doi:10.1038/nature13644

STRUCTURAL BIOLOGY

Corralling a protein-degradation regulator

The crystal structure of the COP9 signalosome, a large protein complex that regulates intracellular protein degradation, reveals how the complex achieves exquisite specificity for its substrates.

RAYMOND J. DESHAIES

Some 20 years ago[1], an enzyme complex was linked to the dramatic changes in development that occur when seedlings push through the soil and encounter sunlight. This complex, named the COP9 signalosome (CSN), is now thought to be common to all animals, plants and fungi. The CSN is involved in protein degradation, but because of its complicated structure, detailed knowledge of how its activity is controlled has remained elusive. In a paper published on *Nature*'s website today, Lingaraju *et al.*[2] report the crystallization of the CSN and determine its structure to a resolution of a remarkable 3.8 ångströms.

The CSN consists of eight protein subunits, CSN1–8, and regulates a family of enzyme complexes called cullin–RING E3 ubiquitin ligases (CRLs)[3], which modify their target proteins by attaching ubiquitin proteins to them. Ubiquitin modifications can have many effects on proteins, from influencing their cellular location to causing their degradation. In fact, the cullin protein that makes up the backbone of each CRL must itself be modified by a ubiquitin-like protein, NEDD8, before it can function as a ubiquitin ligase. The CSN inhibits this activity by detaching NEDD8 from cullin, and can also bind 'deneddylated' CRLs, thereby maintaining CRL inactivity after NEDD8 removal[4–7].

The CSN structure described by Lingaraju and colleagues brings to mind a widely splayed hand on which a small box sits askew, topped by a tomato (Fig. 1). Like a hand, the CSN has five digits (the amino-terminal ends of CSN1, 2, 4, 7, and 3 plus 8) projecting from an organizing centre, the palm. The palm is formed by the 'winged-helix' subdomains of these subunits, which associate to form a horseshoe-shaped structure. Resting on the hand is the box, formed by bundling of the carboxy-terminal ends of each subunit. Sitting atop this platform is the CSN5–CSN6 tomato.

Whereas some aspects of the CSN structure were anticipated from previous work on related proteins, it is a big surprise that the structure obtained by Lingaraju and co-workers is in an inactive configuration. The active site of the CSN is specified by a 'JAMM' domain in the CSN5 subunit. Typically, the active sites of the enzymes in the JAMM family contain a zinc ion (Zn^{2+}) bound by three evolutionarily conserved amino-acid residues (two histidines and an aspartate), with the remaining ligand-binding site of Zn^{2+} occupied by a water molecule that has been activated by another evolutionarily conserved amino acid, glutamate 76 (Glu 76; ref. 8). This activated water molecule detaches ubiquitin or ubiquitin-like proteins from their targets by hydrolysis. Whereas the histidine and aspartate residues of CSN5 are positioned as expected in the CSN structure, the water molecule is replaced by another amino acid, Glu 104. This explains a long-standing puzzle: whereas other JAMM-containing proteins efficiently cleave model substrates, such as ubiquitin with a rhodamine dye attached to its C terminus, purified CSN does so only poorly.

Lingaraju *et al.* tested the role of Glu 104 in CSN regulation by performing enzyme assays on CSN complexes in which Glu 104 was mutated. This mutant cleaved ubiquitin–rhodamine much faster than the natural enzyme, indicating that Glu104–Zn^{2+} binding might keep the CSN in an inactive state when it is free from CRL. Notably, mutation of the adjacent residue, threonine 103, results in defective development of the nervous system in fruit flies[9], which suggests that Glu 104-mediated regulation is required for proper control of CSN activity *in vivo*.

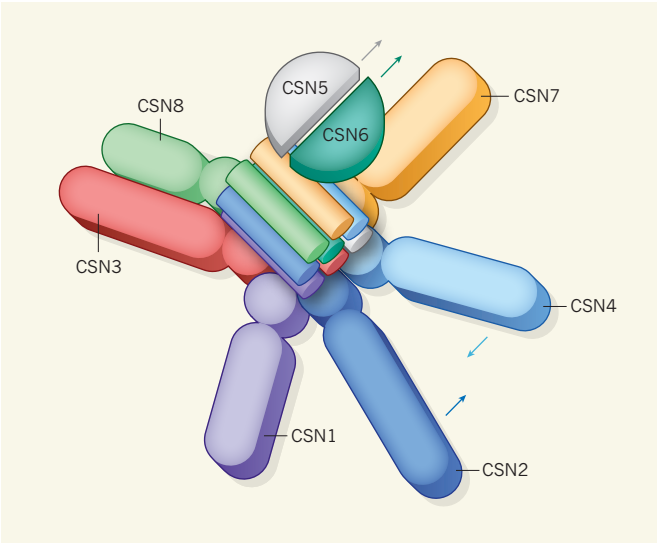

Figure 1 | Structure of the COP9 signalosome (CSN). This enzyme complex is comprised of eight CSN protein subunits. Six subunits make up the base of the CSN, a splayed 'hand' in which the proteins' N-terminal ends are at the fingertips and their winged-helix domains, drawn as circles, assemble to form the palm (partially obscured). The C-terminal ends of each protein are bundled together into a 'box' that sits askew on the hand. The CSN5 and CSN6 subunits associate intimately to form a 'tomato' sitting on the box. Lingaraju *et al.*[2] report that the CSN is inactive until it binds to its target, a cullin–RING E3 ubiquitin-ligase enzyme complex. On binding, the CSN undergoes activating conformational changes, indicated by coloured arrows that represent the movements of the altered subunits. For simplicity, the box is drawn as a uniform bundle, and so does not represent the actual position and length of each C terminus. (Figure adapted from Fig. 1 of ref. 2.)

The inhibited state of unbound CSN raises the obvious question of how the CSN gains its activity on binding CRLs. The authors used computer-modelling studies to compare their crystal structure of free CSN with a structure determined by electron microscopy[7] in which the CSN was bound to a CRL enzyme to which NEDD8 is attached. This comparison showed clearly that, to reconcile the two structures, substantial rearrangements of CSN2, CSN4 and CSN5–CSN6 must occur when the CSN and CRL bind (Fig. 1). In particular, movements in CSN4 and CSN6 must lead to a change in the CSN4–CSN6 interface.

To probe the significance of this interface, Lingaraju and colleagues deleted a β-hairpin loop in CSN6 that contributes to its interaction with CSN4. Surprisingly, the resulting complex, like the Glu 104 mutant, efficiently cleaved ubiquitin–rhodamine. It also deneddylated CRL more than four times faster than did the wild-type enzyme. These observations make it tempting to speculate that CSN4 is the signalosome's CRL sensor, and

that CSN4 movement during CRL binding triggers a cascade of rearrangements transmitted through CSN6 that prise CSN5's Glu 104 residue away from Zn^{2+}, so that Glu 76 can move into position, activating the CSN. However, when the authors made a double mutant lacking both the CSN6 loop and Glu 104, they found it to be more active than either individual mutant, suggesting that these two mutations have independent effects, rather than acting in a linear cascade. Furthermore, the N-terminal region of CSN4 does not seem to make strong contact with CRL[7], indicating that the CSN's CRL sensor may be in another subunit.

This study highlights a crucial lesson on the use of evolutionary conservation to predict enzyme regulation. Comparing the crystal structure of the CSN with those of the JAMM-containing enzymes AMSH-LP (ref. 10) and Rpn11 (refs 11, 12) reveals that, although all three use the same amino acids to coordinate Zn^{2+} and the activated water molecule, their activities are controlled in markedly different ways. AMSH-LP seems to be constitutively active, Rpn11 activity is promoted by rearrangements that bring the enzyme and its target substrate into proximity[13,14], and CSN5 is activated by substrate-driven relief of inhibition. Strikingly, CSN5 is inhibited by distinct mechanisms depending on whether the subunit is on its own[15] or integrated into the CSN. Although some generalizations apply across the JAMM family, it is clear that each member has its own distinctive features.

What lies ahead for research on the CSN? It will be fascinating to examine the structure of different CSN mutants, to work out the mechanism by which binding to CRLs brings about major conformational changes. It would also be wonderful to see a CSN–NEDD8–CRL complex in its full glory, to gain an atomic-level view of the CSN–CRL interface and how it might be influenced by NEDD8 or substrates that bind to CRL. Another question is whether binding of the CSN to neddylated or deneddylated CRL promotes the same conformational change in the CSN.

Binding and kinetic studies of the CSN and the mutated complexes reported by Lingaraju *et al.* should reveal whether the CSN's catalytic rate is determined by the conformational rearrangement that occurs on CRL binding. Furthermore, *in vivo* studies with Glu 104 and CSN6-loop mutants should show why free CSN must be inhibited.

Finally, this structure may help the design of drugs that act on the CSN, which could be an attractive target for the treatment of breast and liver cancer[16,17]. Although detailed characterization of CSN inhibitors has not been reported, my laboratory has identified several candidates through high-throughput screening (PubChem AID652009). The surprising observations reported by Lingaraju *et al.* suggest that it may be possible to inhibit the CSN but spare other JAMM proteins, by interfering with the active-site rearrangement that occurs when the CSN and CRL bind. ■

Raymond J. Deshaies *is in the Division of Biology and Biological Engineering, California Institute of Technology, Pasadena, California 91125, USA.*
e-mail: deshaies@caltech.edu

1. Wei, N., Chamovitz, D. A. & Deng, X.-W. *Cell* **78,** 117–124 (1994).
2. Lingaraju, G. M. *et al. Nature* http://dx.doi.org/10.1038/nature13566 (2014).
3. Lyapina, S. *et al. Science* **292,** 1382–1385 (2001).
4. Cope, G. A. *et al. Science* **298,** 608–611 (2002).
5. Fischer, E. S. *et al. Cell* **147,** 1024–1039 (2011).
6. Emberley, E. D., Mosadeghi, R. & Deshaies, R. J. *J. Biol. Chem.* **287,** 29679–29689 (2012).
7. Enchev, R. I. *et al. Cell Rep.* **2,** 616–627 (2012).
8. Ambroggio, X. I., Rees, D. C. & Deshaies, R. J. *PLoS Biol.* **2,** e2 (2004).
9. Suh, G. S. B. *et al. Neuron* **33,** 35–46 (2002).
10. Sato, Y. *et al. Nature* **455,** 358–362 (2008).
11. Pathare, G. R. *et al. Proc. Natl Acad. Sci. USA* **111,** 2984–2989 (2014).
12. Worden, E. J., Padovani, C. & Martin, A. *Nature Struct. Mol. Biol.* **21,** 220–227 (2014).
13. Śledź, P. *et al. Proc. Natl Acad. Sci. USA* **110,** 7264–7269 (2013).
14. Matyskiela, M. E., Lander, G. C. & Martin, A. *Nature Struct. Mol. Biol.* **20,** 781–788 (2013).
15. Echalier, A. *et al. Proc. Natl Acad. Sci. USA* **110,** 1273–1278 (2013).
16. Adler, A. S. *et al. Cancer Res.* **68,** 506–515 (2008).
17. Lee, Y.-H. *et al. Oncogene* **30,** 4175–4184 (2011).