguyen, D.P., Komander, D. and Chin, J.W. (2010) Engineered diubiquitin synthesis reveals Lys29-isopeptide specificity of an OUT deubiquitinase. Nat. Chem. Biol. 6, 750–757.
[23] Komander, D., Reyes-Turcu, F., Licchesi, J.D., Odenwaelder, P., Wilikinson, K.D. and Barford, D. (2009) Molecular discrimination of structurally equivalent Lys63-linked and linear polyubiquitin chains. EMBO Rep. 10, 466–473.
[24] Rohaim, A., Kawasaki, M., Kato, R., Dikic, I. and Wakatsuki, S. (2012) Structure of a compact conformation of linear diubiquitin. Acta Crystallogr. D68, 102–108.
[25] Fushman, D. and Walker, O. (2009) Exploring linkage dependence of polyubiquitin conformations using molecular modeling. J. Mol. Biol. 395, 803–814.
[26] Sato, Y., Fujita, H., Yoshikawa, A., Yamashita, M., Yamagata, A., Kaiser, S.E., Iwai, K. and Fukai, S. (2011) Specific recognition of linear ubiquitin chains by the Npl4 zinc finger (NZF) domain of the HOIL-1L subunit of the linear ubiquitin chain assembly complex. Proc. Natl. Acad. Sci. U.S.A. 108, 20520–20525.
[27] Ikeda, F., Crosetto, N. and Dikic, I. (2010) What determines the specificity and outcomes of ubiquitin signaling? Cell 143, 677–681.
[28] Strachan, J., Roach, L., Sokratous, K., Tooth, D., Long, J., Garner, T.P., Searle, M.S., Oldham, N.J. and Layfield, R. (2012) Insights into the molecular composition of endogenous unanchored polyubiquitin chains. J. Proteome Res. 11, 1969–1980.
[29] Raasi, S., Orlov, I., Fleming, K.G. and Pickart, C.M. (2004) Binding of polyubiquitin chains to ubiquitin-associated (UBA) domains of HHR23A. J. Mol. Biol. 341, 1367–1379.
[30] Trempe, J.-F., Brown, N.R., Lowe, E.D., Gordon, C., Campbell, I.D., Noble, M.E.M. and Endicott, J.A. (2005) Mechanism of Lys48-linked polyubiquitin chain recognition by the Mud1 UBA domain. EMBO J. 24, 3178–3189.
[31] Ren, X. and Hurely, J.H. (2010) VHS domains of ESCRT-0 cooperates in high-avidity binding to polyubiquitinated cargo. EMBO J. 29, 1045–1054.
[32] Laplantine, E., Fontan, E., Chiaravalli, J., Lopez, T., Lakisic, G., Veron, M., Agou, F. and Israel, A. (2009) NEMO specifically recognizes K63-linked poly-ubiquitin chains through a new bipartite ubiquitin-binding domain. EMBO J. 28, 2885–2895.
[33] Zeng, W., Sun, L., Jiang, X., Chen, X., Hou, F., Adhikari, A., Xu, M. and Chen, Z.J. (2010) Reconstitution of the RIG-I pathway reveals a signaling role of unanchored polyubiquitin chains in innate immunity. Cell 141, 315–330.
[34] Sato, Y., Yoshikawa, A., Mimura, H., Yamashita, M., Yamagata, A. and Fukai, S. (2009) Structural basis for specific recognition of Lys 63-linked polyubiquitin chains by tandem UIMs of RAP80. EMBO J. 28, 2461–2468.
[35] Yun, C., Stanhill, A., Yang, Y., Zhang, Y., Haynes, C.M., Xu, C.-F., Neubert, T.A., Mor, A., Philips, M.R. and Ron, D. (2008) Proteasomal adaptation to environmental stress links resistance to proteotoxicity with longevity in *Caenorhabditis elegans*. Proc. Natl. Acad. Sci. U.S.A. 105, 7094–7099.
[36] Raasi, S. and Pickart, C.M. (2003) Rad23 ubiquitin-associated domains (UBA) inhibit 26S proteasome-catalyzed proteolysis by sequestering lysine 48-linked polyubiquitin chains. J. Biol. Chem. 278, 8951–8959.
[37] Opipari, A.W., Boguski, M.S. and Dixit, V.M. (1990) The A20 cDNA induced by tumor necrosis factor α encodes a novel type of zinc finger protein. J. Biol. Chem. 265, 14706–14708.
[38] Bienko, M., Green, C.M., Crosetto, N., Rudolf, F., Zapart, G., Coull, B., Kannouche, P., Wider, G., Peter, M., Lehmann, A.R., Hofmann, K. and Dikic, I. (2005) Ubiquitin-binding domains in Y-family polymerases regulate translesion synthesis. Science 310, 1821–1824.
[39] Mizuno, E., Kawahata, K., Kato, M., Kitamura, N. and Komada, M. (2003) STAM proteins bind ubiquitinated proteins on the early endosome via the VHS domain and ubiquitin-interacting motif. Mol. Biol. Cell 14, 3675–3689.
[40] Zhang, N., Wang, Q., Ehlinger, A., Randles, L., Lary, J.W., Kang, Y., Haririnia, A., Storaska, A.J., Cole, J.L., Fushman, D. and Walters, K.J. (2009) Structure of the S5a:K48-linked diubiquitin complex and its interactions with Rpn13. Mol. Cell 35, 280–290.
[41] Song, A.-X., Zhou, C.-J., Peng, Y., Gao, X.-C., Zhou, Z.-R., Fu, Q.-S., Hong, J., Lin, D.-H. and Hu, H.-Y. (2010) Structural transformation of the tandem ubiquitin-interacting motifs in Ataxin-3 and their cooperative interactions with ubiquitin chains. PLoS ONE 5, e13202.

[42] Garner, T.P., Strachan, J., Shedden, E.C., Long, J.E., Cavey, J.R., Shaw, B., Layfield, R. and Searle, M.S. (2011) Independent interactions of ubiquitin-binding domains in a ubiquitin-mediated ternary complex. Biochemistry 50, 9076–9087.
[43] Grabbe, C., Husnjak, K. and Dikic, I. (2011) The spatial and temporal organization of ubiquitin networks. Nat. Cell Biol. 12, 295–307.
[44] Lo, Y.C., Lin, S.C., Rospigliosi, C.C., Conze, D.B., Wu, C.J., Ashwell, J.D., Eliezer, D. and Wu, H. (2009) Structural basis for recognition of diubiquitins by NEMO. Mol. Cell 33, 602–615.
[45] Wu, C.J., Conze, D.B., Li, T., Srinivasula, S.M. and Ashwell, J.D. (2006) Sensing of Lys63-linked polyubiquitination by NEMO is a key event in NF-kappaB activation. Nat. Cell Biol. 4, 398–406.
[46] Dynek, J.N., Goncharov, T., Dueber, E.C., Fedorova, A.V., Izrael-Tomasevic, A., Phu, L., Helgason, E., Fairbrother, W.J., Deshayes, K., Kirkpatrick, D.S. and Vucic, D. (2010) C-IAP1 and UbcH5 promote K11-linked polyubiquitination of RIP1 in TNF signaling. EMBO J. 29, 4198–4209.
[47] Kulathu, Y., Akutsu, M., Bremm, A., Hofmann, K. and Komander, D. (2009) Two-sided ubiquitin binding explains specificity of the TAB2 NZF domain. Nat. Struct. Mol. Biol. 16, 1328–1330.
[48] Sato, Y., Yoshikawa, A., Yamashita, M., Yamagata, A. and Fukai, S. (2009) Structural basis for specific recognition of Lys63-linked polyubiquitin chains by NZF domains of TAB2 and TAB3. EMBO 28, 3903–3909.
[49] Ahang, L., Ding, X., Cui, J., Xu, H., Chen, J., Gong, Y.N., Hu, L., Zhou, Y., Ge, J., Lu, Q., Liu, L., Chen, S. and Shao, F. (2011) Cysteine methylation disrupts ubiquitin-chain sensing in NF-κB activation. Nature 481, 204–208.
[50] Licchesi, J.D., Mieszczanek, J., Mevissen, T.E., Rutherford, T.J., Akutsu, M., Virdee, S., El Qualid, F., Chin, J.W., Ovaa, H., Bienz, M. and Komander, D. (2011) An ankyrin-repeat ubiquitin-binding domain determines TRABID's specificity for atypical ubiquitin chains. Nat. Struct. Mol. Biol. 19, 62–71.
[51] Sato, Y., Yoshikawa, A., Yamagata, A., Mimura, H., Yamashita, M., Ookata, K., Nureki, O., Iwai, K., Komada, M. and Fukai, S. (2008) Structural basis for specific cleavage of Lys63-linked polyubiquitin chains. Nature 455, 358–362.
[52] Hirano, S., Kawasaki, M., Ura, H., Kato, R., Raiborg, C., Stenmark, H. and Wakatsuki, S. (2006) Double-sided ubiquitin binding of Hrs-UIM in endosomal protein sorting. Nat. Struct. Mol. Biol. 13, 272–277.
[53] Raiborg, C. and Stenmark, H. (2002) Hrs and endocytic sorting of ubiquitinated membrane proteins. Cell Struct. Funct. 6, 403–408.
[54] Matsumoto, G., Wada, K., Okuno, M., Kurosawa, M. and Nukina, N. (2011) Serine 403 phosphorylation of p62/SQSTM1 regulates selective autophagic clearance of ubiquitinated proteins. Mol. Cell 44, 279–289.
[55] Rajesh, S., Bago, R., Odintsova, E., Muratov, G., Baldwin, G., Sridhar, P., Rajesh, S., Overduin, M. and Berditchevski, F. (2011) Binding to syntenin-1 protein defines a new mode of ubiquitin-based interactions regulated by phosphorylation. J. Biol. Chem. 286, 39606–39614.
[56] Sokratous, K., Roach, L.V., Channing, D., Strachan, J., Long, J., Searle, M.S., Layfield, R. and Oldham, N.J. (2012) Probing affinity and ubiquitin linkage selectivity of ubiquitin-binding domains using mass spectrometry. J. Am. Chem. Soc. 134, 6416–6424.
[57] Sims, J.J., Scavone, F., Cooper, E.M., Kane, L.A., Youle, R.J., Boeke, J.D. and Cohen, R.E. (2012) Polyubiquitin-sensor proteins reveal localization and linkage-type dependence of cellular ubiquitin signaling. Nat. Methods 9, 303–309.