

# Role of Predicted Metalloprotease Motif of Jab1/Csn5 in Cleavage of Nedd8 from Cul1

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COP9 signalosome (CSN) cleaves the ubiquitin-like protein Nedd8 from the Cul1 subunit of SCF ubiquitin ligases. The Jab1/MPN domain metalloenzyme (JAMM) motif in the Jab1/Csn5 subunit was found to underlie CSN's Nedd8 isopeptidase activity. JAMM is found in proteins from archaea, bacteria, and eukaryotes, including the Rpn11 subunit of the 26S proteasome. Metal chelators and point mutations within JAMM abolished CSN-dependent cleavage of Nedd8 from Cul1, yet had little effect on CSN complex assembly. Optimal SCF activity in yeast and both viability and proper photoreceptor cell (R cell) development in *Drosophila melanogaster* required an intact Csn5 JAMM domain. We propose that JAMM isopeptidases play important roles in a variety of physiological pathways.

SCF ubiquitin ligases (1, 2) regulate cellular function through the ubiquitination of numerous substrates, including p27 and IκBα (3). SCF complexes consist of four essential subunits: Cul1, the RING-H2 protein Hrt1/Roc1/Rbx1, Skp1, and a substrate-binding F-box protein (3). In addition, the ubiquitin-like protein Nedd8 is conjugated to Cul1. This modification (neddylation) is essential in fis-

sion yeast (4) and stimulates recruitment of E2 to SCF ubiquitin ligase (5).

The COP9 signalosome (CSN), which is a highly conserved complex related to both the proteasome lid and the eukaryotic initiation factor 3, promotes deneddylation of Cul1 (6, 7). CSN may also regulate a protein kinase that phosphorylates IκBα and p53 (8). CSN has been implicated in a diverse range of

physiological processes, including plant and animal development (9–11), transcription (12), and signaling (13, 14), but the relationship between the biochemical and physiological functions of CSN remains unclear.

Individual deletion of all known de-ubiquitinating enzymes in *Saccharomyces cerevisiae* (15) and mutation of a candidate active-site cysteine in the Csn5/Jab1 subunit of CSN had no effect on deneddylation of Cul1 (15, 16). Thus, to elucidate the mechanism of deneddylation, we sought to identify a motif within CSN that might underlie isopeptidase activity. Iterated PSI-BLAST analysis (17) and sequence alignment revealed a set of eukaryotic and prokaryotic Pad1/Jab1/MPN/Mov34 domain (hereafter referred to as Jab1/MPN domain) proteins previously identified as homologs of Csn5 and Csn6 (18–20). Almost all prokaryotic Jab1/MPN domains possess a His-X-His-X<sub>10</sub>-Asp motif (where X indicates any residue) accompanied by an upstream conserved glutamate (Fig. 1). Al-

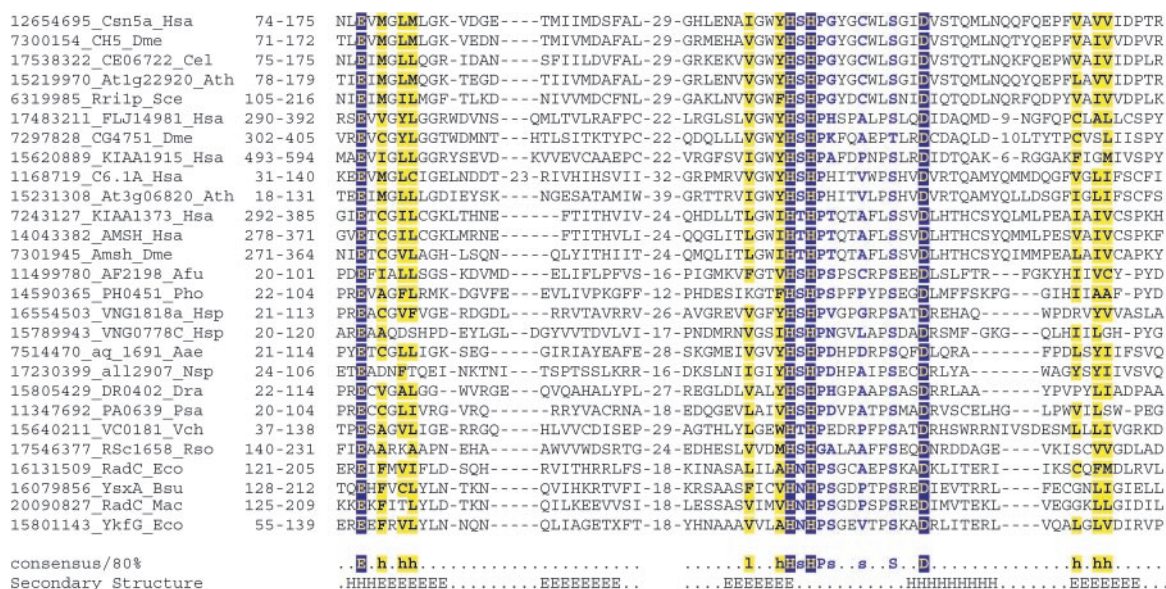
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**Fig. 1.** Alignment of predicted JAMM domains (34). Selected sequences were aligned using T-Coffee (35) and modified manually to ensure correct superposition of the conserved motifs. A more thorough alignment is available (fig. S1). Eukaryotic proteins are grouped to reflect orthologous relationships. Position of the aligned region in the sequence is shown by numbers; poorly conserved spacers are not shown and are designated by numbers. The consensus includes amino acid residues conserved in 80% of the aligned sequences; l indicates aliphatic residues (A, I, L, V; yellow shading), h indicates hydrophobic residues (F, Y, W, A, I, L, V, M; yellow shading), and s indicates small residues (G, A, C, S, D, N, V, P; blue letters). Predicted metal-chelating and catalytic residues are shown in yellow against a dark blue background. Secondary structure prediction was made using the PHD program with the multiple alignment submitted as the query (36); E indicates extended conformation (β-strand), and H indicates α-helix. Each protein is denoted by the GenBank identifier followed by the gene name and



abbreviated species name. Species abbreviations: Hsa, *Homo sapiens*; Dme, *Drosophila melanogaster*; Cel, *Caenorhabditis elegans*; Ath, *Arabidopsis thaliana*; Sce, *Saccharomyces cerevisiae*; Afu, *Archaeoglobus fulgidus*; Pho, *Pyrococcus horikoshii*; Hsp, *Halobacterium* sp.; Aae, *Aquifex aeolicus*; Nsp, *Neostoc* sp.; Dra, *Deinococcus radiodurans*; Psa, *Pseudomonas aeruginosa*; Vch, *Vibrio cholerae*; Rso, *Ralstonia solanacearum*; Eco, *Escherichia coli*; Bsu, *Bacillus subtilis*; Mac, *Methanosarcina acetivorans*.

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though this motif is not found in all eukaryotic Jab1/MPN domain proteins (e.g., Csn6), it is conserved in a wide range of them, including all Csn5 and proteasome Rpn11 orthologs (Fig. 1). His-Asp/Glu residues often serve as ligands to immobilize a catalytic metal ion within the active site of hydrolases, including proteases (21). Given the excep-

tional conservation of residues able to chelate zinc, we hypothesized that the Csn5 JAMM motif comprises a metalloprotease that sustains Cul1 deneddylation (22).

To test this hypothesis, we evaluated the sensitivity of CSN-associated deneddylation activity to metal chelators (6). Presence of 20 mM ethylene diamine tetraacetic acid

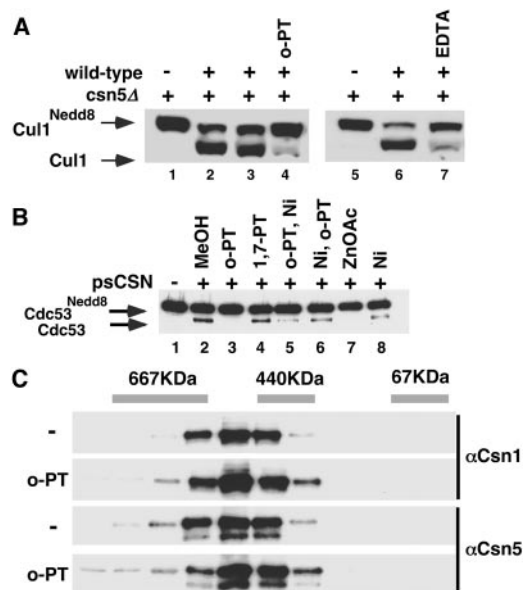
(EDTA) or 1 mM 1,10-phenanthroline (o-PT), but not 1 mM EDTA, blocked the ability of wild-type extract to restore Cul1 deneddylation activity to *csn5Δ* extract (Fig. 2A). Moreover, pretreatment of purified pig spleen CSN (23), but not substrate (15), with o-PT abolished cleavage of immunopurified Cul1-Nedd8 conjugates (Fig. 2B). Inhibition was dependent on metal chelation, because the nonchelating o-PT analog 1,7-phenanthroline had no effect on activity (Fig. 2B). These data mirror those for the LasA metalloproteases (24), suggesting that CSN's deneddylation activity was metal-dependent. Excess nickel added either before or after inactivation of psCSN by o-PT partially restored activity (Fig. 2B). Restoration with zinc was unsuccessful, because CSN, like the zinc metalloproteases thermolysin (25) and carboxypeptidase A (26), was inhibited by even submillimolar levels of free zinc (Fig. 2B). Importantly, o-PT had no obvious effect on the assembly of the CSN complex as judged by size exclusion chromatography (Fig. 2C). Thus, CSN-associated deneddylation activity, but not complex integrity, required metal ions.

We next tested whether the JAMM motif was essential to sustain proper Cul1 neddylation state *in vivo*. *Schizosaccharomyces pombe csn5Δ* extracts contain Cul1 exclusively in the Nedd8-modified form, but a wild-type pattern of Cul1 neddylation is restored upon ectopic expression of FLAG-Csn5 (Fig. 3A) (16). By contrast, FLAG-Csn5 proteins with mutations in the conserved JAMM residues accumulated normally and assembled with Csn1<sup>myc13</sup> (Fig. 3B) and Csn2<sup>myc13</sup> (Fig. 3C) but nevertheless failed to complement the *csn5Δ* phenotype (Fig. 3A).

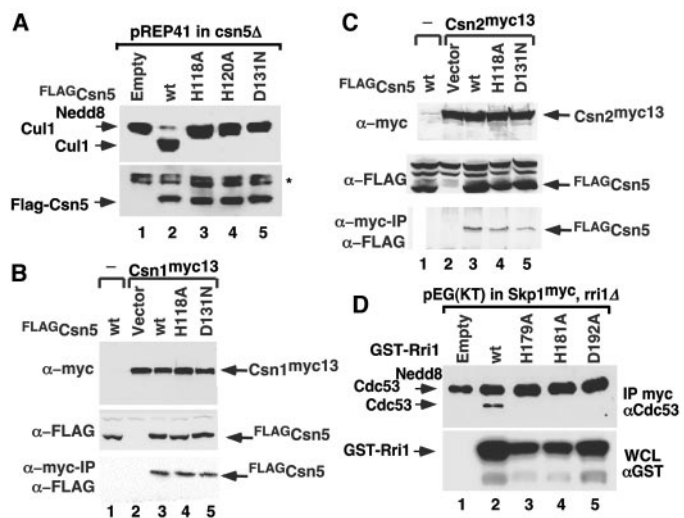
*Saccharomyces cerevisiae* contains both neddylation enzymes (27, 28) and the Csn5 ortholog Rri1 (6). In *rri1Δ* cells, the budding yeast Cul1 ortholog, Cdc53, accumulates exclusively in a neddylated form (Fig. 3D) (6). Although budding yeast lacks obvious orthologs for other CSN subunits, affinity purification revealed candidate partners for Rri1 (29), two of which were essential to sustain a normal pattern of Cdc53 neddylation as judged by immunoblot analysis of deletion mutants (fig. S2). To address the function of Rri1's JAMM motif, we expressed wild-type and JAMM point mutant Rri1 proteins as glutathione *S*-transferase (GST) fusions in *rri1Δ* cells. Expression of wild-type—but not mutant—GST-Rri1 proteins partly restored deneddylated Cdc53 (Fig. 3D). Thus, despite Rri1 being the most divergent Csn5 ortholog identified to date, its JAMM motif was nevertheless required to sustain Cul1 deneddylation.

To address the physiological significance of Rri1-associated isopeptidase activity, we deleted *RRI1* in the temperature-sensitive (*ts*) strains *cdc53-1*, *cdc34-2*, *skp1-12*, and *cdc4-1*. Because neddylation of Cul1 enhances SCF activ-

**Fig. 2.** CSN-dependent deneddylation of Cul1 is sensitive to divalent cation chelators. (A) Deneddylation of *S. pombe* Cul1 is inhibited by both o-phenanthroline (o-PT) and EDTA. *csn5Δ S. pombe* lysates (lane 1 and 5) or supplemented with wild-type (Cul1-myc13) lysates (lanes 2 to 4 and 6 and 7) at 30°C for 30 min. For lanes 3, 4, and 7, both lysates were preincubated for 5 min with 0.5% methanol (MeOH) carrier, 1 mM o-PT, or 20 mM EDTA, respectively. Reactions were evaluated by western blot with antibodies to *S. pombe* Cul1 (Pcu1). (B) psCSN activity is modulated by metal ion concentration. psCSN was incubated for 5 min in the presence of 0.5% MeOH (lane 2), 1 mM o-PT (lanes 3 and 5), 1 mM 1,7-phenanthroline (1,7-PT, lane 4), 1 mM o-PT plus 2 mM NiCl<sub>2</sub> (Ni, lane 6), 1 mM zinc acetate (ZnOAc, lane 7), or 1 mM NiCl<sub>2</sub> (Ni, lane 8) before being mixed with immunopurified Cul1<sup>Nedd8</sup> (37). The sample in lane 5 was adjusted to 2 mM NiCl<sub>2</sub> after o-PT treatment. (C) o-PT does not disrupt CSN. Pig spleen CSN alone or CSN treated with 1 mM o-PT was fractionated by Superose 6 gel filtration. Fractions were analyzed by Western blotting with antibodies to either Csn1 or Csn5. Size markers are indicated above.



**Fig. 3.** Point mutations within JAMM disrupt CSN deneddylation activity but not assembly. (A) Mutation of either the histidine or aspartic acid residues within Csn5's JAMM motif abolishes Cul1 deneddylation activity in fission yeast. Whole-cell lysates (see SOM) from *S. pombe csn5Δ* strains carrying empty vector (lane 1) or vectors encoding FLAG-Csn5 (wt, lane 2), FLAG-Csn5 (H118A), FLAG-Csn5 (H120A), or FLAG-Csn5 (D131N) were analyzed by Western blot with antibodies to *S. pombe* Cul1 (Pcu1, top panel) and FLAG (bottom panel). Asterisk denotes a nonspecific band. (B) and (C) Mutations in JAMM domain of Csn5 do not abolish CSN assembly. Extracts (6) from *S. pombe csn5Δ* strains carrying *csn1-myc13*<sup>+</sup> [(B), lanes 2 to 5] or *csn2-myc13*<sup>+</sup> [(C), lanes 2 to 5] and expressing FLAG-tagged wild-type or mutant Csn5 proteins were evaluated directly by Western blotting with antibodies to the Myc or FLAG tag (top and middle panels), or were first immunoprecipitated with antibodies to the Myc tag followed by Western blotting with antibodies to FLAG (bottom panel). An untagged control is shown in lane 1. (D) Mutations within Rri1's JAMM motif abolish Cul1 deneddylation activity in *S. cerevisiae*. *SKP1<sup>Myc9</sup>rri1Δ* cells were transformed with the Gal-inducible vector pEG(KT) (lane 1) or the same vector encoding GST fusions to Rri1 (lane 2), Rri1(H179A) (lane 3), Rri1(H181A) (lane 4), or Rri1(D192A) (lane 5). Extracts (see SOM) were either analyzed by Western blot analysis with antibodies to GST or were first immunoprecipitated with α-myc resin followed by Western blotting with antibodies to Cdc53.



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ity (30), we anticipated that *rri1Δ* would suppress the ts growth of SCF mutants. Paradoxically, *rri1Δ* exacerbated both cell growth (Fig. 4A) and Sic1 turnover (Fig. 4C) defects when combined with SCF mutations.

Cosuppression of Csn5 in *Arabidopsis* partially stabilizes the SCF reporter substrate

IAA6-LUC (7). However, it has not been established whether this effect was mediated by reduced deneddylation or loss of some other CSN activity (8). To test the specificity of the genetic interactions between *rri1Δ* and SCF mutants, we expressed wild-type and mutant GST-Rri1 in *cdc34-2*, *rri1Δ* and *skp1-12*, *rri1Δ*

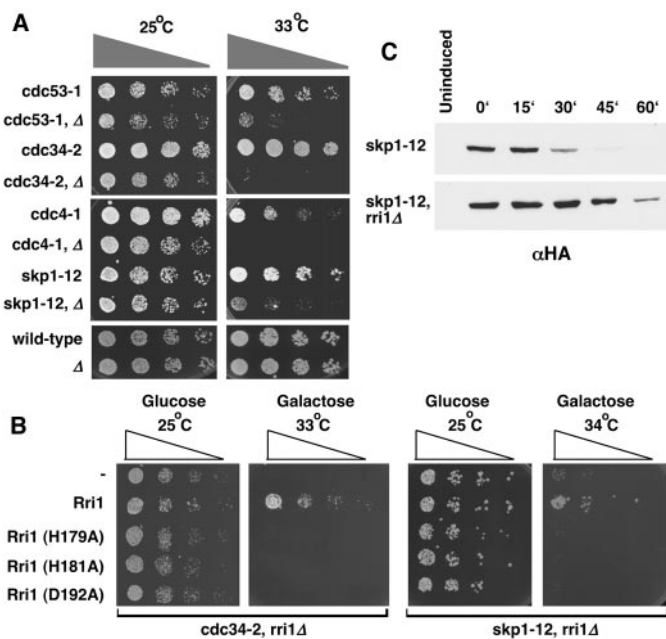
cells. Galactose (Gal)-inducible expression of wild-type GST-Rri1, but not JAMM point mutant GST-Rri1, restored growth to both *cdc34-2 rri1Δ* and *skp1-12 rri1Δ* at the restrictive temperature (Fig. 4B). Thus, the genetic interaction between SCF mutants and *rri1Δ* most likely arose from loss of JAMM-dependent isopeptidase activity. Deletion of *S. cerevisiae Nedd8* (aka *RUB1*) similarly exacerbates the ts growth defect of SCF mutants (27), suggesting that cycles of neddylation and deneddylation are needed to sustain optimal SCF activity.

To begin to address whether JAMM-dependent isopeptidase activity underlies the myriad physiological functions that have been proposed for Csn5 and CSN (8), we tested the role of the Csn5 JAMM motif in *Drosophila melanogaster* development. Mutations in *CSN5* in *Drosophila* result in organismal lethality (11). At the cellular level, a range of specific defects in photoreceptor neuron differentiation have been described (11). Transgenic *csn5-null* (*csn5<sup>Δ/Δ</sup>*) fly larvae carrying a P-element with wild-type (11) or JAMM mutant (D148N) *Drosophila CSN5* cDNAs under the control of the heat shock-dependent *hsp70* promoter were generated. Wild-type Csn5 and Csn5 (D148N) accumulated to similar levels in third instar larvae (31) subjected to periodic heat shocks, whereas no Csn5 protein was detected in unshocked larvae, confirming that all detectable Csn5 in these animals was transgenic (Fig. 5I). Small but equivalent fractions of wild-type Csn5 and Csn5 (D148N) molecules formed CSN complexes upon heat shock-induced expression (15). Inefficient assembly may be due to heat stress, because our analysis of Csn5 in *S. pombe* (Fig. 3, B and C) and Rpn11 in *S. cerevisiae* (32) indicated that mutation of the JAMM motif did not prevent folding of the Jab1/MPN domain.

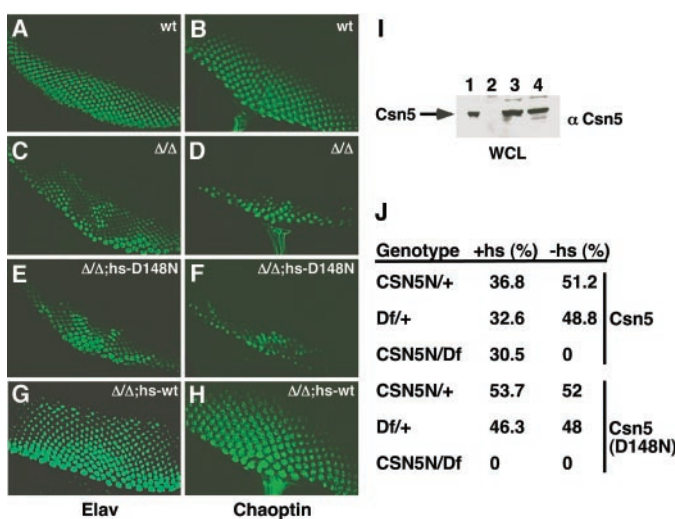
Whereas the lethality of *csn5<sup>Δ/Δ</sup>* animals was rescued by periodic heat shock-induced expression of a wild-type Csn5 transgene, no adult flies were recovered upon equivalent expression of Csn5 (D148N) (Fig. 5J). Furthermore, *CSN5*-dependent expression of the R cell markers *Elav* and *Chaoptin* was restored by expression of wild-type Csn5 (Fig. 5, G and H), but not Csn5 (D148N) (Fig. 5, E and F). These data indicate that the JAMM motif is required for an essential developmental function of Csn5 in vivo.

Csn5 contains a metal-coordinating JAMM motif that was essential for cleavage of Nedd8 from Cul1. Evolutionary and structural considerations (20) predicted a catalytic role for JAMM, and this prediction was supported by genetic and biochemical analyses. However, we failed to detect Nedd8-directed isopeptidase with recombinant Csn5, suggesting an obligate role for complex assembly in enzyme activity. A JAMM motif in the proteasome lid component Rpn11 was likewise required for cleavage

**Fig. 4.** Functional interaction between SCF and Rri1. (A) Deletion of the *RRI1* locus in *S. cerevisiae* enhances the temperature-sensitive phenotype of mutants deficient in SCF. Strains indicated were grown in complete media (YP) with dextrose (Dex) at 25°C to mid-log phase, and serial dilutions of cells were spotted onto YP Dex plates. Cells were grown at 25°C or 33°C, and plates were analyzed after 2 to 3 days growth. (B) Enhancement of the temperature-sensitive phenotype of *cdc34-2 rri1Δ* and *skp1-12 rri1Δ* can be rescued by overexpression of wild-type, but not the JAMM mutant, GST-Rri1. *cdc34-2 rri1Δ* and *skp1-12 rri1Δ* strains were transformed with the Gal-inducible vectors described in Fig. 3D. Strains were grown on YP Dex at 25°C, YP Gal at 33°C for *cdc34-2 rri1Δ*, or YP Gal at 34°C for *skp1-12 rri1Δ*. (C) Deletion of *RRI1* in *skp1-12* impairs turnover of Sic1. *skp1-12* and *skp1-12 rri1Δ* cells carrying a *GAL-SIC1<sup>HA</sup>* vector were transferred to YP Gal to express Sic1<sup>HA</sup> following nocodazole-induced arrest in mitosis. A chase was then initiated by transfer of the cells to YP Dex to silence the GAL promoter, and time points were taken as indicated. Cells lysates (see SOM) were analyzed by western blot analysis with antibodies against the hemagglutinin (HA) epitope.



**Fig. 5.** Mutation of JAMM in *Drosophila* Csn5 does not rescue lethality or R cell differentiation defect in *csn5<sup>Δ/Δ</sup>* animals. (A to H) Developing eye disk of second/third instar wild-type [wt, (A) and (B)], *csn5<sup>Δ/Δ</sup>* [(A) and (B)], *csn5<sup>Δ/Δ</sup>;hs-Csn5(D148N)* [(C) and (D)], *csn5<sup>Δ/Δ</sup>;hs-Csn5(D148N)* [(E) and (F)], and *csn5<sup>Δ/Δ</sup>;hs-Csn5* [(G) and (H)] larvae were stained for the R cell markers *Elav* [(A), (C), (E), and (G)] and *chaoptin* [(B), (D), (F), and (H)]. Genetics and rescue experiments, immunohistochemistry, and histology were performed as described (11). (I) Western blot analysis with anti-Csn5 antibodies (Novus) of whole-cell extracts from second/third instar larvae of wild-type (lane 1), *csn5<sup>Δ/Δ</sup>;hs-Csn5(D148N)* (lanes 2 and 4), and *csn5<sup>Δ/Δ</sup>;hs-Csn5* (lane 3). For lanes 3 and 4, larvae were heat shocked at 37°C for 30 min. Larvae were subsequently harvested 60 min after heat shock and lysed as described (see SOM). (J) Lethality of *csn5<sup>Δ/Δ</sup>* cannot be rescued by JAMM mutant Csn5. Percentage recovery of genotypes (see SOM) of adult progeny obtained from crossing *CSN5-null/+* to *Df/+*; *CSN5-transgene* (wild-type or D148N mutant). Percentages reflect approximately 80 adult progeny scored (see SOM). Abbreviations: CSN5N, CSN5-delete; +, wild-type CSN5; Df, deficiency-spanning CSN5 locus; hs, heat-shock (see SOM).



of ubiquitin conjugates from proteasome substrates (32). There exist other Csn5/Rpn11 homologs in eukaryotes (Fig. 1) and, by extension, we propose the “JAMMIN” hypothesis, which posits that eukaryotic JAMM proteins are isopeptidases that deconjugate Nedd8 or other ubiquitin-like proteins.

*Drosophila* sustained by Csn5 carrying a JAMM domain mutation arrest development as larvae with abnormalities in photoreceptor differentiation, suggesting that at least two functions associated with Csn5—viability and photoreceptor differentiation—require its JAMM-dependent isopeptidase activity. Given that Csn5 has been implicated in c-jun signaling (12), p27 turnover (33), cytokine signaling (14), and growth cone-target interactions (11), it will be interesting to see if isopeptidase activity of Csn5 underlies these diverse processes as well.

All neddylated proteins known are members of the cullin family. It is not clear whether CSN isopeptidase acts exclusively upon cullin-Nedd8 conjugates or cleaves other targets. Regardless, given the large number of F-box proteins and the potential for substantial diversity in the substrates for SCF and other cullin-based ubiquitin ligases, CSN deneddylation activity may play an enormous role in cellular regulation.

References and Notes

1. D. Skowyra, K. L. Craig, M. Tyers, S. J. Elledge, J. W. Harper, *Cell* **91**, 209 (1997).
2. R. M. Feldman, C. C. Correll, K. B. Kaplan, R. J. Deshaies, *Cell* **91**, 221 (1997).
3. R. J. Deshaies, *Annu. Rev. Cell Dev. Biol.* **15**, 435 (1999).
4. F. Osaka *et al.*, *EMBO J.* **19**, 3475 (2000).
5. T. Kawakami *et al.*, *EMBO J.* **20**, 4003 (2001).
6. S. Lyapina *et al.*, *Science* **292**, 1382 (2001).
7. C. Schwechheimer *et al.*, *Science* **292**, 1379 (2001).
8. M. Seeger, C. Gordon, W. Dubiel, *Curr. Biol.* **11**, R643 (2001).
9. N. Wei, X. W. Deng, *Trends Genet.* **15**, 98 (1999).
10. S. Freilich *et al.*, *Curr. Biol.* **9**, 1187 (1999).
11. G. S. Suh *et al.*, *Neuron* **33**, 35 (2002).
12. F. X. Claret, M. Hibi, S. Dhut, T. Toda, M. Karin, *Nature* **383**, 453 (1996).
13. R. Kleemann *et al.*, *Nature* **408**, 211 (2000).
14. E. Bianchi *et al.*, *Nature* **404**, 617 (2000).
15. G. Cope, R. Deshaies, unpublished data.
16. N. D. Zhou *et al.*, *BMC Biochem.* [online] **2**, article #7 (2001). Available at: [www.biomedcentral.com/bmcbiochem](http://www.biomedcentral.com/bmcbiochem).
17. S. F. Altschul *et al.*, *Nucleic Acids Res.* **25**, 3389 (1997).
18. L. Aravind, C. P. Ponting, *Protein Sci.* **7**, 1250 (1998).
19. K. E. Mundt *et al.*, *Curr. Biol.* **9**, 1427 (1999).
20. C. P. Ponting, L. Aravind, J. Schultz, P. Bork, E. V. Koonin, *J. Mol. Biol.* **289**, 729 (1999).
21. N. D. Rawlings, A. J. Barrett, *Methods Enzymol.* **248**, 183 (1995).
22. Like JAMM, metal-dependent peptidases often possess two conserved acidic residues: one that acts as a ligand for zinc and a second that acts as a nucleophile to promote attack by water on the peptide backbone. Based on the common occurrence of Glu as a nucleophile and on the predicted secondary structure of the Jab/MPN domain, we propose that the Glu helps polarize a water molecule for hydrolytic attack and the COOH-terminal His and Asp residues serve as three ligands for zinc.
23. N. Wei, X. W. Deng, *Photochem. Photobiol.* **68**, 237 (1998).

24. A. G. Loewy *et al.*, *J. Biol. Chem.* **268**, 9071 (1993).
25. D. R. Holland, A. C. Hausrath, D. Juers, B. W. Matthews, *Protein Sci.* **4**, 1955 (1995).
26. M. Gomez-Ortiz, F. X. Gomis-Ruth, R. Huber, F. X. Aviles, *FEBS Lett.* **400**, 336 (1997).
27. D. Lammer *et al.*, *Genes Dev.* **12**, 914 (1998).
28. D. Liakopoulos, G. Doenges, K. Matuschewski, S. Jentsch, *EMBO J.* **17**, 2208 (1998).
29. A. C. Gavin *et al.*, *Nature* **415**, 141 (2002).
30. V. N. Podust *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **97**, 4579 (2000).
31. Null mutant survival up to third instar larvae was context dependent. Although Csn5-null larvae rarely survived to third instar at UCLA, we routinely found that these mutants survived to third instar at CalTech.
32. R. Verma *et al.*, *Science* **298**, 611 (2002).
33. K. Tomoda, Y. Kubota, J. Kato, *Nature* **398**, 160 (1999).
34. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

35. C. Notredame, D. G. Higgins, J. Heringa, *J. Mol. Biol.* **302**, 205 (2000).
36. B. Rost, C. Sander, R. Schneider, *Comput. Appl. Biosci.* **10**, 53 (1994).
37. Materials and Methods are available as supporting material on Science Online.
38. We thank D. Wolf, M. Hochstrasser, K. Mundt, and A. Carr for generously providing yeast strains and plasmids, the S. Benzer lab for providing lab equipment and space, and S. Lyapina for psCSN. We thank members of the Deshaies lab for providing helpful insight and discussions. This work was supported by NIH (G.A.C.) and the Howard Hughes Medical Institute.

Supporting Online Material

[www.sciencemag.org/cgi/content/full/1075901/DC1](http://www.sciencemag.org/cgi/content/full/1075901/DC1)  
Materials and Methods  
Figs. S1 and S2  
References and Notes

9 July 2002; accepted 7 August 2002  
Published online 15 August 2002;  
10.1126/science.1075901  
Include this information when citing this paper.

## Role of Rpn11 Metalloprotease in Deubiquitination and Degradation by the 26S Proteasome

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The 26S proteasome mediates degradation of ubiquitin-conjugated proteins. Although ubiquitin is recycled from proteasome substrates, the molecular basis of deubiquitination at the proteasome and its relation to substrate degradation remain unknown. The Rpn11 subunit of the proteasome lid subcomplex contains a highly conserved Jab1/MPN domain-associated metalloisopeptidase (JAMM) motif—EX<sub>n</sub>HXHX<sub>10</sub>D. Mutation of the predicted active-site histidines to alanine (*rpn11AXA*) was lethal and stabilized ubiquitin pathway substrates in yeast. Rpn11<sup>AXA</sup> mutant proteasomes assembled normally but failed to either deubiquitinate or degrade ubiquitinated Sic1 in vitro. Our findings reveal an unexpected coupling between substrate deubiquitination and degradation and suggest a unifying rationale for the presence of the lid in eukaryotic proteasomes.

Proteolysis by the 26S proteasome proceeds by binding of the ubiquitinated substrate protein to the 19S regulatory particle, followed by its unfolding and translocation into the lumen of the 20S core, where it is degraded by the action of the 20S peptidases (1–3). At some point in this process, the ubiquitin targeting signal is detached from the substrate. It is appealing to envision that this deubiquitination is obligatorily coupled to degradation. Such coupling would render the targeting event irreversible, prevent unproductive turn-

over of ubiquitin, and presumably alleviate steric blockade of the 20S core entry portal by the bulky ubiquitin chain, which is linked by isopeptide bonds. When and where substrate deubiquitination takes place, the identity of the deubiquitinating enzyme (DUB), and whether deubiquitination of a substrate is essential for its degradation by the proteasome are unclear (4, 5).

Budding yeast ubiquitinated S-Cdk inhibitor Sic1 (Ub-Sic1) is rapidly degraded by purified 26S proteasomes (3, 6) in a reaction that recapitulates physiological requirements for Sic1 proteolysis (7, 8). To investigate whether degradation of Sic1 is normally accompanied by its deubiquitination, we evaluated the fate of Ub-Sic1 after inhibition of 26S proteolytic activity. Epoxomicin inhibits the proteasome by covalently binding the catalytically active β subunits of the 20S core (9). Purified 26S proteasomes were preincu-

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