

# UBIQUITIN-SPECIFIC PROTEASES function in plant development and stress responses

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## Abstract

**Key message** UBIQUITIN-SPECIFIC PROTEASES play important roles in plant development and stress responses.

**Abstract** Protein ubiquitination and deubiquitination are reversible processes, which can modulate the stability, activity as well as subcellular localization of the substrate proteins. UBIQUITIN-SPECIFIC PROTEASE (UBP) protein family participates in protein deubiquitination. Members of UBPs are involved in a variety of physiological processes in plants, as evidenced by their functional characterization in model plant *Arabidopsis* and other plants. UBPs are conserved in plants and distinct UBPs function in different regulatory processes, although

functional redundancies exist between some members. Here we briefly reviewed recent advances in understanding the biological functions of UBPs protein family in *Arabidopsis*, particularly the molecular mechanisms by which UBPs regulate plant development and stress responses. We believe that elucidation of UBPs function and regulation in *Arabidopsis* will provide new insights about protein deubiquitination and might shed light on the understanding of the mechanistic roles of UBPs in general, which will definitely contribute to crop improvement in agriculture.

**Keywords** Ubiquitin · Ubiquitination/deubiquitination · UBIQUITIN-SPECIFIC PROTEASES · Plant development · Plant stress response and tolerance

**Accession numbers:** Sequence data of AtUBPs related to this review can be found in the Arabidopsis Genome Initiative or GenBank/EMBL databases under the following accession numbers: At2g32780 (AtUBP1), At1g04860 (AtUBP2), At4g39910 (AtUBP3), At2g22310 (AtUBP4), At2g40930 (AtUBP5), At1g51710 (AtUBP6), At3g21280 (AtUBP7), At5g22030 (AtUBP8), At4g10570 (AtUBP9), At4g10590 (AtUBP10), At1g32850 (AtUBP11), At5g06600 (AtUBP12), At3g11910 (AtUBP13), At3g20630 (AtUBP14), At1g17110 (AtUBP15), At4g24560 (AtUBP16), At5g65450 (AtUBP17), At4g31670 (AtUBP18), At2g24640 (AtUBP19), At4g17895 (AtUBP20), At5g46740 (AtUBP21), At5g10790 (AtUBP22), At5g57990 (AtUBP23), At4g30890 (AtUBP24), At3g14400 (AtUBP25), At3g49600 (AtUBP26), and At4g39370 (AtUBP27).

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## Ubiquitination/deubiquitination and UBPs in plants

Ubiquitin (Ub) is a small peptide with 76 amino acids that could be covalently attached to the target proteins via ubiquitination (Hershko and Ciechanover 1998; Pickart and Eddins 2004). Protein ubiquitination is catalyzed by three sequential steps performed by E1 (Ub-activating enzyme), E2 (Ub-conjugating enzyme), and E3 (Ub ligase) respectively (Pickart 2003; Pickart and Eddins 2004). In this way, an isopeptide bond between the C terminus of Ub (Glycine76, G76) and the *ε*-amino group of a target Lysine (K) residue is formed. Since there are seven K residues (K6, K11, K27, K39, K33, K48 and K63) in each Ub, additional isopeptide bonds might be created between G76 in one Ub and any K in a second Ub, resulting in different types of Ub polymers. The peptide bond between Ub G76 and the N terminus of Ub also exists, which is used to generate linear Ub chain (Pickart 2003). Ubiquitination participates in

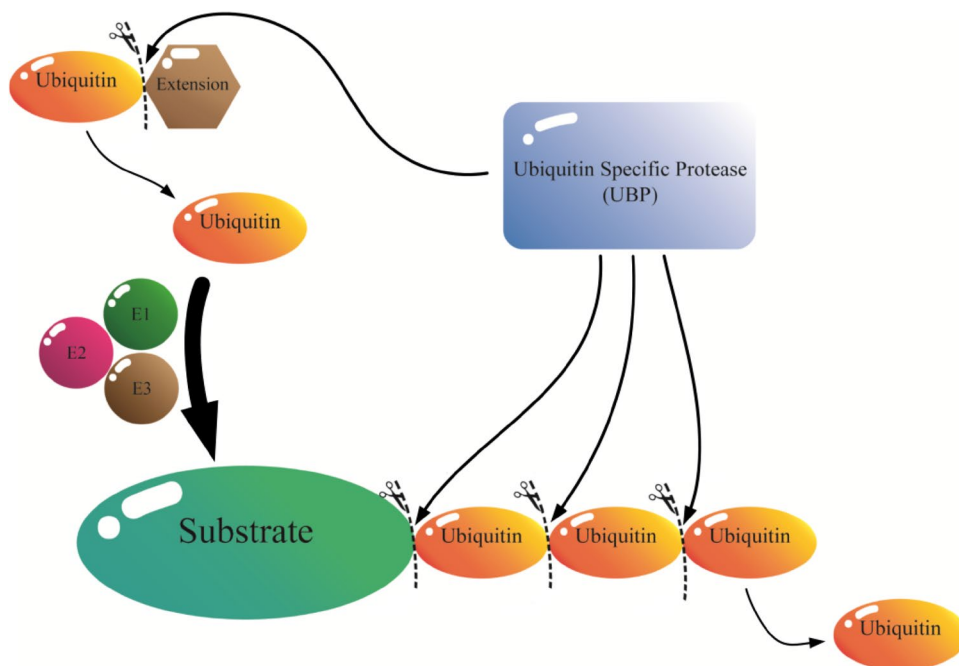
critical cellular processes, and dysfunction of ubiquitination machinery leads to detrimental impacts (Pickart 2003; Zhang 2003).

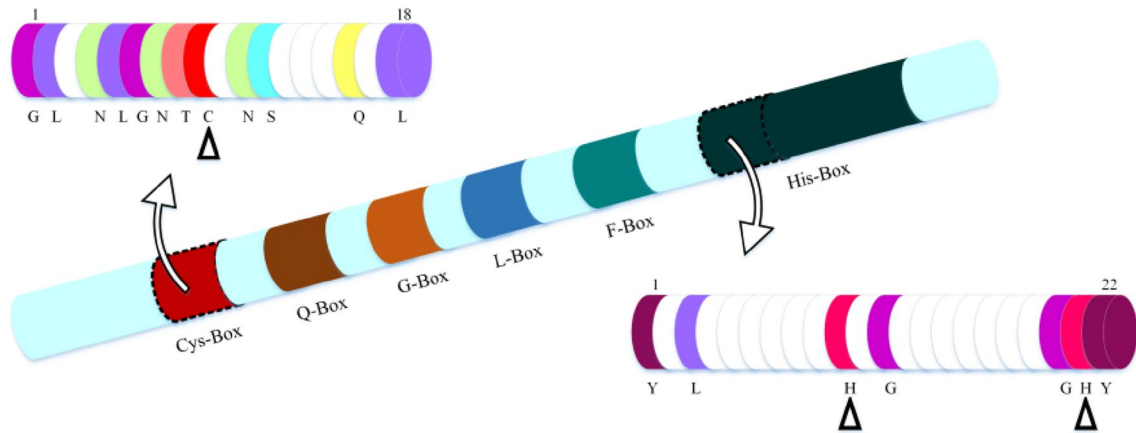
Monoubiquitination mainly modulates protein activity and subcellular location or is involved in establishing “histone code” which plays an important role in transcriptional regulation (Glickman and Ciechanover 2002; Zhang 2003). A recent example for monoubiquitination is IRON-REGULATED TRANSPORTER1 (IRT1), a high-affinity iron transporter functions in iron uptake from the soil at the root surface. IRT1 is monoubiquitinated and this monoubiquitination regulates IRT1 for proper iron uptake to prevent iron toxicity (Barberon et al. 2011). Most polyubiquitinated proteins (K48-linked) are subjected to 26S proteasome-mediated degradation, while under certain circumstance, however, the polyubiquitinated proteins are targeted to non-proteolytic pathways, determined by different types of Ub chains (Hochstrasser 1996; Glickman and Ciechanover 2002; Smalle and Viestra 2004; Chen and Sun 2009; Lim and Lim 2011). K63-linked polyubiquitination has been implicated in endocytosis, DNA damage repair as well as signaling transduction (Lim and Lim 2011; Ulrich 2012; Piper et al. 2014). For example, PIN2 is required for directional cellular efflux of auxin, and RGLG E3 ligase participates in K63-kinked polyubiquitination of PIN2. PIN2 vacuolar sorting and endocytosis depends on K63-linked polyubiquitination, resulting in adaptive growth in plants (Leitner et al. 2012). Conversely, the deubiquitinating enzymes

(DUBs) cleave the conjugated Ub from its substrates and deubiquitination mediated by DUBs has been shown to modulate the stability, activity or destiny of their target proteins (Wilkinson 2000; Fischer 2003; Isono and Nagel 2014). Generally, there exist two major types of DUBs including cysteine proteases and metalloproteases (Verma et al. 2002; Nijman et al. 2005). They mainly possess two basic biochemical activities, one is to generate mature Ub from its precursors which are translated as polyproteins composed of Ub moieties or fusion with ribosomal proteins, and another is to cleave the covalently attached Ub chain from its substrates as well as to hydrolyze the Ub chain into free Ub (Fig. 1; Amerik and Hochstrasser 2004; Nijman et al. 2005).

UBIQUITIN-SPECIFIC PROTEASES (UBPs) belongs to the cysteine proteases DUBs and possess two conserved catalytic motifs, Cys- and His-box (Fig. 2; Hu et al. 2002; Reyesturcu et al. 2009). There are 27 UBPs in *Arabidopsis* and 21 putative UBPs family members in the rice (Yan et al. 2000; Liu et al. 2008; Moon et al. 2009). Phylogenetic analysis revealed that AtUBPs can be further grouped into 14 subfamilies, which function in different regulatory processes including plant growth, development as well as the stress responses (Table 1; Yan et al. 2000; Liu et al. 2008; Isono and Nagel 2014). Here we reviewed the advances in understanding the biological functions of UBPs in *Arabidopsis*, aiming to comprehensively dissect the mechanisms by which AtUBPs modulate the plant development and stress responses.

**Fig. 1** Basic biochemical activities of UBPs. Ub is translated as its precursor with an extension at the C-terminus in plants. UBPs function in generating mature Ub by processing the peptide bond between Ub and the fused extension, or function in cleaving the covalently attached Ub chain from Ub-conjugated substrates as well as hydrolyzing Ub chain into free Ubs





**Fig. 2** Schematic representation of the protein structure of UBPs. UBPs belong to cysteine proteases DUBs and possess a signature catalytic Cys- and His-box. The Cys boxes of the UBPs show high conservation both in sequence and length (18 amino acids) while the His

boxes are more variable. UBPs might also contain several less conserved motifs including Gln (Q), Gly (G), Leu (L), and Phe (F) boxes as well as other additional motifs, indicating their functional diversity of UBPs in plants

**Table 1** Subfamily of UBPs in *Arabidopsis*

Subfamily	Name	Function	References
1	AtUBP1	Canavanine resistance	Yan et al. (2000)
	AtUBP2	Canavanine resistance	Yan et al. (2000)
2	AtUBP3	Pollen tube development	Doelling et al. (2007)
	AtUBP4	Pollen tube development	Doelling et al. (2007)
3	AtUBP5		
	AtUBP8		
	AtUBP9		
	AtUBP10		
	AtUBP11		
4	AtUBP6	Canavanine resistance	Moon et al. (2004)
	AtUBP7		
5	AtUBP12	Plant immunity regulation, flowering, seed development	Ewan et al. (2011), Cui et al. (2013), Derkacheva et al. (2016)
	AtUBP13	Plant immunity regulation, flowering, seed development	Ewan et al. (2011), Cui et al. (2013), Derkacheva et al. (2016)
6	AtUBP14	Organ size regulation, root hairs development, embryogenesis	Hase et al. (2006), Li et al. (2010), Doelling et al. (2001)
7	AtUBP15	Flowering, organ size regulation	Liu et al. (2008), Du et al. (2014)
	AtUBP16	Salt tolerance	Zhou et al. (2012)
	AtUBP17		
	AtUBP18		
	AtUBP19	Embryogenesis	Liu et al. (2008)
8	AtUBP20		
	AtUBP21		
9	AtUBP22		
10	AtUBP23		
11	AtUBP24	ABA signaling	Zhao et al. (2016)
12	AtUBP25		
13	AtUBP26	Flowering, seed development	Schmidz et al. (2009), Luo et al. (2008)
14	AtUBP27	Mitochondrial morphogenesis	Pan et al. (2014)

## Structure, organization and specificity of UBPs in *Arabidopsis*

The capacity of UBP like-DUBs to hydrolyze the attached Ub via a peptide or isopeptide bond was first discovered in plants using a combination of Ub covalent affinity chromatography and anion exchange HPLC (Sullivan et al. 1990). Further analysis revealed that the DUB activity can be performed devoid of ATPs (Sullivan et al. 1990). Ultimately, the enzymes responsible for the UBP-like DUB activity were identified as a protein family consisting of 27 members in *Arabidopsis* (Wilkinson 1997). These AtUBPs typically possess a Cys-box and a His-box. The Cys boxes of the AtUBPs are more conserved in both sequence and length while the His boxes are more variable (Fig. 2; Yan et al. 2000; Liu et al. 2008). Positionally conserved Cys and His residues are essential for the DUB activities and mutations of these amino acids could substantially abolish the DUB activity of AtUBPs (Doelling et al. 2007; Liu et al. 2008; Zhou et al. 2012; Zhao et al. 2016). Furthermore, AtUBPs might also contain several less conserved motifs such as Phe, Gly, Leu, and Gln boxes as well as additional motifs, including zinc fingers (ZnFs), MATH, Ub-like (UBL) domains, and Ub associated (UBA) domains (Yan et al. 2000; Doelling et al. 2007; Liu et al. 2008). The biological functions of these motifs in AtUBPs have not been well understood. However, they might enable AtUBPs to participate in a broad range of physiological processes. For instance, ZnFs in some AtUBPs might mediate the protein–protein interactions following binding a zinc ion by the conserved Cys or His residues (Bonnet et al. 2008). Outside the conserved motifs, low sequence similarity was observed between different AtUBPs, indicating their functional diversity of AtUBPs (Yan et al. 2000; Liu et al. 2008).

The organization of the AtUBP protein family and the relationship between each member were determined with respect to the conserved domains, length as well as sequence similarity (Yan et al. 2000; Liu et al. 2008; Isono and Nagel 2014). These AtUBPs proteins were further grouped into 14 subfamilies and members of each subfamily share conserved motifs, which however might be less conserved among the whole AtUBP protein family (Yan et al. 2000; Liu et al. 2008). In fact, the aforementioned additional motifs such as ZnFs and UBA domains could also serve as signatures in specific subfamily of AtUBPs (Bonnet et al. 2008). Microarray analysis found that AtUBPs exhibit non-identical expression profiles in *Arabidopsis*. Furthermore, expression of each AtUBP displays various patterns in different organs, suggesting their potential regulatory function in specific organs (Liu et al. 2008).

UBPs contribute to Ub pool homeostasis regulation by cleaving Ub from substrates or its precursors (Isono and

Nagel 2014). People have identified several AtUBPs targets, which will promote understanding the specificity and mechanistic roles of UBPs in *Arabidopsis*. Substrate recognition, Ub chain types preference and even their positioning on the target Ub chain might determine the specificity of UBPs. However, the detailed information about the substrate specificity of AtUBPs is limited. Most of human UBPs (which named USPs) are nonspecific and will cleave nearly any Ub chain type, while some members also display distinct specificities (McGouran et al. 2013). Similarly, AtUBPs might cleave various types of Ub chain, but the preference to targets and cleaving efficiency should be different due to particular structure of each type of Ub chain and/or the substrate itself. At present, most AtUBPs function on linear or K48-linked polyubiquitin chains, but no direct evidence has been found that UBPs also work on K63-linked polyubiquitin chains in plants. For example, AtUBP3, 4 and 5 exhibit preference for different linear Ub chain with specific Ub–Ub linkage (Chandler et al. 1997; Raonaik et al. 2000), while AtUBP12 and 13 show activity towards K48-linked diubiquitin (Ub<sub>2</sub>) (Ewan et al. 2011). Since the number of UBPs (even DUBs) is much smaller than that of E3 ligases in plants, each UBP might have more than one substrates (Moon et al. 2004; Nijman et al. 2005). To deal with a broader range of Ub-conjugated proteins, UBPs may not interact with the target proteins directly but interact with the attached Ub chain. In this way, Ub chain type and structure might be critical for UBPs specificity. Taken together, the overall picture of UBPs substrate specificity in *Arabidopsis* remains incomplete.

Accumulating evidences have revealed that UBPs function in plant development and stress responses via genetic or epigenetic regulation. However, the *in vivo* substrates and interacting partners as well as the physiological roles of most UBPs are still poorly defined. Therefore, the molecular functions of UBPs are far from being understood in plants. Here we will briefly review the biological roles of UBPs in various physiological processes in model plant *Arabidopsis*.

### UBPs function in plant development

During the life cycle of flowering plants, their growth and development are tightly controlled through the integration of the physiological status of the seedling and environmental cues. Data have shown that UBPs and deubiquitination might function in regulating plant development in *Arabidopsis*. Targets of several AtUBPs have been characterized, and the detailed molecular mechanisms of AtUBPs involved in plant development now begin to be uncovered.

Embryo development in higher plant seeds is a highly orchestrated process including cell division and



differentiation as well as pattern determination (Meinke 2003). During this process, a large number of proteins are implicated to ensure the single-celled zygote to develop into an organized multicellular embryo, which could produce a whole viable seedling (Meinke 2003). AtUBP14 has been identified as a regulator of embryo development in *Arabidopsis* and displays DUB activity on K48-linked or some linear Ub chains (Doelling et al. 2001). Data showed that growth of the *ubp14* mutant was arrested at embryo stage and accumulated high amount of ubiquitinated proteins, which was consistent to the DUB activity of AtUBP14. In this way, Ub/26S proteasome pathway and AtUBP14 together plays an important role in plant embryogenesis, however, the molecular mechanisms by which AtUBP14 modulates embryo development is elusive (Doelling et al. 2001). In addition, the *ubp19* mutant was also defective in embryogenesis, indicating that AtUBP19 might participate in embryo development in *Arabidopsis* (Liu et al. 2008).

In plant seeds, the development of the embryo and endosperm is highly coordinated (Berger et al. 2006; Lafonplacette and Kohler 2014). The *FIS* genes encode Polycomb group (PcG) proteins and mediate H3K27me3 modification of *PHERES1* (*PHE1*), a regulator involved in seed development (Makarevich et al. 2006). AtUBP26 loss-of-function mutant displayed a weak phenotype of autonomous endosperm and arrested seed development (Luo et al. 2008). *PHE1* was upregulated in the *ubp26* ovules while the H3K27me3 level at the *PHE1* locus decreased, suggesting that AtUBP26 is required for addition of H3K27me3 to *PHE1* (Luo et al. 2008). AtUBP26 might be necessary for the *FIS* PcG function in seeds, however, we cannot exclude the possibility that AtUBP26 regulates the H3K27me3 modulation of *PHE1* locus through a *FIS*-independent manner. Since AtUBP26 can reverse monoubiquitination of H2B (H2Bub1) and *ubp26* mutant accumulates high level H2Bub1 (Sridhar et al. 2007), we propose that H2B deubiquitination at *PHE* locus mediated by AtUBP26 functions in *FIS* PcG-dependent histone H3 methylation and transcriptional regulation. Plants with AtUBP12 and 13 loss-of-functions also developed autonomous endosperm in the absence of fertilization (Derkacheva et al. 2016). AtUBP12 and 13 function as H2A deubiquitinases in plants, and AtUBP12-overexpressing transgenic plants accumulates lower level of H2Aub1, consistent to its DUB activity towards H2A. AtUBP12 and 13 work together with LHP1, a component of plant specific PcG proteins system, in regulating PcG target gene expression. AtUBP12 could bind to PcG target chromatin and H2K27me3 level decreased in *ubp12 ubp13* mutant (Derkacheva et al. 2016).

Seed size is another important morphological character of plants and contributes to plant evolutionary fitness and stress responses (Leishman and Westoby 1994; Gegas et al. 2010; Linkies et al. 2010). The *Arabidopsis da1-1*

mutant was isolated with increased seed and organ size (Li et al. 2008), which possesses a mutation in the Ub receptor DA1 protein, which acts maternally to affect seed size by restricting cell proliferation in the integuments (Li et al. 2008; Xia et al. 2013). SUPPRESSOR2 OF DA1 (SOD2)/AtUBP15 functions as a positive regulator of seed size (Du et al. 2014). *sod2/ubp15* mutant produced small seeds compared to wild type, while overexpression of AtUBP15 resulted in large seeds. AtUBP15 promotes cell proliferation in maternal integuments of ovules as well as in developing seeds. AtUBP15 contains a MYND-type zinc finger domain, which might contribute to protein–protein interaction between AtUBP15 and its partner(s). DA1 interact with and targets AtUBP15 to degradation by the Ub/26S proteasome system (Du et al. 2014). Future work is required to identify the possible E3 ligase that responsible for AtUBP15 ubiquitination. The mechanistic role of AtUBP15 in organ size regulation still needs to be studied.

Leaf cell size is positively correlated with endopolyploidy level in plants (Melaragno et al. 1993; Gonzalez et al. 2010). The mitotic cyclins and cyclin-dependent kinase (CDK) complexes function as essential negative regulators of endocycle (Nowack et al. 2012; Edgar et al. 2014). The anaphase promoting complex/cyclosome (APC/C) selectively ubiquitinates mitotic cyclins, targeting them to degradation via the Ub/26S proteasome, then affect endocycle (Capron et al. 2003; Zielke et al. 2008; Heyman and Veylder 2012). AtUBP14 physically interacts with UV-B-INSENSITIVE4 (UVI4), an inhibitor of APC/C (Hase et al. 2006), and functions antagonistically with CELL CYCLE SWITCH52 A1 (CCS52A1), an activator of APC/C (Larson-Rabin et al. 2009). In this way, APC/C activity might be repressed by AtUBP14, leading to impaired endoreduplication (Xu et al. 2016). AtUBP14 loss-of-function resulted in decreased abundance of cyclin A2;3 (CYCA2;3) and CDKB1;1. Consistently, *ubp14* displays large cotyledon and leaf with higher ploidy level (Xu et al. 2016). The authors reported that UVI4 could not be deubiquitinated by AtUBP14 and the mechanistic link between AtUBP14 and UVI4 is not clear. It is interesting to confirm whether AtUBP14 regulates the ubiquitination status of CYCA2;3 or CDKB1;1 directly. In summary, AtUBP14 functions in regulating endoreduplication and leaf size.

Plant floral transition depends on exact perceptions of both internal and environmental cues at optimal time during their life cycle (Searle 2003; Jung and Muller 2009). The circadian oscillators participate in regulating the expression of *CONSTANS* (*CO*), which subsequently upregulates *FLOWERING LOCUS T* (*FT*) to promote flowering in response to proper day length (Suarezlopez et al. 2001; Turck et al. 2008; Harmer 2009). *FLOWERING LOCUS C* (*FLC*), a MADS-box transcription factor that represses the

expression of multiple genes related to flowering, is also involved in the floral transition and functions as a negative regulator of flowering in plants (Pien et al. 2008; Xu et al. 2008; Michaels and Amasino 1999; Sheldon et al. 1999). AtUBP12 and 13 are involved in regulating circadian clock and photoperiodic flowering. The *ubp12 ubp13* double mutant displays phenotype of early flowering and short periodicity of circadian rhythms. AtUBP12 and 13 regulate photoperiodic flowering possibly through a *CO*-dependent pathway (Cui et al. 2013). Histone H2B ubiquitination status at *FLC* locus has been implicated in its transcriptional regulation (Cao et al. 2008; Schmitz et al. 2009). AtUBP26 loss-of-function resulted in an early-flowering phenotype. *FLC* and its related family members were downregulated in *ubp26* mutant. H2Bub1 level markedly accumulated while H3K36me3 and H3K27me3 level decreased at *FLC* locus in *ubp26* compared to that in wild type (Schmitz et al. 2009). Therefore, AtUBP26 is required for transcriptional activation of *FLC* by deubiquitinating H2Bub1 to maintain the H3K36me3 status, which in turn regulate plant response to internal or environmental signals (Sridhar et al. 2007). It is proposed that the H2B ubiquitination status has essential impacts on the recruitment of histone methyltransferase to corresponding chromatin region, resulting in altered histone H3 methylation. Please refer to the Zhang's review paper for more insights in the possible mechanism (Zhang 2003). Interestingly, AtUBP15 has also been reported to be involved in plant flowering time control, however, the mechanisms is elusive (Liu et al. 2008).

Plant sexual reproduction following flowering depends on delivery of the sperm to the egg in the ovary via the long and polarized pollen tube (Higashiyama and Takeuchi 2015). Pollen tube growth and development are coordinately modulated by a series of regulatory processes (Taylor and Hepler 2003; Hepler et al. 2012; Steinhorst and Kudla 2013; Qu et al. 2015). Molecular cues produced by pollen tube itself or by the female tissues could serve as signals for pollen tube attraction and transmission (Hepler et al. 2001; Kanaoka and Higashiyama 2015). Studies revealed that the homologous AtUBP3 and 4 might function in pollen development and/or transmission (Doelling et al. 2007). The *ubp3 ubp4* pollen often fails to undergo mitosis II and possesses defects in vacuole and endomembrane organization, resulting in failure in fertilization and lethality. In addition, this mutant is also defective in gametogenesis and displays pollen germination defects (Doelling et al. 2007). These results suggested that AtUBP3 and 4 is implicated in pollen tube development, however, the underlying mechanism still needs to be characterized.

Mitochondria are house-keeping organelles with dynamic morphology and function (Friedman and Nunnari 2014). In *Arabidopsis*, the homologous dynamin-related proteins (DRPs), DRP3A and DRP3B, are large GTPases

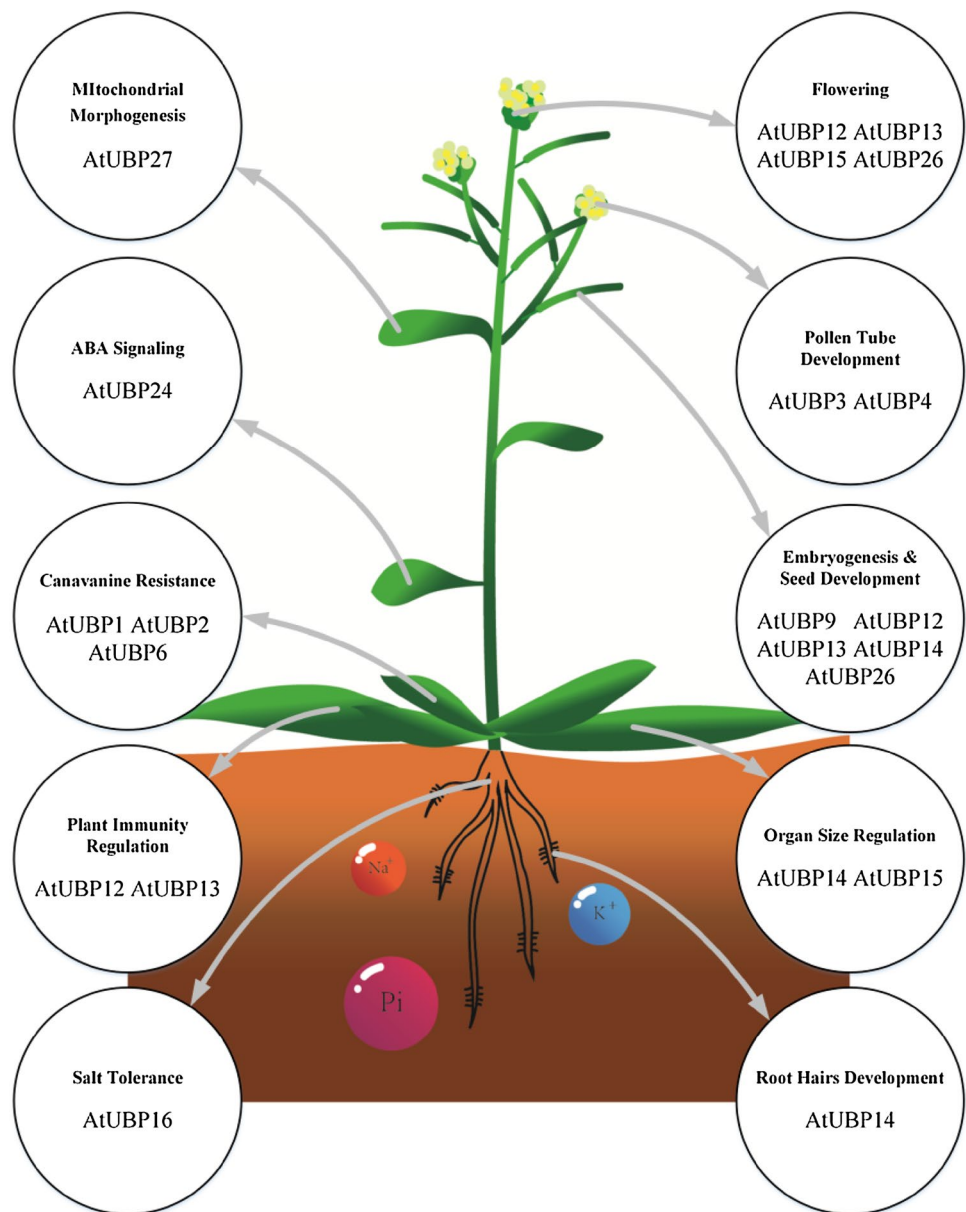
function as the main mitochondrial fission factors and participate in mitochondria morphogenesis (Aung and Hu 2012). Phosphorylation and ubiquitination of DRPs have been reported to function in mitochondrial morphology modulation during mitosis (Kerscher et al. 2006; Wang et al. 2012). AtUBP27 is a mitochondrial-located DUB which might play a role in its morphogenesis or function (Pan et al. 2014). AtUBP27 over-expression results in altered mitochondrial morphology although its loss-of-function mutants do not display obvious phenotypes. AtUBP27 reduces the association of DRP3 proteins with mitochondria, possibly facilitating the recycling of DRP3 proteins from mitochondria to the cytosol (Pan et al. 2014). Since the direct interaction between AtUBP27 and DRP3 was not observed, it is hard to conclude that DRP3 is a target of AtUBP27. In addition, there is no report about ubiquitination of DRP3, which further supports AtUBP27 regulates DRP3 possibly in an indirect manner. It might be interesting to determine whether the respiration in *ubp27* is changed due to altered mitochondrial morphogenesis.

### UBPs function in plant stress responses

As sessile organisms, plants must cope with multiple environmental abiotic or biotic stresses during their life cycle. Ub/26S proteasome system has been implicated in plant stress tolerance regulation, possibly through affecting the stability, activity or subcellular localization of their substrates (Vierstra 1996; Haglund and Dikic 2005; Dreher and Callis 2007). Studies have revealed that UBPs participate in plant salt tolerance, ABA signaling, drought tolerance, plant nutrients deficiency response, toxic amino acid analog resistance as well as plant immunity regulation in *Arabidopsis* (Fig. 3; Yan et al. 2000; Li et al. 2010; Ewan et al. 2011; Zhou et al. 2012; Zhao et al. 2016). With the discovery of specific substrates or target proteins, the regulatory roles of UBPs in plant stress responses will be better understood in the future.

Soil salinity is an important and constantly increasing abiotic stress, which reduces the crop productivity significantly worldwide (Zhu 2001; Hasegawa 2013). Plant cellular ion and redox homeostasis are always disrupted under salt stress condition (Hasegawa 2013; Paul 2003; De Gara et al. 2010). Furthermore, disordered/unfolded proteins which induce endoplasmic reticulum (ER) stress in plants accumulate during salt stress (Urade 2009; Liu et al. 2011). Salt-Overly-Sensitive (SOS) pathway is specifically activated during salt stress to maintain ion homeostasis (Shi et al. 2000; Qiu et al. 2002; Quan et al. 2007; Zhou et al. 2012). It has been reported that mutation of SHM1, the major serine hydroxymethyltransferase isozyme in *Arabidopsis*, leads to ROS over accumulation and the mutant

**Fig. 3** The overall biological functions of UBPs in *Arabidopsis*. Together with ubiquitination machinery, AtUBPs contribute to Ub pool homeostasis regulation and function in various plant development and stress responses via genetic or epigenetic regulation



is sensitive to salt stress and pathogens (Somerville and Ogren 1981; McClung et al. 2000; Moreno et al. 2005; Voll et al. 2006). SHM1 functions in photorespiratory regulation (Jamai et al. 2009). AtUBP16 has been reported to be required for plant salt tolerance by regulating PM  $\text{Na}^+/\text{H}^+$  antiport activity and SHM1 activity (Zhou et al. 2012). AtUBP16 interacts with SHM1 and modulates deubiquitination of SHM1 *in planta*, which regulates its stability and activity (Zhou et al. 2012). The degradation of SHM1 was enhanced in *ubp16* mutant compared with the wild type, implying that AtUBP16 stabilizes SHM1 by removing the conjugated Ub. *ubp16* mutant accumulates lower level of SHM1, but not SHM4 in contrast to wild type. In addition, AtUBP16 affect the proper subcellular localization of SHM1. Ser hydroxymethyltransferase activity is much

lower in the *ubp16* mutant than that in the wild-type. DUB activity of AtUBP16 is required for its function in salt tolerance regulation. These results suggest that deubiquitination of specific Ub-conjugated components of the photorespiratory pathway is critical for maintenance of ion homeostasis and cell death repression during salt stress (Zhou et al. 2012).

Plant hormone abscisic acid (ABA) plays an important role in many physiological processes, including seed maturation, germination and plant stress responses (Cutler et al. 2010). The ABA signaling pathway and its major components have been well characterized (Ma et al. 2009; Park et al. 2009; Raghavendra et al. 2010; Miyakawa et al. 2013). Ubiquitination also functions in ABA signaling and Ub E3 ligases involved in ABA signaling pathway have

been identified such as AIP2, DDA1 and KEG (Zhang et al. 2005; Liu et al. 2011; Luisa et al. 2014). These E3 ligases could ubiquitinate different components of ABA signaling pathway and regulate their stability or activity, resulting in altered plant ABA signal transduction. In contrast, deubiquitination is also involved in ABA response and AtUBP24 is a negative regulator of the ABA signaling in *Arabidopsis* (Zhao et al. 2016). Loss-of-function mutant *ubp24* was hypersensitive to ABA during seedling growth. However, the *ubp24* mutant exhibited reduced ABA sensitivity in the guard cells, which resulted in rapid water loss and elevated sensitivity to drought stress than the wild type. These findings suggest that AtUBP24 might have different targets in ABA signaling pathway. AtUBP24-mediated DUB activity is essential for plant ABA response. Genetic analysis revealed that ABI2 was downstream of AtUBP24. Interestingly, AtUBP24 also participates in salt tolerance in plant, however, the underlying mechanism is still not clear (Zhao et al. 2016).

Plant root hairs mediate the uptake of water and provide access to immobile nutrients (Gilroy and Jones 2000; Lopez-Bucio et al. 2003). The differentiation of root hair depends on the position with regard to the underlying cortical cells and controlled by a coordinated regulatory circuit (Bernhardt et al. 2005; Kwak and Schiefelbein 2007; Ishida et al. 2008; Schiefelbein et al. 2009). Root epidermal pattern is plastic, especially under challenging environment such as immobile nutrients deficiency (Salazarhenao et al. 2016). Upon phosphate (Pi) deficiency, root epidermal cell elongation is reduced, while the frequency of root hairs per unit of root length increases significantly (Sanchezcalderon et al. 2005; Peret et al. 2011). Pi starvation responses is regulated by a conserved Myb transcription factor PHR1 and the Pi deficiency-specific root hairs development might be controlled by the WER cascade (Rubio et al. 2001; Schachtman and Shin 2007). A weak allele of AtUBP14 mutant, *per1*, displays a Pi-specific defect in root hair elongation and epidermal cell differentiation (Li et al. 2010). AtUBP14 is involved in regulating expression of genes that are important for the signaling of Pi deficiency. Interestingly, phenotype of *per1* might not be caused by Pi deficiency, but is rather caused by disturbed Pi signaling. Together, AtUBP14 functions in the adaptive root hair development to Pi deficiency or even general nutrient availability in the environment (Li et al. 2010). However, the relationship between AtUBP14 and WER cascade still needs to be determined in the future.

Canavanine (CAN) is produced as an anti-herbivore compound in certain legumes. Its toxicity depends on the ability to substitute for Arg during translation (Racioppi et al. 1981). CAN can substantially alter the charge and structure of the proteins (Rosenthal 1991; Baetz and Martinola 2014). Plants with AtUBP1 or 2 loss-of-function

mutation were severely stunted with short roots and chlorotic leaves in the presence of CAN in the medium (Yan et al. 2000). It's believed that deubiquitination of specific factors by AtUBP1 and 2 could save them from degradation by the Ub/26S proteasome system, resulting in increased level of the substrates and CAN resistance. The underlying mechanisms and biological functions of AtUBP1 and 2 in CAN resistance need to be elucidated. AtUBP6 has also been reported to be able to restore the CAN resistance of the yeast *Δubp6* mutant (Moon et al. 2004). AtUBP6 contains a UBL domain and a Ca<sup>2+</sup>-dependent CaM-binding domain, which indicates that Ca<sup>2+</sup> signal might play a role in UBPs-mediated CAN resistance in plants (Moon et al. 2004).

Plants also suffer pathogen invasion and efficient defense mechanisms have been evolved in plants which encompass both basal immunity initiated by the recognition of conserved pathogen-associated molecular patterns (PAMPs), named PTI, and pathogen-specific responses triggered via pathogen effectors and plant-specific recognition events, named ETI (Dangl and Jones 2001; Zipfel 2008; Thomma et al. 2011). The hypersensitive response (HR) with a highly localized programmed cell death (PCD) in the infected region is induced during plant defense response, and restricts pathogen spread (Heath 2000). Many components involved in plant immunity including receptor-like kinases such as pattern recognition receptors (PRRs) and transcription factors as well as specific plant hormones have been well characterized (Zipfel 2008). PRRs might be ubiquitinated and targeted to degradation via the Ub/26S proteasome system as a suppression strategy of plant defense by pathogens, indicating that protein ubiquitination and the Ub/26S proteasome system participates in plant immunity regulation (Lu et al. 2011). Two UBPs, AtUBP12 and 13, are functionally redundant and participate in regulating plant immunity against virulent *Pseudomonas syringae* pv *tomato* (*Pst* DC3000) in *Arabidopsis*. AtUBP12 and its *Solanaceous* orthologue NtUBP12 were both identified as negative regulators of the Cf-9-dependent HR. The salicylic acid (SA) signaling pathway is involved in AtUBP12- and 13-mediated plant immunity regulation. AtUBP12/13 and NtUBP12 are functional DUBs and their DUB activity is required for plant immunity and defense response (Ewan et al. 2011). In this way, AtUBP12- and NtUBP12-mediated deubiquitination of specific substrates play important role in plant disease resistance.

## Concluding remarks and perspectives

In plants, ubiquitination functions as a central signaling mechanism for development and stress responses (Moon et al. 2004; Bartel and Cytovsky 2012; Lyzenga and Stone



2012; Marino et al. 2012). In contrast, plant UBPs and their DUB activity also participate in determining the destiny and activity of Ub-tagged substrates by cleaving Ub tags from target proteins (Isono and Nagel 2014). UBPs, together with other DUBs in plants, contribute to modulate the homeostasis of Ub pool during challenging environment, resulting in elevated plant stress tolerance (Fischer 2003). Increasing evidences demonstrated that UBPs function in plant development and stresses responses (Fig. 3). It should be noted that UBPs regulate their substrates not only by determining their stability. As it was shown for AtUBP26, which modulates histone H2B ubiquitination status, affects histone methylation and thus controls gene expression in the corresponding chromatin region (Sridhar et al. 2007). Another example is AtUBP16, which is involved in plant salt tolerance, deubiquitinates SHM1 and regulates its stability as well as subcellular localization (Zhou et al. 2012).

Substrates of most AtUBPs have not been found, thus precluding in-depth mechanistic understanding of UBPs in *Arabidopsis*. To elucidate the biological functions of AtUBPs, the identification of the targets of specific AtUBP is required. Structural studies proved that UBPs often interact with the Ub chain rather than their target proteins directly (Hu et al. 2002). In this way, the identification of AtUBP targets is difficult. Methods combined with interacting protein screening, proteomics investigation as well as plant mutant identification might be used for UBPs substrates determination in the future. Intact multivesicular body (MVB) and its functional component ESCRT are necessary for plasma membrane (PM)-bound cargo proteins transport and autophagosomal degradation (Filimonenko et al. 2007; Rusten et al. 2007; Lee et al. 2009; Zelazny et al. 2011). Two metalloprotease type DUBs, AtAMSH1 and 3, interact with ESCRT-III subunit and function in regulating endocytosis and autophagosomal degradation, then play a critical role in autophagy-mediated plant development and stress response (Katsiarimpa et al. 2013). Although AtUBPs and AtAMSHs belongs to different DUB subfamilies, we cannot exclude the possibility that some AtUBPs could also associate with MVBs (possibly through ESCRT) and participate in endocytosis as well as autophagic degradation regulation. To address this hypothesis will provide new evidences for understanding the biological functions of AtUBPs.

The other important question needs to be resolved is how the function of UBPs to be regulated. To address this question, UBP-interacting partners screening and determination of possible post-translational modifications (PTMs) on UBPs should be performed. DUBs can be regulated both at transcriptional and posttranslational level and UBPs might be regulated in a similar way (Huang and Cochran 2013). For example, circadian control of the expression

of AtUBP12 and 13 has been observed (Cui et al. 2013). In addition, future studies should focus on the balance between the two opposite processes, ubiquitination and deubiquitination, in plants and the resulted substrates fate and activity regulation. Elucidation of the biological functions of UBPs in plants will definitely shed light on understanding plant adaption towards changing environment and crop improvement in agriculture.

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