International Journal of Plant Developmental Biology ©2007 Global Science Books

The Charming Complexity of CUL3

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ABSTRACT

Ubiquitination is a fascinating regulatory tool for various biological processes, mostly for the control of rapid and selective degradation of important regulatory proteins involved in cell cycle and development, among others. The superfamily of cullin-RING finger protein complexes is the largest known class of E3 ubiquitin ligases and several substrates have been described in different organisms. In plants, cullins can be grouped into at least four subfamilies, and each subfamily associates with a specific class of substrate receptors that often belong to larger protein families. Consequentially, the corresponding complex interaction patterns indicate that numerous substrate proteins are ubiquitinated by plant E3 ligases. In this review we recapitulate recent findings on a newly identified plant E3 ligase family that contains class 3 cullins (CUL3) and BTB/POZ proteins as their corresponding substrate adaptors. Here, three main aspects will be described: 1) the molecular composition of CUL3-based E3 ligases, 2) BTB/POZ domain containing proteins and their role in substrate recognition, and 3) comparison of plant and other eukaryotic CUL3-based E3 ligases to provide an outlook on potential roles of this specific E3 ligase family in higher plants. By focusing on these points, the review will provide a perspective on the impact of CUL3-based E3 ligases on plant development.

Keywords: Ubiquitin Proteasome Pathway, E3 ligases, BTB

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CULLIN-BASED E3 LIGASES AND THE UBIQUITIN PROTEASOME PATHWAY

Cullin-based E3 ligases have been the focus of intensive research within the last decade. They are central scaffolding subunits of multimeric E3 ligases which assist in the transfer of ubiquitin (UBQ) moieties to substrate proteins. E3 ligases function as regulators through which many proteins are tagged for degradation via the UBQ proteasome pathway what can result in recognition by the 26S proteasome, a higher order protein complex of around 1 MDa in size with chaperone and protease activities (for a general overview see Dreher and Callis 2007).

The process of ubiquitination is highly conserved in eukaryotes and comprises three basic steps: UBQ is first activated in an ATP-dependent manner by an E1 UBQ activaating enzyme that transfers the UBQ to an E2 UBQ conjugating enzyme which in turn can interact with an E3 ligase to allow ubiquitination of a target protein at a conserved lysine residue. This final step is crucial and can result in mono- or poly-ubiquitination of the target (**Fig. 1A**). Mono-ubiquitination causes activation or translocation but not degradation of the modified protein. For example, histones and often transcription factors are mono-ubiquitinated with a positive effect on RNA polymerase II activities and transcription of specific target genes (Carter *et al*. 2005; Pavri *et al*. 2006). In addition, attachment of a single UBQ to various integral membrane proteins at their cytosolic domains is required for their sorting into multivascular bodies (Reggiori and Pelham 2001; Katzmann *et al*. 2004). Alternatively, polyubiquitin chains can be built up by the E3 ligase and these may have alternative attachment sites at lysine (K) residues 6, 29, 48, or 63 of the next connected UBQ. UBQ-K-29 and UBQ-K-48 are well established recognition signals by the 26S proteasome leading to proteolysis of the modified protein (Chastagner *et al*. 2006). In contrast K-6 and K-63 chains appear not to have a destabilizing effect but rather have been implicated in non-proteolytic pathways like kinase activation, DNA damage tolerance, and like mono-ubiquitination with protein trafficking and synthesis (Galan and Haguenauer-Tsapis 1997; Sun and Chen 2004; Wen *et al*. 2006). Although K-6 and K-29 chains have only been described in yeast and animals it is likely that both chain formations are also present in plants based on the high degree of conservation of both the UBQ proteins and the UBQ conjugation machinery.

Received: 2 February, 2007. Accepted: 18 February, 2007.

Invited Mini-Review

BASIC COMPOSITION OF COMMON PLANT CULLIN-BASED E3 LIGASES

Three related cullin-based E3 ligases are known in plants that contain the cullins 1, 3a/3b or 4. All these cullins are scaffold proteins in E3 ligase complexes of similar composition in so far that in all cases the cullin assembles with a RING (Really Interesting New Gene)-finger protein RBX1 in its C-terminal part and with a substrate adaptor in its N-terminal part (**Fig. 1B**). Here, two α-helices, H2 and H5, are critical for interaction between the cullin and its substrate adaptor which has been shown by crystallization of human cullin-based E3 ligases and mutational analysis of corresponding plant E3s (Xu *et al*. 2003; Goldenberg *et al*. 2004; references in Figueroa *et al*. 2005; Angers *et al*. 2006).

The best understood E3 ligase in higher plants is the SCF (Skp1-Cullin-F-box-protein) - complex that consists of RBX1, the cullin CUL1, a SKP1 (*Saccharomyces cerevisiae* suppressor of kinetochore protein 1) homolog, and a substrate adaptor protein with an F-box domain (Gray *et al*. 2002). The RING-finger protein is required to bind E2, whereas the SKP1-homologue builds up a heterodimer with the F-box protein to assemble with the cullin. The second class is the BRC3 (BTB-RBX1-Cul3) E3 ligase with CUL3 as the cullin subunit. BRC3 E3s have a similar composition like SCF-complexes but use BTB (Broad complex, Tramtrack, Bric-à-Brac) -proteins as substrate adaptors (Wang *et al*. 2004; Figueroa *et al*. 2005). Only very recently, composition of a CUL4-based E3 ligase DRC4 (DDB1-RBX1-CUL4) has been described in *Arabidopsis thaliana* that recruits the DNA-Damaged Binding proteins DDB1a and DDB1b as substrate adaptors (Bernhardt *et al*. 2006; Chen *et al*. 2006). In all three cases, specificity of the different E3 ligase complexes is principally defined by their individual substrate adaptors. Especially SCF- and BRC3-E3s are characteristic for their high diversity of substrate adaptors. For example, in *Arabidopsis* around 700 F-box proteins and more than 100 BTB-proteins are predicted (Gagne *et al*. 2002; Stogios *et al*. 2005; http://www.ebi.ac.uk/interpro). Although for most of these proteins a precise function and complex assembly is missing, these numbers indicate that both classes of E3 ligases are prone to target various cellular proteins to the ubiquitination machinery.

REGULATORY MECHANISMS TO CONTROL CULLIN-BASED E3 LIGASE ACTIVITIES: RUB1/NEDD8-MODIFICATION, CSN AND THE CAND1 PROTEIN

An interesting feature of CUL1, CUL3 and CUL4-based E3 ligases is their modification with a protein related to UBQ called RUB1 in plants or NEDD8 (Neural-precursor-cell-Expressed and Developmentally Down regulated 8) in animals and humans (reviewed in Chiba and Tanaka 2004). Conjugation of a RUB-protein to a cullin requires a machinery similar to the UBQ-conjugating pathway which in *Arabidopsis* is represented by the E1-like heteromer AXR1/ECR1 (Auxin Resistant 1/Enzyme Conjugating RUB1) and an E2-like protein RCE1 (RUB1-Conjugating Enzyme 1) (Dharmasiri *et al*. 2003) (**Fig. 1A**). RUB-modification occurs on a single lysine residue, which is located at the very C-terminal highly conserved winged-helix domains of the cullin (for CUL1 (K682), for CUL3a/b (K678), and potentially K738 in CUL4) (Hellmann *et al*. 2003; Weber *et al*. 2005).

The effect of RUB-modification is still not fully understood. Although it does not lead to instability of the modified cullin it clearly affects activity of cullin-based E3 ligases since defects in RUB-modification stabilize target proteins (Schwechheimer *et al*. 2001). Since RCE1 directly binds to RBX1, Dhamasiri *et al*. (2003) proposed that the RUB-conjugation enzyme competes for binding with E2s from the ubiquitin conjugation pathway which can affect E3 activities. Alternatively, it was speculated that RUB-modification of cullins is required for association of the RING-

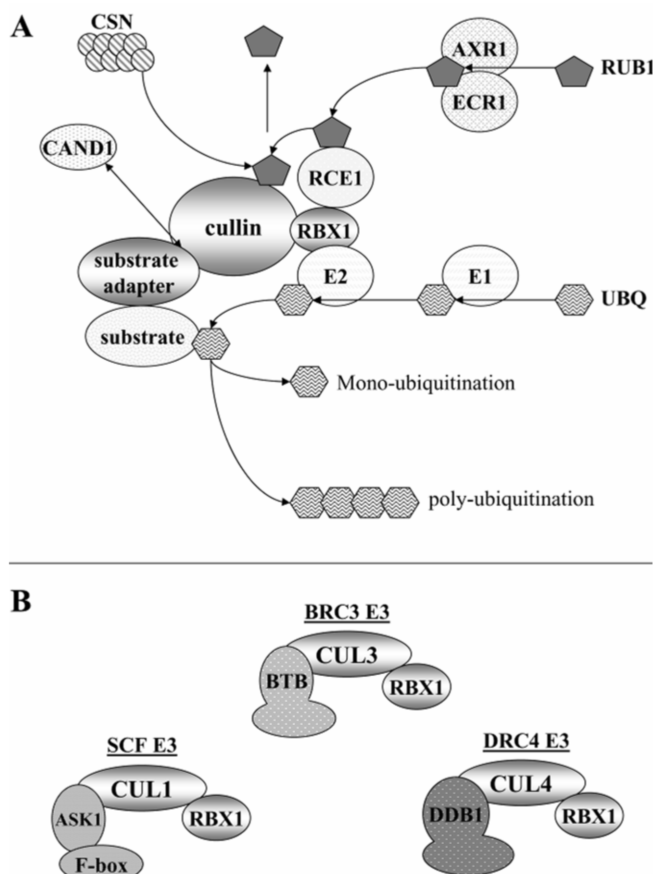

Fig. 1 Schematic models for cullin-based E3 ligase composition and interconnected pathways. (**A**) E3 ligases receive UBQ via an E1/E2-dependent UBQ-conjugation pathway and transfer the UBQ to a substrate protein. Cullin-based E3 ligases (cullin-RBX1-substrate adaptor) are targets for a RUB-conjugation pathway that affect activity of the E3 ligase. RUB-moieties can be removed by the COP9 signalosome (CSN). CAND1 competes with substrate adaptors for binding to the cullin. (**B**) Schematic drawing of the three RBX1-cullin based E3 ligases in plants. From left to right: SCF-complexes contain dimeric substrate adaptors of a SKP1-homologue (in Arabidopsis ASK) and an F-box protein, BRC3 and DRC4 E3s use the monomeric substrate adaptors BTB/POZ-protein and DDB1, respectively.

finger protein to the cullin since the RUB-modification site is closely located to the cullin-RBX1 binding sites (Wu *et al*. 2000; Chiba and Tanaka 2004). Removal of the RUB-protein from the cullin, however, is as crucial as RUB-conjugation which was demonstrated by work on the COP9 signalosome (CSN) (Schwechheimer *et al*. 2001). Here, the CSN5 subunit has RUB-de-conjugating activity (**Fig. 1A**). Although more cullin protein is RUB-modified in *csn5* mutants, proteasome dependent substrate turnover is strongly reduced (Schwechheimer *et al*. 2001; Dohmann *et al*. 2005). This observation suggested that it is the dynamic cycling of cullin RUB-modification and -removal on the cullin which is crucial for normal E3 activity (Schwechheimer *et al*. 2001; Dohmann *et al*. 2005).

Findings in plants and animals also imply that cullin-based E3 ligase activity is strongly affected by the concerted interplay of CAND1 (Cullin Associated and Neddylation-Dissociated 1), CSN activity and the RUB-conjugation pathway. CAND1 preferentially interacts with an unmodified cullin at its N-terminal and C-terminal regions and competing with the substrate adaptor subunit (Alonso-Peral *et al*. 2006) (**Fig. 1A**). It is proposed that this interaction with CAND1 removes the now unmodified cullin from the CSN complex and leaves an inactive E3 ligase by dissociation of cullin and substrate adaptor subunits. In turn, RUB-modification of the cullin would favour re-assembly of the substrate adaptor subunit to the cullin and allows the ubiquitination process to resume (Cope and Deshaies 2003). This

cycling of disassembly and assembly provides an attractive concept for how substrate adaptors can target substrates to the E3 ligase for ubiquitination.

In plants CAND1 interaction was only demonstrated for *Arabidopsis* CUL1 and CUL4 (Alonso-Peral *et al*. 2006; Moon *et al.* 2007). However, in humans binding of CAND1 to CUL3 has also been shown (Lo and Hannink 2006), based on the high homology between human/animal and plant cullins it is likely that CAND1 acts as regulator of plant BRC3 E3 ligases, too.

An interesting finding was recently made by Wimuttisuk and Singer (2007) who demonstrated that human CUL3 proteins assemble to heterodimers linked by NEDD8. The authors could show that such a heterodimer is present *in vivo* and is required for ubiquitination of the substrate protein cyclin E. This opens up a new, fascinating perspective on the meaning of RUB-modification and might also allow the possibility that via RUB/NEDD8 higher order cullin-containing E3 ligases are build up that not only consist of the same but also of different cullin members.

FUNCTION OF BRC3 E3S IN PLANTS

Whereas, SCF-complexes have been well explained in various developmental and physiological processes such as embryogenesis, and phytohormone and light signal transduction pathways (Dreher and Callis 2007), there is limited knowledge about plant CUL3-based E3 ligases. First clues about complex composition of this specific type of E3 ligase stems from work in *Caenorhabditis elegans* in which CUL3 was described to recruit BTB-proteins as substrate adaptors (Figueroa *et al*. 2003; Pintard *et al*. 2003; Xu *et al*. 2003). This was followed by the identification of several CUL3-interacting BTB-proteins in plants which contain either just the BTB-motif or have an additional domain like MATH (Meprin And Traf Homology), armadillo, or tetratricopeptide (TPR) repeats, respectively (Wang *et al*. 2004; Dieterle *et al*. 2005; Gingerich *et al*. 2005; Weber *et al*. 2005). While in plants a variety of CUL3-interacting BTB-proteins were successfully identified, knowledge about substrate proteins still remains poor. At this point the only good candidate is ACS5 which belongs to the 1-aminocyclopropane-1-carboxylic acid synthase (ACS) family that catalyzes a final step in ethylene biosynthesis (Wang *et al*. 2004). Wang and co-workers could demonstrate that the BTB protein ETO1 (Ethylene Overproducer 1) interacts with ACS5 via its TPR motif and with CUL3 via its BTB domain. Most importantly, however, proteasomal degradation of ACS5 is strongly reduced in an *eto1* mutant background indicating that ETO1 acts as a negative regulator of ACS5 activity (Wang *et al*. 2004). Although *in planta* activity of a CUL3-based E3 ligase activity is still lacking, the work from Wang *et al*. (2004) provided strong evidence for a $BRC3^{ETO1}$-dependent degradation of ACS5. Besides this involvement in ethylene biosynthesis, CUL3 has also been implicated in phytochrome A dependent red light response showing reduced inhibition of hypocotyl growth under far-red light conditions and changed *COP1* expression (Dieterle *et al*. 2005). The mutant is also slightly late-flowering with more rosette leaves at the onset of flowering (Dieterle *et al*. 2005). Although loss of CUL3a has only minor impact on plant development, loss of both CUL3 proteins is dramatic causing arrest of embryogenesis mostly at the heart stage and also affecting endosperm development (Figuoera *et al*. 2005; Thomann *et al*. 2005). As mentioned above, *Arabidopsis* contains more than 100 BTB proteins (Interpro database). However, for most of these the molecular functioning, assembly with other proteins and their biological roles are hardly understood. So far biological function has been assigned to NPH (Non-phototrophic hypocotyl)-3 (blue light signal transduction; Motchoulski and Liscum 1999), ETO1 (Wang *et al*. 2004), NPR (Non-expressor of PR genes)-1 (salicylic and jasmonic acid signal transduction; Rochon *et al*. 2006), POB (POZ-BTB protein)-1 (sugar metabolism; Thelander *et al*. 2002), RPT (Root Phototropism)-2 (phototropic response and stomatal opening; Inada *et al*. 2004), ARIA (Arm-Repeat Protein Interacting with ABF2) (absciscic acid signalling; Kim *et al*. 2004), BOP (Blade-on-Petiole) (lateral organ development; Norberg *et al*. 2005), and the BT (BTB-TAZ) (transcriptionnal regulation, control of telomerase activity; Ren *et al*. 2007) proteins. Yet, except for ETO1 (Wang *et al*. 2004), it is uncertain whether any of these are part of a BRC3 complex that targets substrates for ubiquitination. Since specificity of the E3 ligase is defined by its substrate adaptors, it is crucial for the understanding of plant CUL3-E3 ligase function to discuss this protein family in greater detail.

BTB-CONTAINING PROTEINS: IDENTIFICATION AND CROSS-SPECIES DISTRIBUTION

Originally, the BTB/POZ domain was identified as a protein-protein interaction motif in poxvirus non-DNA-binding proteins and in zinc finger transcription regulators *Bric à Brac*, *Tramtrack* and *Broad-Complex* from *Drosophila melanogaster.* The BTB domain can mediate both self association and interaction with non-BTB structures such as CUL3 proteins (Pintard *et al*. 2003; Wang *et al*. 2004; Weber *et al*. 2005). Based on crystal structure analyses, a BTB fold was also discovered in three other protein families: Skp1, ElonginC (transcription elongation factor subunit C), and voltage-gated potassium channel T1 (T1-Kv) proteins (Stogios *et al*. 2005). ElonginC is a subunit of the cullin-based VHL (von-Hippel-Lindau) E3 ligase, although this specific class of E3 has not been described in plants. However, the presence of the BTB fold in BTB-, SKP1- and elonginC- proteins indicates a more general motif for cullin-substrate adaptor assembly.

Analyzing the InterPro database (http://www.ebi.ac.uk/interpro) it becomes palpable that the motif is scarce in bacteria with members found for example in *Leptospora* and *Clostridium* strains and in archeae there is the unique example of the chlamydia-related symbiont *Acanthamoeba* sp. UWE25 having 43 BTB-leucine-rich repeat proteins. Surprisingly there are a variety of viruses such as *Vaccinia* and *Myxoma* encoding for BTB-domain containing proteins, and it will be interesting to elucidate what the precise function of these proteins in their hosts are. BTB-proteins are common in plants, nematodes, fungus, insects and vertebrates. For example the InterPro database predicts more than 100 BTB proteins in *D. melanogaster*, over 200 in *Oryza sativa*, and more than 300 in mouse and human. With only few exceptions, such as MATH- and armadillo-BTB proteins, the architecture of BTB-proteins is defined by a single BTB domain located at the protein's N-terminus, followed by a middle linker region and a secondary C-terminal motif often present as a set of tandem repeats (**Fig. 2**). It is the secondary motifs that define the classes of BTB protein. Based on these secondary motifs, around 11 different classes of BTB-domain proteins can be found in *Arabidopsis*, and even 18 in *O. sativa* (Gingerich *et al*. 2005; Interpro database) (**Fig. 2**). Recently, Stogios *et al*. (2005) compared 17 fully sequenced eukaryotic genomes for the presence of BTB-domain containing proteins. Interestingly, Stogios and co-workers found that many protein motifs which are found universally across plants and vertebrates become species-specific when associated with a BTB domain. For instance, *Arabidopsis* encodes for 21 BTB-NPH3 proteins that are presumably all involved in blue light signal transduction (Motchoulski and Liscum 1999), and this specific class of proteins is lacking in vertebrates. BTB-ankyrin proteins are present in yeast and plants but appear to be absent from *C. elegans*, mouse and human. *Arabidopsis* and presumably plants in general do not encode for any BTB-BACK-Kelch (BBK) proteins which are abundant in mouse and human (Stogios *et al*. 2005). Except for the plant-specific NPH3 motif, however, ankyrin is a widespread motif in both plants, vertebrates and *C. elegans*, and kelch proteins are present in plants. Hence, these different domain combinations clearly demonstrate domain shuffling and follow lineage-specific

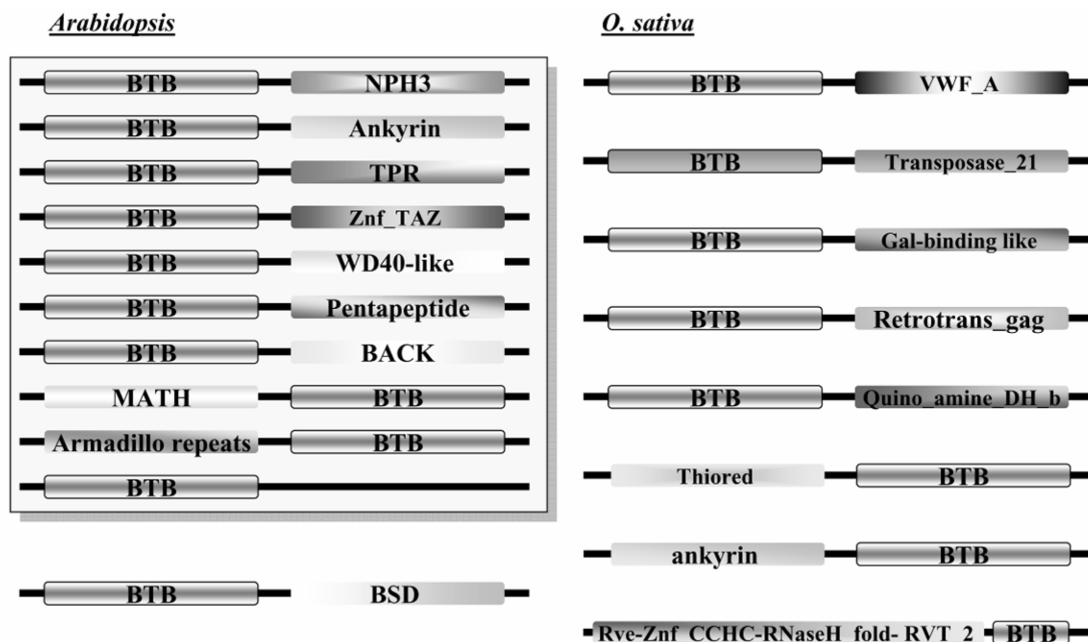

Fig. 2 Schematic drawing of BTB-domain proteins from *Arabidopsis* and *O. sativa* with secondary domains. Gray shaded box marks BTB-domain proteins in *Arabidopsis* which are also found with a similar domain composition in *O. sativa*. Proteins drawn outside the gray box were not found in either *Arabidopsis* or rice. *BACK*, BTB/Kelch-associated; *BSD*, BTF2-like transcription factors, Synapse-associated proteins and DOS2-like proteins; *Gal-binding like*, Galactose binding like; *MATH*, Meprin and TRAF homology; *NPH3*, Non-phototropic hypocotyl 3-like; *Retrotrans_gag*, Retrotransposon gag domain-containing protein; *Quino_amine_DH_b*, Quinoprotein amine dehydrogenase, beta chain-like; *RNaseH_fold*, Polynucleotidyl transferase, Ribonuclease H fold; *Rve*, Retroviral integrase, catalytic region; *RVT_2*, Reverse transcriptase, RNA-dependent DNA polymerase; *Thiored*, Thioredoxin-related; *TPR*, Tetratricopeptide TPR; *Transposase_21*, En/Spm-like transposon proteins; *VWF_A*, von Willebrand factor, type A; *Znf_CCHC*, Zinc finger, CCHC-type; *ZNF_TAZ*, Zinc finger, TAZ-type (protein domain compositions are presented based on predictions from the InterPro database (http://www.ebi.ac.uk/interpro).d DDB1, respectively.

expansion as evolutionary adaptation mechanisms in eukaryotes to engage new protein functions. In this context, Thomas (2006) proposed an interesting hypothesis that the high-diversity of substrate adaptors of E3 ligases like BTB-proteins and also F-box proteins represent an innate immunity system as a result of an 'adaptive evolution (positive selection) of host genes that mediate pathogen recognition and defence'. Thus a good part of the substrate adaptors might target foreign pathogen proteins from bacteria or viruses for degradation via the ubiquitin proteasome pathway. In turn, viruses and bacteria could have adapted to this mechanism which might explain the presence of BTB-proteins in viruses or recently described mechanisms of *Agrobacterium tumefaciens* using an F-box protein and the ubiquitin proteasome pathway to mediate integration of its T-DNA into the genomic DNA of the plant (Tzfira *et al*. 2004).

INTERACTION SURFACES OF THE BTB FOLD

Overall, the long evolutionary history and the diversity of domain-compilations already refer to the important role of the BTB interaction module in many different biological processes such as transcriptional regulation, cytoskeleton modelling, tetramerization/ion channel gating, and targeting of proteins for degradation as subunits of ubiquitin E3 ligases (for an overview see Perez-Torrado *et al*. 2006). To understand the diversity of interaction patterns the BTB-fold is capable of mediating, a reflection on the BTB fold binding profile is required. Stogios *et al*. (2005) defined the core sequence of the BTB fold as approximately 95 amino acids organized in five α-helices and three β-strands with a high degree of conservation in its overall tertiary structure. The N-terminus forms an α-helix hairpin (A1/A2) next to an exposed three stranded β-sheet (B1-3). This is followed by a third α-helix (A3) and an extension of inconsistent length and formation, ending at the carboxyl terminus with a second helix hairpin module (A4/A5). Outside this core fold, the four investigated BTB-fold containing protein families strongly differ showing only a low grade of sequence similarities to each other. This degree of variation allows family-specific types of protein-protein associations.

For example the T1 domains show no N- or C-terminal additions but contain a loop structure in the connection of A3 and A4 and form homotetramers (Kreusch *et al*. 1998). In comparison, the N-terminal extension of BTB-ZF proteins plays a role in creating the surface for dimerization with other BTB-proteins and a fifth β-strand in the A3-A4 connection gives the orientation by assembling with the partner protein β1 strand to an antiparallel sheet (Ahmad *et al*. 2003). In contrast Skp1 proteins do not dimerize with other Skp1 proteins but appear only to assemble as a single protein to cullins of SCF complexes. They have both a C-terminal extension of two α-helices that is involved in the interaction with F-box proteins and an additional stretch between A3 and A4 that contains one more α-helix without any assembling functions (Botuyan *et al*. 2001).

The work from Stogios *et al*. (2005) also showed that as variable as any of these extensions are in the four investigated BTB-superfamilies, the amino acid sequence of the core BTB fold itself is already highly variable. Only 15 of these core positions are lineage independently significantly conserved and 12 of these are buried when the protein is in the monomer form. Other residues and motifs show conservation only within the families. For example Skp1 proteins share a common motif in the B3 β-sheet ´PxPN´ that mediates the specific interaction with CUL1 (Goldenberg *et al*. 2004), whereas the T1 family exhibits at this site a ´FFDR´ motif for tetramerization (Stogios *et al*. 2005). Mutations of the *C. elegans* protein MEL26 (BTB-MATH) in analogous residues (M243, I245, D247) abolished the interaction with CUL3 (Xu *et al*. 2003), but the residues are not family specific. Additionally, in the A4 α-helix in Skp1 proteins a ´NY´ motif is immediately conserved and also responsible for the CUL1 interaction. Moreover, mutagenesis of the corresponding amino acids in the BTB-MATH-protein MEL26 disrupted assembling with CUL3 (Xu *et al*. 2003). This, however, was not observed in the *Arabidopsis* MEL26 or-

tholog BPM1 (Weber *et al*. 2005). To complicate the prediction of important residues mediating protein-protein interaction, the BTB-domain alone can mediate assembly with CUL3 (e.g. KIAA1309; Furukawa *et al*. 2003) but there are also examples of the BTB-domain requiring interplay with a second motif to interact with the cullin (e.g. SPOP (Speckle-type POZ protein; Kwon *et al*. 2006). However, these results point out the importance of both highly conserved interaction surfaces and (family) specific motifs that are determining both specificity and the wide range of the BTB interaction patterns; and it becomes evident that it is a team play of distributed residues in the BTB-fold with occasionally secondary motifs contributing for assembly with other proteins.

ANIMAL BRC3 ACTIVITIES AND SUBSTRATE TARGETING AS MODEL FOR PLANTS

It remains open whether BTB-proteins can function as monomers or if dimerization is required for activity as experimental proof is, to our knowledge, missing which demonstrates that BTB proteins are active as monomers. Yet, for many BTB proteins in mammals, *Drosophila* and *C. elegans* such as the BTB-Zinc-finger proteins PLZF (Promyelocytic Leukemia Zinc-Finger) and BCL6, or the BTB-Kelch protein Keap1, the dimeric forms were proven to be functionally required (Robinson and Cooley 1997; Melnick *et al*. 2000; McMahon *et al*. 2006). For example, transcriptional regulation activity of BCL-6 requires dimerization via the BTB domain to recruit nuclear co-repressor proteins like SMRT (Silencing Mediator of Retinoid and Thyroid Receptor) and N-CoR (Nuclear receptor Co-Repressor) as a critical step in transcriptional regulation (Ahmad *et al*. 2003). Comparably, transcriptional repression activity of PLZF is obligatorily connected with a dimerizing BTB-domain (Melnick *et al*. 2000), and mammalian Keap1 assembles as a dimer with CUL3 proteins to recruit the substrates Nrf2 via its kelch motif (McMahon *et al*. 2006). Here, Keap1 acts as an oxidative stress sensor guiding the antioxidant transcription factor Nrf2 for degradation via the 26S proteasome (Furukawa and Xiong 2005). Hence, given the high degree of conservation of the BTB-domain's interaction patterns, it is conceivable that dimerization represents indeed a general scheme for functionality of BTB-proteins. Particularly, the finding that Keap1 is active as a dimer in a $BRC3^{Keap1}$ E3 ligase provides new insights in E3 functioning and can also serve as a model system for plant BRC3s.

An intriguing aspect about BRC3 E3s appears to be that at least some members are able to facilitate both mono- and poly-ubiquitination of substrates. For instance, human $BRC3^{SPOP}$, with SPOP as the substrate adaptor, targets Daxx, a multifunctional protein that is involved in apoptotic processes and chromatin modelling, for poly-ubiquitination and degradation via the 26S proteasome (Kwon *et al*. 2006). On the other hand, the same $BRC3^{SPOP}$ E3 ligase can also interact with the variant histone MacroH2A without destabilizing the protein. Rather, $BRC3^{SPOP}$ mono-ubiquitinates MacroH2A as a critical step for heterochromatin establishment and stable X-chromosome silencing (Hernández-Muñoz *et al*. 2005). Thus, $BRC3^{SPOP}$ can serve as an opposite model for plant BRC3 E3s in which i) a single E3 ligase can target varying substrates by employing the same substrate adaptor and ii) such an E3 ligase can have opposite effects on substrate activity and/or stability by either mono- or poly-ubiquitinating the target protein.

INTERPLAY OF BRC3 AND OTHER CULLIN-BASED E3 LIGASES

In the last years it has become more and more evident that BRC3 E3 ligases directly share regulatory pathways or compete for the same substrates with other cullin-based E3 ligases. This shall be delineated in brief by some examples from animals and plants. CyclinE is degraded by two different mechanisms in mammalian and insect cells: on the one hand, the SCF^{Fbw7} E3 ligase recognizes the phosphorylated form of Cdk (Cyclin-dependent Kinase)-2 bound Cyclin E for ubiquitination, whereas a Cul3-based E3 ligase targets unbound Cyclin E for ubiquitination, independently of phosporylation (Singer *et al*. 1999). Similarly mammalian cyclin D1 has been shown *in vitro* to be a target for ubiquitination by both SCF and BRC3 E3 ligases (Maeda *et al*. 2001).

Another example is the *D. melanogaster* transcription factor Ci (*Cubitus interruptus*) which is important during eye development in the Hedgehog signalling pathway and can be the target of two different E3 ligases (Mistry *et al*. 2004). In undifferentiated cells anterior of the morphogenic furrow the phosphorylated full-length Ci155, which functions as an activator, is targeted by the SCF^{Slimb} E3 ligase leading to a truncated version Ci75, which acts as a repressor (Smelkinson and Kalderon 2006). In posterior cells, however, complete degradation of the non-phosphorylated Ci155 is mediated by the $BRC3^{HIB}$ E3 ligase (Jiang 2006).

BRC3 E3 ligase activities are also connected with other cullin-based E3s: both BRC3 and CUL4-based DRC4 E3 ligases affect the transcription factor c-Jun. Here, c-Jun is a direct target for proteasomal degradation mediated by a $DRC4^{COP1-DET1}$ E3 ligase (Wertz *et al*. 2004). But c-Jun activity is also indirectly controlled by $BRC3^{SPOP}$ affecting Daxx stability. Daxx positively regulates a Jun NH_2-terminal kinase cascade which in turn phosphorylates and activates c-Jun and other transcription factors (Kwon *et al*. 2006). The best example in plants for interconnection of BRC3 with another E3 ligase is given for ethylene. Ethylene signal transduction requires the CUL1-based $SCF^{EBF1/EBF2}$-dependent degradation of the transcription factors EIN3/EIL (Potuschak *et al*. 2003), and ethylene biosynthesis is controlled by the previously described $BRC3^{ETO1}$ E3 ligase (Wang *et al*. 2004).

OUTLOOK

The established works described in this review already demonstrate that BRC3 E3 ligases participate in major developmental and physiological processes. Although the basic composition of CUL3-based E3 ligases appears to be solved, it remains open as to what extent the annotated BTB-domain proteins interact with the cullin. F-box proteins have been predicted to number more than 700 in *Arabidopsis* and very few of these have been demonstrated to participate in an SCF-complex. Similarly the more than 100 BTB-proteins in *Arabidopsis* and over 200 in rice represent a challenge for the future to answer basic questions such as: are BTB proteins in general part of an E3 ligase? What are their precise biological roles? What are the main targets for BRC3 activities in plants? And do BRC3 activities lead to mono- and/or poly-ubiquitination of affected substrates? In the upcoming years the work on this protein family in context with E3 ligase activities promises to uncover interesting roles for the individual BRC3 E3 ligases in relationship to their specific regulatory networks and to contribute important new understandings to plant science.

ACKNOWLEDGEMENTS

We would like to thank Sutton Mooney for critical reading and advice.

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