

## Extra Views

# Recruiting Substrates to Cullin 4-Dependent Ubiquitin Ligases by DDB1

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Received 11/17/04; Accepted 11/22/04

Previously published online as a *Cell Cycle* E-publication:  
<http://www.landesbioscience.com/journals/cc/abstract.php?id=1396>

## KEY WORDS

ubiquitin-proteasome system, ubiquitin ligase, cullin, CUL4, DNA repair, DDB1, Cdt1

## ABSTRACT

The ubiquitin-proteasome system is the major pathway by which cells target proteins for degradation in a specific manner. The E3 ubiquitin ligase, which brings targeted proteins (substrates) and activated ubiquitin in close proximity, enabling covalent conjugation of ubiquitin to the substrate, is an essential component of this system. Of the E3 ligases, the cullin (CUL) ligases are of high interest because of their capacity to form multiple distinct E3 complexes to ubiquitinate a potentially large number of substrates. Of the six closely related cullins, very little is known about how specific substrates are recruited to CUL4-dependent ligases. A recent paper in *Nature Cell Biology* may shed some light on this issue as well as on the function of DDB1, a damaged-DNA binding protein that has long been associated with DNA repair.

Timely and efficient destruction of proteins in the cell is critical for normal function. The ubiquitin-proteasome system is the major pathway by which the cell targets proteins for degradation in a specific manner. Ubiquitin, a small protein highly conserved from yeast to mammals, is covalently conjugated to proteins via a cascade of three enzymatic activities.<sup>1</sup> First, an E1 ubiquitin activating enzyme in an ATP-dependent manner binds to ubiquitin via a high-energy thioester bond. Then, the ubiquitin is passed to one of several E2 ubiquitin conjugating enzymes. The E2 then interacts with an E3 ubiquitin ligase, which brings the substrate in close proximity with the E2, allowing the conjugation of ubiquitin to a lysine on the substrate. Multiple ubiquitins may be conjugated, in polyubiquitin chains and/or on multiple lysines. Once a polyubiquitin chain has been formed, the targeted protein is transferred to the 26S proteasome for degradation.

E3 ligases can be divided into two major families. The HECT family of E3s contains a domain homologous to the E6-associated protein (E6AP) carboxyl terminus that can form thioester linkages with ubiquitin. Multiple cellular proteins with diverse structures contain the HECT domain (five in budding yeast and ~30 in humans), implicating many substrates and physiological functions for the HECT family of E3 ligases.<sup>2</sup> The RING family of E3s contains either an intrinsic RING finger domain or an associated RING subunit essential for ubiquitin ligase activity. The cullins make up a large and unique group of RING-type E3 ligases by binding with one of the small RING-finger proteins known as ROCs (RING of Cullins, also known as Rbx or Hrt).<sup>3</sup> Unlike other single-polypeptide E3 ligases, each cullin (six in *C. elegans* and humans, five in *Drosophila*, and three in *S. pombe* and *S. cerevisiae*) forms many multisubunit complexes to target a potentially large number of specific substrates for ubiquitination. The assembly of these multi-subunit cullin-dependent ubiquitin ligase complexes, and thus the ubiquitination of substrates, is tightly regulated: all cullins are negatively regulated by CAND1, a 120 kDa protein that consists of 27 tandem HEAT (huntingtin-elongation-A subunit-TOR) repeats and prevents binding of the adaptor protein and thus substrates to the complex. To assemble productive ligase-substrate complexes, cullins are covalently modified by a small ubiquitin-like modifier, Nedd8, to dissociate CAND1.<sup>4-6</sup> Once the polyubiquitinated substrate is degraded by the proteasome, the COP9 signalosome presumably removes the conjugated Nedd8,<sup>7</sup> allowing CAND1 to bind cullins again and resume inhibition. This seemingly complex regulation is essential to ensure the assembly of individual cullin-ROC cores, which are likely present in the cell as rate-limiting factors, into multiple distinct substrate-cullin-ROC complexes.

A unique and remarkable feature of cullin-dependent ligases is the assembly of individual cullins into multiple ligases via a conserved, yet distinct N-terminal domain. CUL1, the first cullin to be studied and the best understood, recruits proteins via an adaptor and a specificity factor: SKP1 binds directly and simultaneously to CUL1 and one of a series of

F-box-containing proteins (diagramed in Fig. 1).<sup>8-10</sup> F-box proteins, estimated to be more than 60 in mammals, recruit specific substrates to the CUL1 complex, such as p27 by SKP2, or I $\kappa$ B $\alpha$  by  $\beta$ -TrCP. CUL2 and CUL5, on the other hand, recruit substrates via a heterotrimeric adaptor complex, containing Elongins B and C and one of a number of number of SOCS-box-containing proteins.<sup>11</sup> The SOCS box is a 40-residue protein motif initially identified in the suppressor of cytokine signaling family of proteins, and mammalian genomes encode more than 40 SOCS proteins. There is also some difference in substrate specificity between CUL2 and CUL5, though the mechanism by which this occurs remains to be determined. Just as amazing as these three cullins, which each ubiquitinate multiple substrates, CUL3 utilizes a similar N-terminal domain to bind with the BTB domain, a 100 residue protein motif first identified in the *Drosophila* Broad-Complex C (BR-C), Tramtrack (Ttk) and Bric-a-brac (Bab) proteins.<sup>12-15</sup> There are more than 200 BTB-containing proteins present in mammals, suggesting a potentially large number of substrates for CUL3-ROC1 ligases.

How does CUL4 recognize its substrates? There are two closely related Cul4s in mammals, Cul4A and Cul4B, and one in flies, worms, plants and fission yeast, but none in budding yeast. Loss-of-function of the *Cul4* gene resulted in elongated cells with decondensed chromosomes in fission yeast<sup>16</sup> and massive DNA rereplication in *C. elegans* embryos with a correlative accumulation of the DNA replication licensing factor, CDT1,<sup>17</sup> suggesting a function of Cul4 in controlling DNA replication and genomic stability. The recent publication in *Nature Cell Biology* provides further support to this notion and may shed some light on the substrate recruiting mechanism of Cul4-dependent ligases.<sup>18</sup> The key finding of the paper is that the damage-specific DNA binding protein, DDB1, directly recruits CDT1 to the CUL4-ROC1 ligase for ubiquitination in response to DNA damage. Three lines of studies presented in this paper collectively support a role of DDB1 as a substrate recruiting factor for CUL4, analogously to CUL1-SKP1, CUL2-Elongin C and CUL3- BTB interactions. DDB1 binds with CUL4 abundantly and nearly stoichiometrically, DDB1 binds to the N-terminal helices of CUL4A that correspond to the helices in CUL1 and CUL3 that interact with SKP1 and BTB, respectively, and DDB1 and CAND1 interact with CUL4 in a mutually exclusive manner.

How exactly does DDB1 recruit substrates to CUL4: like SKP1, which recruits substrates indirectly through another specificity factor, the F-box protein, or like BTB proteins, which bind to substrates directly (Fig.1)? Together with several recent studies on this critical and fascinating issue, it seems possible that CUL4 may have a 'hybrid' property of recruiting substrates directly, as in the case of CDT1, and through another specificity factor. DDB1 is a large (127 kDa, 1140 amino acids) protein that has a predicted structure consisting almost entirely of WD-like repeats.<sup>19</sup> CUL4 has been

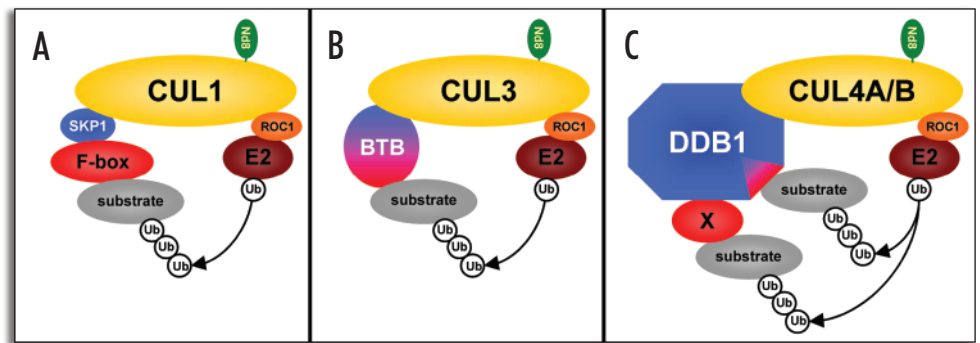


Figure 1. Cullin-dependent ubiquitin ligases utilize multiple subunits to recruit substrates. (A). Cullin 1 (yellow) recruits substrates (grey) via an adaptor, SKP1 (blue) and a specificity factor, one of a series of F-box proteins (red). The RING finger protein ROC1 (or ROC2) (orange) recruits one of several E2 ubiquitin conjugating enzymes (brown), which then conjugate ubiquitin onto the substrate. Ned8 conjugation (green) is required for ligase activity. (B). Cullin 3 recruits substrates via one of possibly over 200 adaptors, containing a BTB domain (blue) and another domain for binding the substrate (red). Otherwise, it functions in an identical manner to Cullin 1. (C). Cullins 4A and 4B may recruit substrates via mechanisms similar to both cullins 1 and 3. The large adaptor protein DDB1 may bind substrates directly (red triangle), such as CDT1, or may recruit substrates via a series of specificity factors (X, red), such as the substrate c-Jun via DET1 and COP1.

linked with ubiquitination of the transcription factor c-Jun via additional proteins, DET1 and COP1.<sup>20</sup> In paramyxovirus infected cells, CUL4 has also been linked with the ubiquitination of the signal transducers and activators of transcription STAT1 and STAT3, bridged by the viral V protein.<sup>21-23</sup> Additionally, human CUL4A has also been reported to stimulate ubiquitination of the HOXA9 homeodomain protein,<sup>24</sup> although it was not clear whether DDB1 or an additional factor(s) is required. Moreover, two other known DDB1 binding proteins, DDB2 and CSA (Cockayne Syndrome protein A), both contain multiple WD repeats. There is no obviously recognizable motif common to DET1, HOXA9, CDT1, V protein, DDB2 and CSA that would point to a shared binding domain in DDB1.

Is it possible that CUL4, as other cullins, ubiquitinates multiple substrates, not involving multiple specificity factors that each contain a short protein motif, but instead relying on the large size and deep capacity of DDB1 to interact with multiple proteins? Time will tell, probably soon.

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