Cell PRESS

On the Structural Model of the COP9 Signalosome

Ning Wei[1,*]
[1]Department of MCDB, Yale University, New Haven, CT 06520, USA
*Correspondence: ning.wei@yale.edu
DOI 10.1016/j.str.2008.12.004

In this issue, Sharon et al. (2009) determined the subunit organization of the COP9 signalosome by mass spectrometry. The structural features uncovered from this analysis are consistent with the multifunctionality of the complex and suggest tantalizing new clues about the inner workings of the CSN.

The architecture of the eight-subunit COP9 signalosome (CSN) has been an intriguing topic ever since the revelation of its full composition a decade ago. While the CSN is best known as the isopeptidase that cleaves the covalently linked Nedd8/Rub1 modification from its cullin substrate (deneddylation), it is puzzling why this protease is made into a multisubunit protein complex. The catalytic center of this reaction resides in the Csn5 subunit (Cope et al., 2002), but it is active only when Csn5 is integrated as part of the CSN holocomplex. In fact, Csn5 also exists outside the CSN complex, where it functions independently of deneddylation (Wei et al., 2008). In addition, a number of other subunits have also been reported to form small or mini-CSN subcomplexes. Unfortunately small CSN subcomplexes are poorly defined, as there is no report on the number of the small complexes and their compositions. As a result, the relevance of the small CSN subcomplexes remains a matter of debate: on one hand, these subcomplexes potentially explain CSN's multi-functionality; on the other hand, they could merely be a fractionation artifact that bears no functional relevance. The difficulty in characterizing a small CSN subcomplex lies in its low abundance and, more importantly, the lack of structural insight into the subunit organization of the CSN.

Conventional crystallography on CSN is exceedingly difficult. Not until the end of last year was the first crystal structure of a single subunit, Csn7, resolved (Dessau et al., 2008). Prior to that, attempts to understand the CSN (subunit) structure were limited to pair-wise subunit interaction analyses using methods such as yeast-two-hybrid or in vitro binding assays. These studies at best can only provide fragmented information about individual subunits that is far insufficient for us to piece together a complete picture of CSN.

In this issue of *Structure*, Michal Sharon and her colleagues in Carol Robinson's group from the University of Cambridge and Ning Zheng's group from the University of Washington undertook the investigation of the CSN complex by an emerging mass spectrometry approach (MS). This method can identify intact complex or subcomplexes with information on the exact subunit composition in each assembly. An in vitro reconstituted eight-subunit human CSN complex, active in deneddylation, was used in the study. Along with the intact complex as the predominant species, complexes missing one subunit or smaller subcomplexes were also found, even under mild MS conditions. To define subunit arrangement in a complex, the intact complex was placed under "harsh" conditions to induce dissociation of subcomplexes and to "strip" off peripheral subunit(s).

The data from these combined strategies have led to a model of the CSN with the following topological features. The complex is composed of two symmetrical modules: Csn1/2/3/8 and Csn4/5/6/7, which are connected by interaction of the core subunits Csn1 and Csn6 (see Figure 5B in Sharon et al. [2009]). The two modules can be folded, allowing pair-wise interactions between the corresponding subunits of the two modules: Csn1/6; Csn2/5; Csn3/4; and Csn7/8. Within each four-subunit module, Can1/3/8 and Csn4/6/7 trimers appear to be more stable and compact, both binding to additional subunits Csn2 and Csn5, respectively. Interestingly, Csn6, which is assigned as a core component that plays a key role in complex stability, does not exist in fission yeast. Therefore, either the fission yeast CSN assumes a different structure, or, alternatively, the structural role of human Csn6 is replaced by duplication of another subunit, such as Csn5, in fission yeast CSN.

The CSN is known to be a cousin of the lid subcomplex of the 26S proteasome. The two complexes have very similar subunit makeup, share remarkable sequence homologies between the corresponding subunits, and even exhibit similar biochemical activities. While CSN deconjugates Nedd8, an ubiquitin-like protein, by Csn5-dependent metalloprotease mechanism, the lid deconjugates ubiquitin by the same mechanism through Rpn11, the Csn5 homolog in the lid (Cope et al., 2002). It is therefore surprising that the spatial arrangements of the subunits in the two complexes are quite different. The lid is composed of two asymmetric modules, and the core subunits linking the two modules do not correspond to those in CSN (Sharon et al., 2006). In addition, the authors noted that the CSN appears less stable than the lid. Whether the instability feature can apply to endogenous CSN in the cell may need further confirmation; one should also take into account that the study on the lid was performed on an endogenous complex isolated from yeast cells, while the CSN was a reconstituted complex. It is possible that certain posttranslational modifications, which the reconstituted CSN does not have, may affect complex stability and influence subcomplex dissociation. It would be interesting to conduct similar examinations on an endogenous CSN complex and to compare it with the reconstituted CSN.

The study on CSN structural organization by Sharon et al. (2009) has a number of significant implications with respect to how CSN may work to execute its functions. One of the most notable observations is the frequent detections of 7-subunit complexes lacking Csn2,

 ©2009 Elsevier Ltd All rights reserved

Csn5, or Csn8 generated from the intact complex. This indicates that the associations of these components with the core of the complex are labile. Interestingly, despite its peripheral position, Csn5 cannot be reassembled with the ΔCsn5 7-subunit complex in vitro, but it can be reassembled with Csn4/6/7 trimer. It seems that Csn5 can integrate into the complex only through the step-wise assembly process. Mouse knockout studies showed that Csn2, 5, and 8 are necessary for biogenesis of mammalian CSN (Lykke-Andersen et al., 2003; Tomoda et al., 2004; Menon et al., 2007). However the observation that a stable 7-subunit complex can be derived from the intact complex immediately prompts the following question: is it possible that subunits, such as Csn2, Csn5, Csn8, are important for biogenesis of the CSN, but once the complex is assembled, these subunits may leave the complex without dramatic harm?

If dissociation of a single subunit from the holocomplex indeed occurs in vivo rather than a mark of instability of a reconstituted complex, this finding could open up many possibilities with regard to functional versatility of the CSN. First, it would lend further support to the idea that some of the CSN subunits have independent functions outside CSN, particularly Csn5 and Csn2, where there is already compiling evidence (see Wei et al., 2008). Second, what would be the function of the 7-subunit CSN that lacks Csn5? Certainly, it could not be active in deneddylation. Unlike mini-CSN subcomplexes, a 7-subunit CSN complex has not been reported so far.

The concept that CSN is a modular assembly is perhaps one of the most significant conclusions to the mechanism of the CSN. Not only does it provide an explanation to many observations of mini-CSN subcomplexes in the cell, but, more importantly, it offers a concrete model based on which the function and the composition of a particular subcomplex can be tested in vivo. With regard to the specific partitions of the modules (Csn1/2/3/8 and Csn4/5/6/7), one cannot help wondering whether the way subcomplexes dissociate could be influenced by how they were reconstituted, as the intact complex was reconstituted from two subcomplexes (Csn1/2/3 and Csn4/6/7) and two individual subunits (Csn5 and Csn8). Still, the proposed modular model represents a significant step forward in our comprehension of the CSN structure. It will certainly stimulate new hypothesis and new experiments toward understanding how CSN works.

REFERENCES

Cope, G.A., Suh, G.S., Aravind, L., Schwarz, S.E., Zipursky, S.L., Koonin, E.V., and Deshaies, R.J. (2002). Science *298*, 608–611.

Dessau, M., Halimi, Y., Erez, T., Chomsky-Hecht, O., Chamovitz, D.A., and Hirsch, J.A. (2008). The Plant Cell *20*, 2815–2834.

Lykke-Andersen, K., Schaefer, L., Menon, S., Deng, X.W., Miller, J.B., and Wei, N. (2003). Mol. Cell. Biol. *23*, 6790–6797.

Menon, S., Chi, H., Zhang, H., Deng, X.-W., Flavell, R., and Wei, N. (2007). Nat. Immunol. *8*, 1236–1245.

Sharon, M., Taverner, T., Ambroggio, X.I., Deshaies, R.J., and Robinson, C.V. (2006). PLoS Biol. *4*, e267.

Sharon, M., Mao, H., Boeri Erba, E., Stephens, E., Zheng, N., and Robinson, C.V. (2009). Structure *17*, this issue, 31–40.

Tomoda, K., Yoneda-Kato, N., Fukumoto, A., Yamanaka, S., and Kato, J.Y. (2004). J. Biol. Chem. *279*, 43013–43018.

Wei, N., Serino, G., and Deng, X.W. (2008). Trends Biochem. Sci. *33*, 592–600.

Journey to the Ends of the Arf

James M. Gruschus,[1] Pei-Wen Chen,[2] Ruibai Luo,[2] and Paul A. Randazzo[2,*]
[1]National Heart, Lung and Blood Institute, National Institutes of Health, Bethesda, MD, USA
[2]Laboratory of Cellular and Molecular Biology, National Cancer Institute, Bethesda, MD 20892, USA
*Correspondence: randazzp@mail.nih.gov
DOI 10.1016/j.str.2008.12.002

In this issue, Liu et al. (2009) used NMR to provide the most complete information to date on the structure of Arf1 and the role of myristate in GDP/GTP exchange. Unanticipated details lead to speculation about functions for the N and C termini of Arfs.

Arf GTP-binding proteins are regulators of membrane traffic and actin remodeling, and have been recently implicated in the invasive and metastatic behavior of cancer cells as well as the metabolism of growth factor receptors. Arf function depends on controlled binding and hydrolysis of GTP. The association of Arfs with membranes is also critical to their function. Membrane association depends on a myristoylated N-terminal amphipathic α-helix. Until Liu et al. (2009), all structural work has used Arf proteins that lack either the myristate or the myristoylated N-terminal helix.

Liu et al. (2009) examine yeast Arf1 (yArf1), which can be prepared in the myristoylated form from bacteria coexpressing yArf1 and N-myristoyltransferase. yArf1 is 74% identical to human Arf1. Consistent with the highly conserved function of Arf proteins, human Arfs can rescue $arf1^{-}arf2^{-}$ yeast (Kahn et al., 1991), yArf1 can be used as a substrate for mammalian Arf GAPs, and mammalian Arf1 is a substrate for yeast Arf GAPs. Most or all of what we learn from yArf

 ©2009 Elsevier Ltd All rights reserved