

Function and regulation of protein neddylation



'Protein Modifications: Beyond the Usual Suspects' Review Series

Gwénaél Rabut⁺ & Matthias Peter⁺⁺

Swiss Federal Institute of Technology Zürich (ETH), Institute of Biochemistry, Zürich, Switzerland

Neddylation is the post-translational protein modification that is most closely related to ubiquitination. However, ubiquitination is known to regulate a myriad of processes in eukaryotic cells, whereas only a limited number of neddylation substrates have been described to date. Here, we review the principles of protein neddylation and highlight the mechanisms that ensure the specificity of neddylation over ubiquitination. As numerous neddylation substrates probably remain to be discovered, we propose some criteria that could be used as guidelines for the characterization of neddylated proteins.

Keywords: cullin; NEDD8; neddylation; Rub1

EMBO reports (2008) 9, 969–976. doi:10.1038/embor.2008.183

See Glossary for abbreviations used in this article.

Introduction

Ubiquitination is a prominent post-translational protein modification that regulates most cellular functions in eukaryotes (reviewed in Glickman & Ciechanover, 2002; Haglund & Dikic, 2005; Weissman, 2001). It consists of the covalent attachment of the small protein ubiquitin to target proteins, thereby modifying their biochemical properties and protein partners (Hicke *et al*, 2005). Besides ubiquitination, several related protein-modification systems function in eukaryotes (Kerscher *et al*, 2006; Kirkin & Dikic, 2007). In particular, the NEDD8 protein—also known as Rub1 in *Saccharomyces cerevisiae*—is the closest relative to ubiquitin and can similarly be conjugated to substrate proteins in a process known as neddylation. Here, we review the characteristic features of protein neddylation and deneddylation. Moreover, we summarize the currently proposed NEDD8 substrates and suggest some criteria that might help to identify new neddylated proteins (Sidebar A).

Conservation and functional importance of neddylation

NEDD8 was initially identified as a gene that is highly expressed in the embryonic mouse brain (Kumar *et al*, 1992). It was, however, soon realized that *NEDD8* is highly conserved in most eukaryotes (plants, slime molds, fungi and animals; Burroughs *et al*, 2007; Kumar *et al*, 1993; Rao-Naik *et al*, 1998) where it is expressed in most, if not all, tissues (Carrabino *et al*, 2004; Hori *et al*, 1999; Kumar *et al*, 1993; Rao-Naik *et al*, 1998), suggesting an important function of NEDD8 in eukaryotic cells. Indeed, neddylation is essential for the viability of most model organisms, including *Schizosaccharomyces pombe*, *Caenorhabditis elegans*, *Drosophila*, *Arabidopsis* and mouse (Dharmasiri *et al*, 2003; Jones & Candido, 2000; Kurz *et al*, 2002; Osaka *et al*, 2000; Ou *et al*, 2002; Tateishi *et al*, 2001), with the notable exception of *S. cerevisiae* (Lammer *et al*, 1998; Liakopoulos *et al*, 1998). In addition, deregulated neddylation might be involved in the aetiology of some human diseases such as neurodegenerative disorders (Dil Kuazi *et al*, 2003; Mori *et al*, 2005) and cancers (Chairatvit & Ngamkitidechakul, 2007; Salon *et al*, 2007). Indeed, MLN4924, which is a general inhibitor of neddylation (Langston *et al*, 2007), shows substantial activity in a broad range of preclinical tumour models, raising the possibility that components of the neddylation pathway might be promising therapeutic targets. It is therefore important to characterize which cellular proteins are neddylated, how this modification affects their function, and how neddylation is catalysed and regulated (Sidebar B).

NEDD8 processing and activation

Similar to ubiquitin, NEDD8 is attached to its substrates by an isopeptide linkage between its carboxy-terminal glycine (Gly) 76 and a lysine of the target protein. However, *NEDD8* genes from all organisms encode non-conjugatable precursors that contain one or more additional residues beyond Gly 76 that need to be cleaved by C-terminal hydrolases (Fig 1). This reaction is catalysed by UCH-L3 (Yuh1 in *S. cerevisiae*; Linghu *et al*, 2002; Wada *et al*, 1998), which can also process ubiquitin precursors (Frickel *et al*, 2007; Johnston *et al*, 1999; Wada *et al*, 1998). However *UCH-L3* knockout mice are viable (Kurihara *et al*, 2000), indicating that there must be other NEDD8-processing enzymes in mammals. Indeed, NEDP1 (also known as DEN1 or SENP8)—a protein with similarity to SUMO proteases that is conserved in *S. pombe*, plants and animals, but not in *S. cerevisiae*—has been shown to catalyse the processing of NEDD8

Swiss Federal Institute of Technology Zürich (ETH), Institute of Biochemistry, 8093 Zürich, Switzerland

⁺Corresponding author. Tel: +41 44 633 4587; Fax: +41 44 632 1269; E-mail: gwénael.rabut@bc.biol.ethz.ch

⁺⁺Corresponding author. Tel: +41 44 633 6586; Fax: +41 44 632 1269; E-mail: matthias.peter@bc.biol.ethz.ch

Submitted 9 June 2008; accepted 25 August 2008; published online 19 September 2008

Glossary

Apc2	anaphase promoting complex 2
APP	amyloid β precursor protein
APPBP1	APP binding protein 1
BCA3	breast cancer-associated gene 3
CAND1	cullin-associated and neddylation-dissociated 1
c-Cbl	casitas B-lineage lymphoma
CSN	COP9 signalosome
Cul	cullin
Dcn1	defective in cullin neddylation 1
EGFR	epidermal growth factor receptor
Mdm2	murine double minute gene 2
NEDD8	neural precursor cell expressed developmentally downregulated protein 8
NEDP1	NEDD8 protease 1
NUB1	NEDD8 ultimate buster-1
Parc	p53-associated parkin-like cytoplasmic protein
PFUCH54	Plasmodia falciparum ubiquitin carboxy-terminal hydrolase with a molecular mass of 54 kDa
pVHL	von Hippel–Lindau protein
Rbx	RING box
RING	really interesting new gene
SUMO	small ubiquitin-like modifier
UBA3	ubiquitin-like modifier activating enzyme 3
Ubc	ubiquitin-conjugating
UCH-L3	ubiquitin carboxyl-terminal hydrolase L3

precursors (Mendoza *et al*, 2003; Wu *et al*, 2003) with remarkable specificity (Gan-Erdene *et al*, 2003; Shen *et al*, 2005).

After its processing, NEDD8 is activated through an ATP-dependent mechanism catalysed by an activating enzyme, E1, which creates a high-energy intermediate (Huang *et al*, 2004a). It is then transferred to a conjugating enzyme, E2 (Huang *et al*, 2007), that shuttles activated NEDD8 to a ligase, E3, which then ensures specific conjugation of NEDD8 to its substrates (Fig 1). Both NEDD8 E1 and E2 are conserved from yeast to humans (for a review, see Parry & Estelle, 2004). The NEDD8 E1 activity is fulfilled by a heterodimer of APPBP1 and UBA3, which are homologous to the amino-terminal and C-terminal domains of the ubiquitin-activating enzyme, respectively (Liakopoulos *et al*, 1998; Osaka *et al*, 1998; Walden *et al*, 2003a). Contrary to ubiquitin, which can be transferred by multiple E2s, available evidence indicates that Ubc12 functions as the unique E2 of the NEDD8 pathway (Liakopoulos *et al*, 1998). Indeed, despite the high sequence similarity (76%) and structural similarity between NEDD8 and ubiquitin (Whitby *et al*, 1998), Ubc12 is exclusively loaded with NEDD8. This insulation of the NEDD8 pathway is achieved by multiple mechanisms. First, the interaction between Ubc12 and the NEDD8 E1 involves a unique N-terminal extension in Ubc12 (Huang *et al*, 2004a, 2007), which prevents mischarging by the ubiquitin E1 (Huang *et al*, 2008). Second, a conserved basic residue in UBA3 acts as a selectivity gate to block misactivation of ubiquitin by the NEDD8 E1: it collides with Arg72 in ubiquitin but not with the corresponding alanine residue in NEDD8 (Souphron *et al*, 2008; Walden *et al*, 2003b). Conversely, Arg72 is the main determinant responsible for preferential activation of ubiquitin over NEDD8 by the ubiquitin E1 (Lee & Schindelin, 2008; Whitby *et al*, 1998). It is noteworthy that ubiquitin activation is not as specific as NEDD8 activation, as

Sidebar A | Criteria for the characterization of neddylation substrates

Minimal criteria for the identification of genuine NEDD8 substrates

- (i) NEDD8 is covalently attached to the substrate *in vivo*
- (ii) Neddylation occurs under endogenous conditions *in vivo*
- (iii) Neddylation depends on specific components of the neddylation machinery *in vivo*

Criteria for further characterization of NEDD8 substrates

- (iv) Determination of the neddylated lysine residues
- (v) Characterization of the phenotype of non-neddylatable mutants
- (vi) Identification of a specific NEDD8 ligase *in vivo*
- (vii) Reconstitution of the neddylation reaction *in vitro*
- (viii) Identification of a NEDD8 isopeptidase *in vivo*

NEDD8 can be activated by the ubiquitin E1, transferred to ubiquitin E2s and incorporated into polyubiquitin chains *in vitro* (Whitby *et al*, 1998). However, this reaction is inefficient and its consequences *in vivo* have not been investigated.

Neddylated proteins and their E3s

The first identified targets of NEDD8 were Cdc53 in *S. cerevisiae* (Lammer *et al*, 1998; Liakopoulos *et al*, 1998) and CUL4A in human cells (Osaka *et al*, 1998). Both are members of the cullin family of proteins—which has between three and six members in all eukaryotes from yeast to humans—suggesting that neddylation is an important mechanism for regulating cullin function. Indeed, all yeast and mammalian cullins—*S. cerevisiae*: Cdc53, Cul3 and Rtt101 (Laplaza *et al*, 2004); *S. pombe*: Pcu1, Pcu3 and Pcu4 (Osaka *et al*, 2000; Zhou *et al*, 2001); and mammalian: CUL1, CUL2, CUL3, CUL4A, CUL4B and CUL5 (Hori *et al*, 1999; Jones *et al*, 2008)—are neddylated on a conserved lysine in their C-terminal domain *in vivo* (Table 1). This lysine is conserved in the vertebrate specific cullin-related proteins PARC and CUL7 (Pan *et al*, 2004), which are probably also neddylated (Jones *et al*, 2008), although neddylation of Cul7 is controversial (Skaar *et al*, 2007). By contrast, Apc2—a subunit of the anaphase-promoting complex that shows similarity to cullins (Zheng *et al*, 2002a)—is not neddylated (Pan *et al*, 2004).

Cullins function as scaffolds for the assembly of multisubunit ubiquitin E3s. They interact tightly with a RING-domain protein—Rbx1 or Rbx2 (Ohta *et al*, 1999)—which recruits charged ubiquitin E2s into the complex and catalyses the ubiquitination of cullin substrates (Seol *et al*, 1999). By analogy, it has been proposed that Rbx1 could also function as a NEDD8 E3 for cullins (Kamura *et al*, 1999). Indeed, Rbx1 interacts with both cullins and Ubc12 (Dharmasiri *et al*, 2003; Morimoto *et al*, 2003), is required for cullin neddylation in insect cells (Kamura *et al*, 1999; Megumi *et al*, 2005) and is sufficient for Cul1 neddylation *in vitro* (Morimoto *et al*, 2003). However, Dcn1—a protein conserved from yeast to humans—is also required for efficient neddylation of several cullins *in vivo* (Kurz *et al*, 2005). Dcn1 interacts directly with Cdc53, Rbx1 and Ubc12, and stimulates Cdc53 neddylation *in vitro* when Ubc12 is present in limiting amounts (Kurz *et al*, 2008; Yang *et al*, 2007). Moreover, a cullin mutant that efficiently binds to Rbx1 but fails to interact with Dcn1 is not efficiently neddylated (Kurz *et al*, 2008), suggesting that Rbx1 is not sufficient for cullin neddylation *in vivo*. Further experiments are required to determine how Dcn1

Sidebar B | In need of answers

- (i) Which proteins are neddylated *in vivo* and how does this modification regulate their activity?
- (ii) What are the components, in particular NEDD8 ligases, which function with Ubc12 to neddylate specific targets *in vivo*?
- (iii) What are the functions of NEDD8 isopeptidases?
- (iv) What are the signals that regulate protein neddylation and deneddylation?
- (v) Can cells discriminate between neddylated and mono-ubiquitinated targets?
- (vi) Can NEDD8 form chains *in vivo*?

promotes cullin neddylation and whether it requires functional Rbx1 for its activity.

It has become evident that cullins are not the only class of proteins modified by NEDD8 (Table 1), indicating that neddylation might regulate many cellular processes. Interestingly, several neddylated proteins seem to be either substrates or components of ubiquitin E3s, revealing an intriguing relationship between ubiquitination and neddylation. For example, the tumour suppressor protein p53 and its relative p73 are both neddylated and ubiquitinated on several lysines by the RING-domain protein Mdm2, which also self-neddylates (Watson *et al*, 2006; Xirodimas *et al*, 2004). Similarly, the RING-domain protein c-Cbl can neddylate and ubiquitinate the EGFR upon its stimulation (Oved *et al*, 2006). pVHL, which is a well characterized component of a Cul2-based ubiquitin E3, is also neddylated by an uncharacterized E3 (Stickle *et al*, 2004). Finally, several ribosomal proteins can be modified by NEDD8 (Xirodimas *et al*, 2008) and ribosomes are also regulated by ubiquitination (Kraft *et al*, 2008). BCA3 (Gao *et al*, 2006) and the APP intracellular domain (Lee *et al*, 2008) are the only neddylated proteins identified so far that have not been implicated in an ubiquitination pathway.

Despite several proteomic approaches (Jones *et al*, 2008; Li *et al*, 2006; Norman & Shiekhatar, 2006; Xirodimas *et al*, 2008), it is still unclear how many other proteins are modified by NEDD8. These studies confirmed that cullins are abundant NEDD8 substrates, but failed to identify other previously characterized neddylated proteins except p53 (Li *et al*, 2006), indicating that non-cullin NEDD8 substrates are only weakly expressed and/or modified in steady-state conditions. Although this does not preclude an important function of their neddylation, it calls for careful characterization of putative NEDD8 targets. We propose a minimum of three criteria that should be shown for genuine NEDD8 substrates (Sidebar A). Covalent attachment of NEDD8 to its target (criterion i) should be detectable under endogenous conditions (criterion ii). Moreover, neddylation should be dependent on *bona fide* components of the neddylation machinery (criterion iii) such as the NEDD8 E1 or Ubc12—the sole E2 currently known to function specifically with NEDD8. Although this criterion has only been shown for neddylation of cullins (Collier-Hyams *et al*, 2005; Liakopoulos *et al*, 1998), and in part for p53 and pVHL (Table 1; Russell & Ohh, 2008; Xirodimas *et al*, 2004), it is important to exclude fortuitous misactivation of NEDD8 by the ubiquitin E1 and misloading on an ubiquitin E2 *in vivo*. Note, however, that this criterion cannot be demonstrated using the *Ubc12-C111S* mutant because overexpression of this construct depletes free NEDD8 (Wada

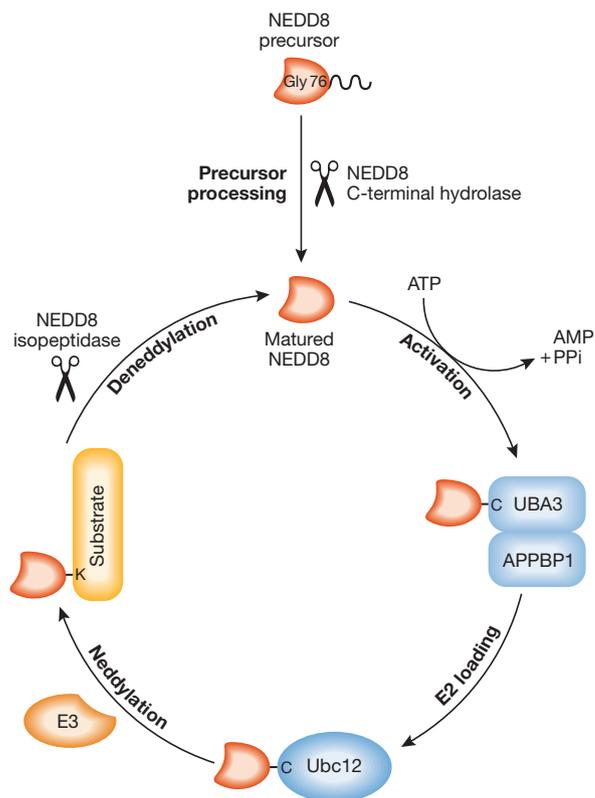


Fig 1 | Neddylation pathway. Schematic representation of the main steps of the neddylation pathway, including NEDD8 precursor processing, activation by the E1 (UBA3–APPBP1 heterodimer), loading onto the E2 (Ubc12), conjugation to a substrate by an E3 and recycling of NEDD8 by an isopeptidase. APPBP1, APP binding protein 1; NEDD8, neural precursor cell expressed developmentally downregulated protein 8; UBA3, ubiquitin-like modifier activating enzyme 3; Ubc, ubiquitin-conjugating.

et al, 2000), thereby preventing specific and adventitious substrate neddylation. Additional criteria to further characterize NEDD8 substrates include the identification of the neddylated lysine residues (criterion iv), ideally with an associated mutant phenotype if neddylation is prevented (criterion v). Identification of a NEDD8 E3 (criterion vi) and reconstitution of substrate neddylation *in vitro* (criterion vii) underline the fact that the modification is, indeed, specific and direct. Finally, identification of a deneddylation activity (see below) provides further support for a reversible modification (criterion viii).

NEDD8 isopeptidases

Protein neddylation is reversed by NEDD8 isopeptidases in a process known as deneddylation. The best characterized NEDD8 isopeptidase is CSN5, a subunit of the COP9 signalosome (CSN), which deneddylates cullins (reviewed in Cope & Deshaies, 2003; Schwechheimer, 2004; Wei & Deng, 2003). The CSN is conserved from yeast to humans (Schwechheimer, 2004; Wei & Deng, 2003) and its activity is essential for viability in metazoans (Cope & Deshaies, 2003). Genetic evidence in several organisms has revealed that the CSN promotes cullin activity, indicating that cycles of neddylation and deneddylation are required for correct

Table 1 | Proteins reported to be neddylated

Neddylated protein	Neddylated residue(s)	NEDD8 ligase	NEDD8 isopeptidase	Proposed consequences of neddylation	Criteria for NEDD8 substrates ^a	References
Cullins and the related proteins Parc and Cul7 ^b (endogenous)	A single conserved lysine in the C-terminal domain	Rbx1 and/or Dcn1	COP9 signalosome (<i>in vivo</i>)	Induces a conformational switch that prohibits cullin interaction with their inhibitor CAND1 and activates their ubiquitin ligase activity	i–viii	Bosu & Kipreos, 2008; Hori <i>et al</i> , 1999; Jones <i>et al</i> , 2008; Kurz <i>et al</i> , 2008; Pan <i>et al</i> , 2004; Parry & Estelle, 2004; Duda <i>et al</i> , 2008
p53 (endogenous)	Mainly Lys 370, Lys 372 and Lys 373	Mdm2 and SCF ^{FBXO11}	NEDP1 (<i>in vivo</i> , on overexpression)	Inhibits p53 transcriptional activity	i–vii	Abida <i>et al</i> , 2007; Xirodimas <i>et al</i> , 2004
p73 (endogenous)	–	Mdm2	NEDP1 (<i>in vivo</i> , on overexpression)	Inhibits p73 transcriptional activity	i, ii, vi	Watson <i>et al</i> , 2006
Mdm2 (endogenous)	–	Mdm2 (self-neddylation)	–	–	i, ii, vi	Xirodimas <i>et al</i> , 2004
pVHL (endogenous)	Lys 159	–	–	Prohibits pVHL interaction with Cul2-containing complexes, which allows its interaction with fibronectin and the assembly of extracellular matrix	i–v	Russell & Ohh, 2008; Stickle <i>et al</i> , 2004
BCA3 (endogenous)	Multiple lysines	–	NEDP1 (<i>in vivo</i>)	Promotes BCA3 interaction with the histone deacetylase SIRT1 and suppresses NF-κB-dependent transcription	i, ii, iv, v, viii	Gao <i>et al</i> , 2006
EGFR (endogenous)	Multiple lysines in the kinase domain	c-Cbl	–	Stimulates EGFR ubiquitination and recruitment of the endocytic machinery for EGFR downregulation	i, ii, vi	Oved <i>et al</i> , 2006
APP intracellular domain (AICD) (overexpressed NEDD8 and APP)	Multiple lysines in the intracellular domain	–	–	Inhibits AICD interaction with the transcription coactivator Fe65 and transcriptional activity of the complex	i, iv, v	Lee <i>et al</i> , 2008
L11 and other ribosomal proteins (overexpressed NEDD8 or L11)	–	–	NEDP1 (<i>in vitro</i> and <i>in vivo</i> , on overexpression)	Promotes ribosomal protein stability	i	Xirodimas <i>et al</i> , 2008

^aSee text and Sidebar A for the description of these criteria; ^bneddylation of Parc and Cul7 has not yet been studied extensively. APP, amyloid precursor protein; BCA3, breast cancer-associated gene 3; CAND1, cullin-associated and neddylation-dissociated 1; c-Cbl, casitas B-cell lymphoma; Cul, cullin; Dcn1, defective in cullin neddylation 1; EGFR, epidermal growth factor receptor; L11, large ribosomal subunit protein L11; Mdm2, murine double minute 2; NEDD8, neural precursor cell expressed developmentally downregulated protein 8; NEDP1, NEDD8 protease 1; NF-κB, nuclear factor-κB; Parc, p53-associated parkin-like cytoplasmic protein; pVHL, von Hippel–Lindau protein; Rbx1, RING box 1; SCF, Skp1–Cullin 1–F-box; SIRT1, sirtuin 1.

cullin function *in vivo* (Bosu & Kipreos, 2008; Cope & Deshaies, 2003; Pintard *et al*, 2003). As CSN inactivation destabilizes many subunits of cullin-based ubiquitin ligases (reviewed in Bosu & Kipreos, 2008; Wu *et al*, 2006), it is thought that, at least in some cases, the deneddylation activity of the CSN, as well as the CSN-associated deubiquitinating enzyme Ubp12, protect components of cullin-based ubiquitin ligases from autocatalytic degradation.

The cysteine protease NEDP1—which can process NEDD8 precursors (see above)—also functions as a specific NEDD8 isopeptidase. Ala 72 in NEDD8 (Arg 72 in ubiquitin) is an important—but not the sole—determinant of NEDP1 selectivity for NEDD8 over ubiquitin (Reverter *et al*, 2005; Shen *et al*, 2005). Compared with

the CSN complex, NEDP1 is inefficient at deneddyating cullins (Wu *et al*, 2003; Yamoah *et al*, 2005); however, it can process most neddylated proteins either *in vitro* or upon overexpression *in vivo* (Table 1 and references therein; see also Mendoza *et al*, 2003). Deletion of the NEDP1 *S. pombe* homologue, *nep1*, leads to an increase of the total cellular NEDD8 conjugates and to a cell-cycle defect, which has not been associated with increased neddylation of a specific NEDD8 substrate (Zhou & Watts, 2005). Similarly, NEDP1 knockdown in HeLa cells increases the fraction of neddylated BCA3 (Gao *et al*, 2006), but no other phenotypes have been reported so far. In the absence of specific NEDP1 deneddylation substrates, it is possible that the NEDP1 isopeptidase functions

primarily in a salvage pathway to remove adventitiously formed NEDD8 conjugates.

Finally, other proteases show dual specificity for ubiquitin and NEDD8. These include USP21 (Gong *et al*, 2000), Ataxin-3 (Ferro *et al*, 2007), PfUCH54 (Artavanis-Tsakonas *et al*, 2006), UCH-L3 (see above) and UCH-L1 (Hemelaar *et al*, 2004), which is a close homologue of UCH-L3 that, however, cannot process NEDD8 precursors (Wada *et al*, 1998). Importantly, the *in vivo* targets of these proteases remain to be investigated.

Direct effects of protein neddylation

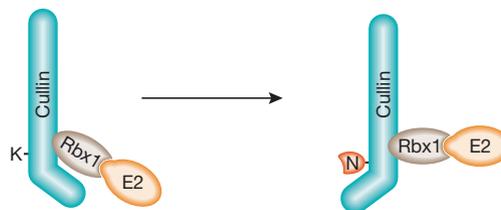
Similar to other post-translational modifications, neddylation of proteins modifies their three-dimensional surface and, hence, their biochemical properties. As illustrated in Fig 2, its direct effects can be classified into three categories from which further consequences such as changes in subcellular localization or enzymatic activity might follow.

First, NEDD8 attachment can induce conformational changes of its targets. It has long been observed that neddylation of cullins stimulates their ubiquitin E3 activity *in vitro* (Kawakami *et al*, 2001; Podust *et al*, 2000; Read *et al*, 2000), although the molecular mechanism has remained elusive. In the unneddylated state, the cullin C-terminal domain forms a groove in which the RING domain of Rbx1 is embedded (Zheng *et al*, 2002a). This conformation constrains the molecular movements of Rbx1 and positions the ubiquitin E2—which interacts with the RING domain of Rbx1—away from its ubiquitination substrates (Zheng *et al*, 2002a). Crystallographic data have now revealed that the cullin C-terminal domain undergoes a substantial conformational change upon neddylation that ‘frees’ the RING domain of Rbx1 and allows it to adopt multiple orientations that stimulate substrate ubiquitination *in vitro* (Duda *et al*, 2008). Notably, the catalytic effect of neddylation can be mimicked by deleting or mutating the residues of the unneddylated cullin C-terminal domain that are in contact with the RING domain of Rbx1 (Yamoah *et al*, 2008; Duda *et al*, 2008). Therefore, cullin neddylation induces a conformational switch in its C-terminal domain that relieves Rbx1 from an auto-inhibitory mechanism (Fig 2A). Interestingly, EGFR neddylation has similarly been proposed to alter the conformation of its cytoplasmic domain in a manner that exposes previously buried lysine residues for further modifications (Oved *et al*, 2006).

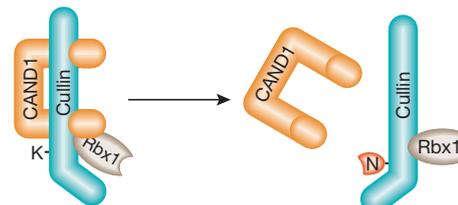
As a second possibility, neddylation can be incompatible with the interaction of some protein partners (Fig 2B). For example, the cullin inhibitor CAND1 preferentially binds to unneddylated cullins (Goldenberg *et al*, 2004; Liu *et al*, 2002; Zheng *et al*, 2002b). Indeed, the conformational switch induced by Cul1 neddylation not only activates Rbx1, but also prevents it from binding to CAND1 (Fig 2B; Duda *et al*, 2008). Similarly, neddylation of pVHL is incompatible with its incorporation in Cul2-containing complexes and therefore stimulates its Cul2-independent function (Russell & Ohh, 2008). In a variation of this theme, neddylation can compete with other post-translational modifications. For example, p53 and EGFR have been shown to be neddylated and ubiquitinated on overlapping lysines (Oved *et al*, 2006; Xirodimas *et al*, 2004), and excessive neddylation of EGFR can inhibit its ubiquitination (Oved *et al*, 2006).

Finally, neddylation can stimulate the recruitment of NEDD8-interacting proteins. For example, NEDD8 can interact directly with the ubiquitin E2 Ubc4 and this interaction has been proposed to participate in the activation of cullin-based ubiquitin ligases (Sakata

A Induce conformational changes



B Preclude certain interactions



C Provide a novel binding surface

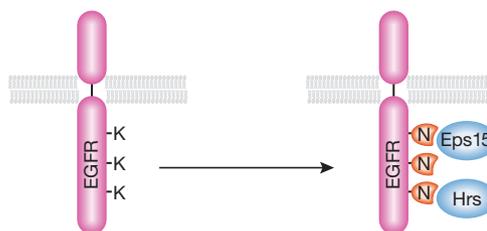


Fig 2 | Direct effects of neddylation. By remodelling the three-dimensional surface of its substrates, neddylation can achieve the following: (A) induce conformational changes (for example, neddylation of cullins allows their C-terminal domain and Rbx1 to adopt catalytically active conformations); (B) preclude association with certain partners or compete with other posttranslational modifications (for example, cullin neddylation is incompatible with CAND1 interaction); and (C) provide a novel binding surface to recruit new partners (for example, neddylated EGFR might recruit endocytic proteins such as Eps15 or Hrs). CAND1, cullin-associated and neddylation-dissociated 1; EGFR, epidermal growth factor receptor; Eps15, EGFR pathway substrate 15; Hrs, hepatocyte growth factor–regulated tyrosine kinase substrate; Rbx1, RING box.

et al, 2007). However, in light of the structural data, such a mechanism is unlikely without additional conformational changes, as the NEDD8 surface interacting with Ubc4 is engaged in binding the C-terminal domain of neddylated cullins (Duda *et al*, 2008). In another example, NUB1, which was identified in a yeast two-hybrid screen as a NEDD8-interacting protein (Kito *et al*, 2001), has been shown to trigger proteasomal degradation of neddylated but not ubiquitinated proteins (Kamitani *et al*, 2001). However, its physiological targets have not been identified and the significance of these observations remains unknown. Finally, neddylated EGFR likely recruits proteins of the endocytic machinery (Fig 2C) in a manner similar to mono-ubiquitinated EGFR, and both modifications cooperate to induce EGFR downregulation (Oved *et al*, 2006). This observation indicates that neddylation and mono-ubiquitination can have partly overlapping or synergistic functions.

Perspectives

Protein neddylation has emerged as an essential post-translational protein modification in the cell. However, as we still do not know how many NEDD8 E3s are functioning under physiological conditions, the extent of the neddylation proteome and its functions remain unclear. Given the strict specificity of NEDD8-activation mechanisms, it is surprising that some NEDD8 E3s are also ubiquitin E3s and that the currently known functions of protein neddylation are tightly associated with ubiquitination. Future research will be needed to investigate whether certain E3s are strictly using Ubc12 to neddylation their substrates or whether neddylation pathways always work in concert with ubiquitination.

ACKNOWLEDGEMENTS

We are grateful to Z.Q. Pan and B. Schulman for sharing their unpublished results. We thank members of the Peter Laboratory for critical reading of the manuscript, in particular R. Dechant, T. Kurz, S. Leidel and N. Meyer. Work in the Peter laboratory is supported by the Swiss National Science Foundation, Oncosuisse and the Swiss Federal Institute of Technology Zürich; G.R. is supported by a Human Frontier Science Programme Organization long-term fellowship.

REFERENCES

Abida WM, Nikolaev A, Zhao W, Zhang W, Gu W (2007) FBXO11 promotes the neddylation of p53 and inhibits its transcriptional activity. *J Biol Chem* **282**: 1797–1804

Artavanis-Tsakonas K, Misaghi S, Comeaux CA, Catic A, Spooner E, Duraisingh MT, Ploegh HL (2006) Identification by functional proteomics of a deubiquitinating/deNeddylating enzyme in *Plasmodium falciparum*. *Mol Microbiol* **61**: 1187–1195

Bosu DR, Kipreos ET (2008) Cullin-RING ubiquitin ligases: global regulation and activation cycles. *Cell Div* **3**: 7

Burroughs AM, Balaji S, Iyer LM, Aravind L (2007) Small but versatile: the extraordinary functional and structural diversity of the β -grasp fold. *Biol Direct* **2**: 18

Carrabino S, Carminati E, Talarico D, Pardi R, Bianchi E (2004) Expression pattern of the *JAB1/CSN5* gene during murine embryogenesis: colocalization with NEDD8. *Gene Expr Patterns* **4**: 423–431

Chairatvit K, Ngamkitidechakul C (2007) Control of cell proliferation via elevated NEDD8 conjugation in oral squamous cell carcinoma. *Mol Cell Biochem* **306**: 163–169

Collier-Hyams LS, Sloane V, Batten BC, Neish AS (2005) Cutting edge: bacterial modulation of epithelial signaling via changes in neddylation of cullin-1. *J Immunol* **175**: 4194–4198

Cope GA, Deshaies RJ (2003) COP9 signalosome: a multifunctional regulator of SCF and other cullin-based ubiquitin ligases. *Cell* **114**: 663–671

Dharmasiri S, Dharmasiri N, Hellmann H, Estelle M (2003) The RUB/Nedd8 conjugation pathway is required for early development in *Arabidopsis*. *EMBO J* **22**: 1762–1770

Dil Kuazi A, Kito K, Abe Y, Shin RW, Kamitani T, Ueda N (2003) NEDD8 protein is involved in ubiquitinated inclusion bodies. *J Pathol* **199**: 259–266

Duda et al (2008) Structural insights into NEDD8 activation of cullin-RING ligases: conformational control of conjugation. *Cell* **134**: 995–1006

Ferro A et al (2007) NEDD8: a new ataxin-3 interactor. *Biochim Biophys Acta* **1773**: 1619–1627

Frickel EM, Quesada V, Muething L, Gubbels MJ, Spooner E, Ploegh H, Artavanis-Tsakonas K (2007) Apicomplexan UCHL3 retains dual specificity for ubiquitin and Nedd8 throughout evolution. *Cell Microbiol* **9**: 1601–1610

Gan-Erdene T, Nagamalleswari K, Yin L, Wu K, Pan ZQ, Wilkinson KD (2003) Identification and characterization of DEN1, a deneddylase of the ULP family. *J Biol Chem* **278**: 28892–28900

Gao F, Cheng J, Shi T, Yeh ET (2006) Neddylation of a breast cancer-associated protein recruits a class III histone deacetylase that represses NF κ B-dependent transcription. *Nat Cell Biol* **8**: 1171–1177

Glickman MH, Ciechanover A (2002) The ubiquitin-proteasome proteolytic pathway: destruction for the sake of construction. *Physiol Rev* **82**: 373–428

Goldenberg SJ, Cascio TC, Shumway SD, Garbutt KC, Liu J, Xiong Y, Zheng N (2004) Structure of the Cand1-Cul1-Roc1 complex reveals regulatory mechanisms for the assembly of the multisubunit cullin-dependent ubiquitin ligases. *Cell* **119**: 517–528

Gong L, Kamitani T, Millas S, Yeh ET (2000) Identification of a novel isopeptidase with dual specificity for ubiquitin- and NEDD8-conjugated proteins. *J Biol Chem* **275**: 14212–14216

Haglund K, Dikic I (2005) Ubiquitylation and cell signaling. *EMBO J* **24**: 3353–3359

Hemelaar J et al (2004) Specific and covalent targeting of conjugating and deconjugating enzymes of ubiquitin-like proteins. *Mol Cell Biol* **24**: 84–95

Hicke L, Schubert HL, Hill CP (2005) Ubiquitin-binding domains. *Nat Rev Mol Cell Biol* **6**: 610–621

Hori T, Osaka F, Chiba T, Miyamoto C, Okabayashi K, Shimbara N, Kato S, Tanaka K (1999) Covalent modification of all members of human cullin family proteins by NEDD8. *Oncogene* **18**: 6829–6834

Huang DT, Walden H, Duda D, Schulman BA (2004a) Ubiquitin-like protein activation. *Oncogene* **23**: 1958–1971

Huang DT, Miller DW, Mathew R, Cassell R, Holton JM, Roussel MF, Schulman BA (2004b) A unique E1-E2 interaction required for optimal conjugation of the ubiquitin-like protein NEDD8. *Nat Struct Mol Biol* **11**: 927–935

Huang DT, Hunt HW, Zhuang M, Ohi MD, Holton JM, Schulman BA (2007) Basis for a ubiquitin-like protein thioester switch toggling E1-E2 affinity. *Nature* **445**: 394–398

Huang DT, Zhuang M, Ayrault O, Schulman BA (2008) Identification of conjugation specificity determinants unmasks vestigial preference for ubiquitin within the NEDD8 E2. *Nat Struct Mol Biol* **15**: 280–287

Johnston SC, Riddle SM, Cohen RE, Hill CP (1999) Structural basis for the specificity of ubiquitin C-terminal hydrolases. *EMBO J* **18**: 3877–3887

Jones D, Candido EP (2000) The NED-8 conjugating system in *Caenorhabditis elegans* is required for embryogenesis and terminal differentiation of the hypodermis. *Dev Biol* **226**: 152–165

Jones J, Wu K, Yang Y, Guerrero C, Nillegoda N, Pan ZQ, Huang L (2008) A targeted proteomic analysis of the ubiquitin-like modifier nedd8 and associated proteins. *J Proteome Res* **7**: 1274–1287

Kamitani T, Kito K, Fukuda-Kamitani T, Yeh ET (2001) Targeting of NEDD8 and its conjugates for proteasomal degradation by NUB1. *J Biol Chem* **276**: 46655–46660

Kamura T, Conrad MN, Yan Q, Conaway RC, Conaway JW (1999) The Rbx1 subunit of SCF and VHL E3 ubiquitin ligase activates Rub1 modification of cullins Cdc53 and Cul2. *Genes Dev* **13**: 2928–2933

Kavakami T et al (2001) NEDD8 recruits E2-ubiquitin to SCF E3 ligase. *EMBO J* **20**: 4003–4012

Kerscher O, Felberbaum R, Hochstrasser M (2006) Modification of proteins by ubiquitin and ubiquitin-like proteins. *Annu Rev Cell Dev Biol* **22**: 159–180

Kirkin V, Dikic I (2007) Role of ubiquitin- and Ubl-binding proteins in cell signaling. *Curr Opin Cell Biol* **19**: 199–205

Kito K, Yeh ET, Kamitani T (2001) NUB1, a NEDD8-interacting protein, is induced by interferon and down-regulates the NEDD8 expression. *J Biol Chem* **276**: 20603–20609

Kraft C, Deplazes A, Sohrmann M, Peter M (2008) Mature ribosomes are selectively degraded upon starvation by an autophagy pathway requiring the Ubp3p/Bre5p ubiquitin protease. *Nat Cell Biol* **10**: 602–610

Kumar S, Tomooka Y, Noda M (1992) Identification of a set of genes with developmentally down-regulated expression in the mouse brain. *Biochem Biophys Res Commun* **185**: 1155–1161

Kumar S, Yoshida Y, Noda M (1993) Cloning of a cDNA which encodes a novel ubiquitin-like protein. *Biochem Biophys Res Commun* **195**: 393–399

Kurihara LJ, Semenova E, Levorse JM, Tilghman SM (2000) Expression and functional analysis of Uch-L3 during mouse development. *Mol Cell Biol* **20**: 2498–2504

Kurz T, Pintard L, Willis JH, Hamill DR, Gonczy P, Peter M, Bowerman B (2002) Cytoskeletal regulation by the Nedd8 ubiquitin-like protein modification pathway. *Science* **295**: 1294–1298

Kurz T, Ozlu N, Rudolf F, O'Rourke SM, Luke B, Hofmann K, Hyman AA, Bowerman B, Peter M (2005) The conserved protein DCN-1/Dcn1p is required for cullin neddylation in *C. elegans* and *S. cerevisiae*. *Nature* **435**: 1257–1261

Kurz T, Chou YC, Willems AR, Meyer-Schaller N, Hecht ML, Tyers M, Peter M, Sicheri F (2008) Dcn1 functions as a scaffold-type E3 ligase for cullin neddylation. *Mol Cell* **29**: 23–35

Lammer D, Mathias N, Laplaza JM, Jiang W, Liu Y, Goebel M, Estelle M (1998) Modification of yeast Cdc53p by the ubiquitin-related protein rub1p affects function of the SCFCdc4 complex. *Genes Dev* **12**: 914–926

- Langston SP, Olhava EJ, Vyskocil S (2007) Inhibitors of E1 activating enzymes. United States Patent 20070191293. Cambridge, Massachusetts, USA: Millennium Pharmaceuticals Inc
- Laplaza JM, Bostick M, Scholes DT, Curcio MJ, Callis J (2004) *Saccharomyces cerevisiae* ubiquitin-like protein Rub1 conjugates to cullin proteins Rtt101 and Cul3 *in vivo*. *Biochem J* **377**: 459–467
- Lee I, Schindelin H (2008) Structural insights into E1-catalyzed ubiquitin activation and transfer to conjugating enzymes. *Cell* **134**: 268–278
- Lee MR, Lee D, Shin SK, Kim YH, Choi CY (2008) Inhibition of APP intracellular domain (AICD) transcriptional activity via covalent conjugation with Nedd8. *Biochem Biophys Res Commun* **366**: 976–981
- Li T, Santockyte R, Shen RF, Tekle E, Wang G, Yang DC, Chock PB (2006) A general approach for investigating enzymatic pathways and substrates for ubiquitin-like modifiers. *Arch Biochem Biophys* **453**: 70–74
- Liakopoulos D, Doenges G, Matuschewski K, Jentsch S (1998) A novel protein modification pathway related to the ubiquitin system. *EMBO J* **17**: 2208–2214
- Linghu B, Callis J, Goebel MG (2002) Rub1p processing by Yuh1p is required for wild-type levels of Rub1p conjugation to Cdc53p. *Eukaryot Cell* **1**: 491–494
- Liu J, Furukawa M, Matsumoto T, Xiong Y (2002) NEDD8 modification of CUL1 dissociates p120(CAND1), an inhibitor of CUL1-SKP1 binding and SCF ligases. *Mol Cell* **10**: 1511–1518
- Megumi Y *et al* (2005) Multiple roles of Rbx1 in the VBC-Cul2 ubiquitin ligase complex. *Genes Cells* **10**: 679–691
- Mendoza HM, Shen LN, Botting C, Lewis A, Chen J, Ink B, Hay RT (2003) NEDP1, a highly conserved cysteine protease that deNEDDylates cullins. *J Biol Chem* **278**: 25637–25643
- Mori F, Nishie M, Piao YS, Kito K, Kamitani T, Takahashi H, Wakabayashi K (2005) Accumulation of NEDD8 in neuronal and glial inclusions of neurodegenerative disorders. *Neuropathol Appl Neurobiol* **31**: 53–61
- Morimoto M, Nishida T, Nagayama Y, Yasuda H (2003) Nedd8-modification of Cul1 is promoted by Roc1 as a Nedd8-E3 ligase and regulates its stability. *Biochem Biophys Res Commun* **301**: 392–398
- Norman JA, Shiekhhattar R (2006) Analysis of Nedd8-associated polypeptides: a model for deciphering the pathway for ubiquitin-like modifications. *Biochemistry* **45**: 3014–3019
- Ohta T, Michel JJ, Schottelius AJ, Xiong Y (1999) ROC1, a homolog of APC11, represents a family of cullin partners with an associated ubiquitin ligase activity. *Mol Cell* **3**: 535–541
- Osaka F, Kawasaki H, Aida N, Saeki M, Chiba T, Kawashima S, Tanaka K, Kato S (1998) A new NEDD8-ligating system for cullin-4A. *Genes Dev* **12**: 2263–2268
- Osaka F *et al* (2000) Covalent modifier NEDD8 is essential for SCF ubiquitin-ligase in fission yeast. *EMBO J* **19**: 3475–3484
- Ou CY, Lin YF, Chen YJ, Chien CT (2002) Distinct protein degradation mechanisms mediated by Cul1 and Cul3 controlling Ci stability in *Drosophila* eye development. *Genes Dev* **16**: 2403–2414
- Oved S *et al* (2006) Conjugation to Nedd8 instigates ubiquitylation and down-regulation of activated receptor tyrosine kinases. *J Biol Chem* **281**: 21640–21651
- Pan ZQ, Kentsis A, Dias DC, Yamoah K, Wu K (2004) Nedd8 on cullin: building an expressway to protein destruction. *Oncogene* **23**: 1985–1997
- Parry G, Estelle M (2004) Regulation of cullin-based ubiquitin ligases by the Nedd8/RUB ubiquitin-like proteins. *Semin Cell Dev Biol* **15**: 221–229
- Pintard L, Kurz T, Glaser S, Willis JH, Peter M, Bowerman B (2003) Neddylation and deneddylation of CUL-3 is required to target MEI-1/Katanin for degradation at the meiosis-to-mitosis transition in *C. elegans*. *Curr Biol* **13**: 911–921
- Podust VN, Brownell JE, Gladysheva TB, Luo RS, Wang C, Coggins MB, Pierce JW, Lightcap ES, Chau V (2000) A Nedd8 conjugation pathway is essential for proteolytic targeting of p27Kip1 by ubiquitination. *Proc Natl Acad Sci USA* **97**: 4579–4584
- Rao-Naik C, delaCruz W, Laplaza JM, Tan S, Callis J, Fisher AJ (1998) The rub family of ubiquitin-like proteins. Crystal structure of *Arabidopsis* rub1 and expression of multiple rubs in *Arabidopsis*. *J Biol Chem* **273**: 34976–34982
- Read MA *et al* (2000) Nedd8 modification of cul-1 activates SCF(β TrCP)-dependent ubiquitination of I κ B α . *Mol Cell Biol* **20**: 2326–2333
- Reverter D, Wu K, Erdene TG, Pan ZQ, Wilkinson KD, Lima CD (2005) Structure of a complex between Nedd8 and the Ulp/Senp protease family member Den1. *J Mol Biol* **345**: 141–151
- Russell RC, Ohh M (2008) NEDD8 acts as a ‘molecular switch’ defining the functional selectivity of VHL. *EMBO Rep* **9**: 486–491
- Sakata E, Yamaguchi Y, Miyauchi Y, Iwai K, Chiba T, Saeki Y, Matsuda N, Tanaka K, Kato K (2007) Direct interactions between NEDD8 and ubiquitin E2 conjugating enzymes upregulate cullin-based E3 ligase activity. *Nat Struct Mol Biol* **14**: 167–168
- Salon C, Brambilla E, Brambilla C, Lantuejoul S, Gazzeri S, Eymin B (2007) Altered pattern of Cul-1 protein expression and neddylation in human lung tumours: relationships with CAND1 and cyclin E protein levels. *J Pathol* **213**: 303–310
- Schwechheimer C (2004) The COP9 signalosome (CSN): an evolutionary conserved proteolysis regulator in eukaryotic development. *Biochim Biophys Acta* **1695**: 45–54
- Seol JH *et al* (1999) Cdc53/cullin and the essential Hrt1 RING-H2 subunit of SCF define a ubiquitin ligase module that activates the E2 enzyme Cdc34. *Genes Dev* **13**: 1614–1626
- Shen LN, Liu H, Dong C, Xirodimas D, Naismith JH, Hay RT (2005) Structural basis of NEDD8 ubiquitin discrimination by the deNEDDylating enzyme NEDP1. *EMBO J* **24**: 1341–1351
- Skaar JR, Florens L, Tsutsumi T, Arai T, Tron A, Swanson SK, Washburn MP, DeCaprio JA (2007) PARC and CUL7 form atypical cullin RING ligase complexes. *Cancer Res* **67**: 2006–2014
- Souphron J, Waddell MB, Paydar A, Tokgoz-Gromley Z, Roussel MF, Schulman BA (2008) Structural dissection of a gating mechanism preventing misactivation of ubiquitin by NEDD8’s E1. *Biochemistry* **47**: 8961–8969
- Stickle NH, Chung J, Klco JM, Hill RP, Kaelin WG Jr, Ohh M (2004) pVHL modification by NEDD8 is required for fibronectin matrix assembly and suppression of tumor development. *Mol Cell Biol* **24**: 3251–3261
- Tateishi K, Omata M, Tanaka K, Chiba T (2001) The NEDD8 system is essential for cell cycle progression and morphogenetic pathway in mice. *J Cell Biol* **155**: 571–579
- Wada H, Kito K, Caskey LS, Yeh ET, Kamitani T (1998) Cleavage of the C-terminus of NEDD8 by UCH-L3. *Biochem Biophys Res Commun* **251**: 688–692
- Wada H, Yeh ET, Kamitani T (2000) A dominant-negative UBC12 mutant sequesters NEDD8 and inhibits NEDD8 conjugation *in vivo*. *J Biol Chem* **275**: 17008–17015
- Walden H, Podgorski MS, Schulman BA (2003a) Insights into the ubiquitin transfer cascade from the structure of the activating enzyme for NEDD8. *Nature* **422**: 330–334
- Walden H, Podgorski MS, Huang DT, Miller DW, Howard RJ, Minor DL Jr, Holton JM, Schulman BA (2003b) The structure of the APPBP1-UBA3-NEDD8-ATP complex reveals the basis for selective ubiquitin-like protein activation by an E1. *Mol Cell* **12**: 1427–1437
- Watson IR, Blanch A, Lin DC, Ohh M, Irwin MS (2006) Mdm2-mediated NEDD8 modification of TAp73 regulates its transactivation function. *J Biol Chem* **281**: 34096–34103
- Wei N, Deng XW (2003) The COP9 signalosome. *Annu Rev Cell Dev Biol* **19**: 261–286
- Weissman AM (2001) Themes and variations on ubiquitylation. *Nat Rev Mol Cell Biol* **2**: 169–178
- Whitby FG, Xia G, Pickart CM, Hill CP (1998) Crystal structure of the human ubiquitin-like protein NEDD8 and interactions with ubiquitin pathway enzymes. *J Biol Chem* **273**: 34983–34991
- Wu JT, Chan YR, Chien CT (2006) Protection of cullin-RING E3 ligases by CSN-UBP12. *Trends Cell Biol* **16**: 362–369
- Wu K *et al* (2003) DEN1 is a dual function protease capable of processing the C terminus of Nedd8 and deconjugating hyper-neddylation CUL1. *J Biol Chem* **278**: 28882–28891
- Xirodimas DP, Saville MK, Bourdon JC, Hay RT, Lane DP (2004) Mdm2-mediated NEDD8 conjugation of p53 inhibits its transcriptional activity. *Cell* **118**: 83–97
- Xirodimas DP, Sundqvist A, Nakamura A, Shen L, Botting C, Hay RT (2008) Ribosomal proteins are targets for the NEDD8 pathway. *EMBO Rep* **9**: 280–286
- Yamoah K, Wu K, Pan ZQ (2005) *In vitro* cleavage of Nedd8 from cullin 1 by COP9 signalosome and deneddyase 1. *Methods Enzymol* **398**: 509–522
- Yamoah K, Oashi T, Sarikas A, Gazdoiu S, Osman R, Pan ZQ (2008) Autoinhibitory regulation of SCF-mediated ubiquitination by human cullin 1’s C-terminal tail. *Proc Natl Acad Sci USA* **105**: 12230–12235
- Yang X, Zhou J, Sun L, Wei Z, Gao J, Gong W, Xu RM, Rao Z, Liu Y (2007) Structural basis for the function of DCN-1 in protein neddylation. *J Biol Chem* **282**: 24490–24494

- Zheng N *et al* (2002a) Structure of the Cul1–Rbx1–Skp1–F boxSkp2 SCF ubiquitin ligase complex. *Nature* **416**: 703–709
- Zheng J *et al* (2002b) CAND1 binds to unneddylated CUL1 and regulates the formation of SCF ubiquitin E3 ligase complex. *Mol Cell* **10**: 1519–1526
- Zhou C, Seibert V, Geyer R, Rhee E, Lyapina S, Cope G, Deshaies RJ, Wolf DA (2001) The fission yeast COP9/signalosome is involved in cullin modification by ubiquitin-related Ned8p. *BMC Biochem* **2**: 7
- Zhou L, Watts FZ (2005) Nep1, a *Schizosaccharomyces pombe* deneddylating enzyme. *Biochem J* **389**: 307–314



Gwénaél Rabut (left) & Matthias Peter

EMBO Protein Modifications: Beyond the Usual Suspects

Previous issues of EMBO reports include:

Variations on complexity

Frank Gannon & Nonia Pariente
doi:10.1038/embor.2008.85

Atypical ubiquitin chains: new molecular signals

Fumiyo Ikeda & Ivan Dikic
doi:10.1038/embor.2008.93

Polyglutamylolation: a fine-regulator of protein function?

Carsten Janke, Krzysztof Rogowski & Juliette van Dijk
doi:10.1038/embor.2008.114

The O-linked N-acetylglucosamine modification in cellular signalling and the immune system

Alexander Golks & Danilo Guerini
doi:10.1038/embor.2008.129

The Atg8 and Atg12 ubiquitin-like conjugation systems in macroautophagy

Jiefei Geng & Daniel J. Klionsky
doi:10.1038/embor.2008.163

See future issues for other reviews in the series:

The expanding field of poly(ADP-ribosyl)ation reactions

Antoinette Hakmé, Heng-Kuan Wong, Françoise Dantzer & Valérie Schreiber

Urm1 at the crossroads of modifications

Sebastian Leidel, Patrick G.A. Pedrioli & Kay Hofmann