Structure
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Structural Insights into the COP9 Signalosome and Its Common Architecture with the 26S Proteasome Lid and eIF3

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SUMMARY

The evolutionary conserved COP9 signalosome (CSN), a large multisubunit complex, plays a central role in regulating ubiquitination and cell signaling. Here we report recombinant insect cell expression and two-step purification of human CSN and demonstrate its functional assembly. We further obtain a three-dimensional structure of both native and recombinant CSN using electron microscopy and single particle analysis. Antibody labeling of CSN5 and segmentation of the structure suggest a likely subunit distribution and the architecture of its helical repeat subunits is revealed. We compare the structure of CSN with its homologous complexes, the 26S proteasome lid and eIF3, and propose a conserved architecture implying similar assembly pathways and/or conserved substrate interaction modes.

INTRODUCTION

The COP9 signalosome (CSN) is an eight subunit protein complex, CSN1–8 (Deng et al., 2000), conserved in eukaryotes (Wei and Deng, 2003, and references therein). It regulates diverse cellular events including cell cycle progression, gene expression, and DNA repair (Wolf et al., 2003).

The best understood function of CSN biochemically is the deneddylation of cullin ring E3 ligases (Lyapina et al., 2001). Their activity is modulated by attachment of Nedd8 (a ubiquitin-like protein) via an isopeptide bond to a conserved lysine of the cullin subunit (Liakopoulos et al., 1998). Neddylation induces a significant change in the position of the RING finger subunit relative to cullin (Duda et al., 2008), greatly enhancing both E2 recruitment and ubiquitin transfer (Saha and Deshaies, 2008). This can also lead to autoubiquitination and destruction of substrate recognition subunits. Thus, CSN-mediated deneddylation not only decreases the activity of cullin ring ligases on bona fide substrates (Yang et al., 2002) but also protects them from self-destruction and, somewhat paradoxically, functions as a positive regulator in vivo (Schwechheimer et al., 2001; Wolf et al., 2003).

The isopeptidase activity of CSN is located in the zinc-coordinating JAMM/MPN+ domain of CSN5 (Cope et al., 2002). CSN6 also has an N-terminal MPN (Mpr1-Pad1 N-terminal) domain, which, however, lacks metal binding and catalytic activity. The other six subunits have C-terminal PCI (proteasome, COP9, and eIF3) domains. Structurally these domains can be subdivided into an N-terminal helical repeat followed by a globular winged helix fold (Dessau et al., 2008). The PCI-containing subunits are assumed to play a scaffolding role (Tsuge et al., 2001).

There are two further macromolecular complexes that share the composition of six PCI plus two MPN domain subunits—the 26S proteasome lid (Glickman et al., 1998) and the eukaryotic translation initiation factor eIF3 (Hinnebusch, 2006). Each CSN subunit can be assigned a corresponding homologous subunit from the lid. There is also a close functional resemblance: Rpn11 of the lid also has an isopeptidase activity in its JAMM/MPN+ motif. Furthermore, both CSN and lid act as binding platforms for other proteins or protein complexes. The compositionally more divergent eIF3 functions as a scaffold during initiation of protein synthesis and has no known enzymatic activity.

Structural insights into CSN prior to this work have been limited. An electron microscopy (EM) study (Kapelari et al., 2000) compared 2D class averages of CSN and the lid. Subunit interactions within intact CSN have been explored by native mass spectrometry (MS) (Sharon et al., 2009). The authors showed that a reconstituted human CSN has a total mass of 321 kDa and contains a single copy of each subunit. CSN falls apart into two subcomplexes, CSN1/2/3/8 and CSN4/5/6/7, coupled by an interaction between CSN1 and CSN6.

To gain structural understanding of the organization and function of CSN we analyzed both native and recombinant human complexes using negative stain EM and single particle analysis. The obtained CSN structure allows the first comparison with the lid and eIF3 revealing a conserved basic architecture.

RESULTS

Recombinant CSN Expression and Purification

CSN is known to interact with a large number of proteins and protein complexes in vivo and has a tendency to copurify with Usp15 or certain kinases from human cells (Hetfeld et al., 2005). This potential heterogeneity might adversely affect single particle analysis of molecular images obtained by EM. To address this and to enable structural analysis of CSN subcomplexes we have developed a two-step procedure to purify recombinant CSN. All full-length human CSN subunits were assembled into a single MultiBac bacmid (Berger et al., 2004) and coexpressed in High Five insect cells. Affinity purification

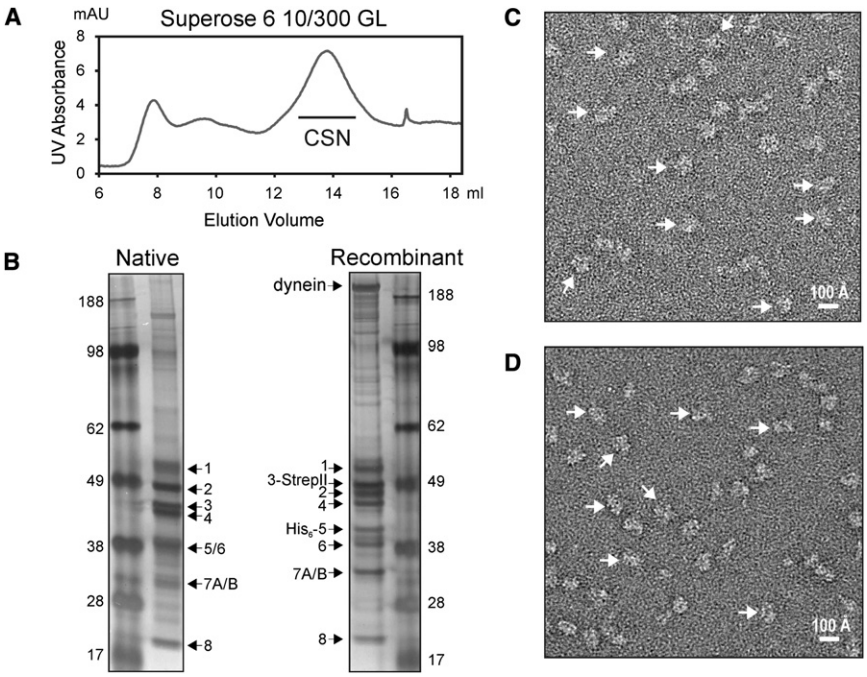

Figure 1. Comparison of Recombinant and Native CSN Samples

(A) Size-exclusion chromatography profile of affinity-purified recombinant CSN.

(B) Silver-stained SDS-PAGE gel of native (left) and recombinant (right) samples. Respective CSN subunits are labeled with 1–8.

(C and D) Typical micrograph fields of recombinant (C) and native (D) CSN. Individual molecular images are indicated by arrows.

using C-terminal StrepII tag on CSN3 followed by size-exclusion chromatography yielded sufficiently pure, intact complex (~0.1 mg per liter of insect cell culture). The symmetrical appearance of the gel-filtration peak (Figure 1A) indicates a homogeneous population. A comparison of native and recombinant samples on silver stained SDS-PAGE (Figure 1B) demonstrates similar relative subunit intensity. The only identified contaminant in the purified CSN sample is dynein (Z. Zhang, personal communication), but we did not detect any of the aforementioned substoichiometric interaction partners. Calibrated size exclusion chromatography (see Figure S1A available online) is consistent with a molecular mass of ~500 kDa for the peak fraction (Figure 1A).

Enzymatic Activity

We purified recombinantly expressed full-length human Cul1-Rbx1, which was subsequently neddylated in vitro (Figure S2A). The recombinant and the native samples were shown to deneddylate Cul1 (Figures S2B and S2C).

Structural Analysis of Recombinant CSN

Recombinant CSN preparations were negatively stained and imaged in a Tecnai F20 electron microscope: a typical field of molecular images is shown in Figure 1C. Single molecular views were manually selected resulting in a data set of 5556 particles. Reference-free class averages (Figure S3A) were obtained using refine2d from EMAN (Ludtke et al., 1999). A selection of reference-free class averages was subjected to angular reconstitution to determine their relative orientations and a 3D map was calculated by weighted back projection. This and subsequent analysis were performed without applying symmetry. The resulting ab initio 3D model exhibited similarity to the 26S proteasome lid (da Fonseca and Morris, 2008; P.C. da Fonseca and E.P.M., unpublished data) as discussed below. As a validation of the angular reconstitution model, we performed 3D analysis of the recombinant CSN data set using a model of the 26S proteasome lid (see below) as a starting point. Several rounds of refinement resulted in a very similar 3D structure (Figure S4) to that obtained by angular reconstitution but with slightly higher resolution and better angular distribution.

Structural Analysis of Native CSN

Molecular images of native CSN (Figure 1D) obtained as described for the recombinant complex were of similar appearance to those obtained with recombinant CSN (Figure 1C). Reference-free classes calculated from 7877 molecular images using refine2d were also in close agreement with those obtained from recombinant CSN (Figure S3A). In each case, the majority of the initial class averages appear divided into two lobes separated by a central region of stain accumulation reminiscent of the description by Kapelari et al. (2000). A 3D structure was calculated from native CSN using the structure of recombinant CSN as an initial reference. As a validation step, the proteasome lid model was also used as a starting point. In both cases the analysis converged after multiple rounds of refinement to the same structure. Refined class averages, corresponding surface views, and reprojections of the resulting native CSN structure are shown in Figure 2A. The native CSN structure is very similar to that obtained from recombinant CSN with no evidence of additional density, which might arise from CSN-associated proteins (Figure S3B). Since the native CSN structure is derived from a larger number of molecular images and is characterized by a more complete range of angular views (Figure S5B) it is used for the detailed interpretation that follows.

CSN Structure and Mass

The structure of CSN contoured at a 3σ threshold measures 100 × 140 × 150 Å and is relatively flat and somewhat elongated with good connectivity (Figures 2B–2F). The level of detail corresponds well with the estimated resolution of ~25 Å (Figure S5A).

The volume of the CSN map contoured at a 3σ threshold is ~380 nm^3, which corresponds to a mass of ~320 kDa. This is significantly less than the calibrated gel filtration mass estimates of 450–500 kDa for both recombinant (Figure S1A) and native (e.g., Menon et al., 2005) samples. However, it is supported by