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The COP9 signalosome regulates cell proliferation of *Dictyostelium discoideum*

Daniel Rosel[a,b], Alan R. Kimmel[a,*]

[a]*Laboratory of Cellular and Developmental Biology, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20892-8028, USA*
[b]*Department of Cell Physiology and Biochemistry, Charles University, Prague, Czech Republic*

Abstract

Regulated protein destruction involving SCF (Skp1/Cullin/F-box, E3 ubiquitin ligase) complexes is required for multicellular development of *Dictyostelium discoideum*. Dynamic modification of cullin by nedd8 is required for the proper action of SCF. The COP9 signalosome (CSN), first identified in a signaling pathway for light response in plants, functions as a large multi-protein complex that regulates cullin neddylation in eukaryotes. Still, there is extreme sequence divergence of CSN subunits of the yeasts in comparison to the multicellular plants and animals. Using the yeast two-hybrid system, we have identified the CSN5 subunit as a potential interacting partner of a cell surface receptor of *Dictyostelium*. We further identified and characterized all 8 CSN subunits in *Dictyostelium discoideum*. Remarkably, despite the ancient origin of *Dictyostelium*, its CSN proteins cluster very closely with their plant and animal counterparts. We additionally show that the *Dictyostelium* subunits, like those of other systems are capable of multi-protein interactions within the CSN complex. Our data also indicate that *CSN5* (and *CSN2*) are essential for cell proliferation in *Dictyostelium*, a phenotype similar to that of multicellular organisms, but distinct from that of the yeasts. Finally, we speculate on a potential role of CSN in cullin function and regulated protein destruction during multicellular development of *Dictyostelium*.
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Keywords: CSN proteins; Protein–protein interactions; RNAi; Proteasome; Cullin; *Dictyostelium*

Introduction

Regulated protein degradation is fundamental to many aspects of eukaryotic growth and development. The SCF (Skp1/Cullin/F-box, E3 ubiquitin ligase) complex functions with the ubiquitin/proteasomal pathway to specify many of these degradative processes; the SCF complex first marks proteins with ubiquitin moieties which serve as recognition targets for destruction by the 26S proteasomal complex. Recently an additional component, the COP9 signalosome, has been identified that is essential for the regulated function of SCF complexes (see Chang and Schwechheimer, 2004; Richardson and Zundel, 2005; Wei and Deng, 2003).

COP9 signalosome (CSN) proteins were first identified in *Arabidopsis* during screens for constitutive photomorphogentic (COP) lethals (Wei and Deng, 1992). Further analyses defined the CSN protein complex in an essential pathway to repress photomorphogenesis (Chamovitz et al., 1996). An equivalent protein complex was subsequently characterized in mammals during purification of the 26S proteasome (Seeger et al., 1998). Many essential CSN-dependent

*Corresponding author. Tel.: +301 496 3016; fax: +301 496 5239.
E-mail address: ark1@helix.nih.gov (A.R. Kimmel).

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functions have now been described for a wide variety of cellular and developmental processes in plants and metazoa (Chang and Schwechheimer, 2004; Richardson and Zundel, 2005; Wei and Deng, 2003).

The CSN holocomplex is composed of 8 subunits, each is highly related to a corresponding protein of the lid component of the 26S proteasome (Chang and Schwechheimer, 2004; Richardson and Zundel, 2005; Wei and Deng, 2003), suggesting an ancient evolutionary relationship among the complexes. Additional relatedness has been noted with proteins of the eukaryotic translation initiation factor 3 (eIF3) complex. Indeed, 6 of the CSN subunits (CSN 1, 2, 3, 4, 7, and 8), possess a common PCI (proteasome/COP9/initiator factor) domain signature; CSN5 and 6 have a MPN/MOV34/JAB domain that is also found in the corresponding proteins of the 26S proteasome and eIF3 complexes. Of the CSN subunits, CSN2 and CSN5 are the most conserved through the eukarya, and while CSN-like functions are recognized in the yeasts, the individual components are highly diverged, or perhaps even absent (Chang and Schwechheimer, 2004; Richardson and Zundel, 2005; Wei and Deng, 2003).

The broad functions for CSN activity have been mainly attributed to its role in ubiquitin/proteasomal-mediated protein degradation. Cullin, an integral part of SCF, requires a nedd8 peptide tag for its function. However, dynamic aspects of neddylation/deneddylation are essential for cullin function (Schwechheimer and Deng, 2001). Thus, re-cycling and stabilization of the SCF complex requires deneddylation of the cullins; deneddylation is mediated principally by the CSN complex. Consequently, loss of CSN increases cellular neddylation and decreases cullin levels in the yeasts, animals, and plants (Chang and Schwechheimer, 2004; Richardson and Zundel, 2005; Wei and Deng, 2003). The CSN5 subunit appears essential for deneddylation, and it has been suggested that this activity may parallel the de-ubiquitinylation factor observed for the lid component of the 26S proteasome (Chang and Schwechheimer, 2004; Richardson and Zundel, 2005; Wei and Deng, 2003).

In addition to its role as a regulator of SCF, the CSN complex may also play an indirect role in protein degradation. The complex exhibits an associated kinase activity, and phosphorylation by CSN of several transcription factors will regulate their stabilities by modulating patterns of ubiquitinylation (Bech-Otschir et al., 2001). Indeed, CSN5 was first identified in mammals as a binding protein of the c-Jun activation domain and has been alternatively referred to as JAB (Claret et al., 1996). CSN5 may have monomeric functions that are independent of the CSN and may interact uniquely with both nuclear and membrane proteins (Bianchi et al., 2000). Although the other CSN subunits do not appear to function as monomers, CSN complexes have been identified that are smaller than the holocomplex and that contain only a subset of the 8 core subunits (Bemis et al., 2004; Yang et al., 2000). However, their unique roles have not been defined. In this context, the phenotypes arising from loss-of-function mutations in genes for the individual CSN components need not be identical (Mundt et al., 2002).

Dictyostelium has proven an interesting system for the study of regulated protein destruction and development (Ennis et al., 2000, 2003; Mohanty et al., 2001; Nelson et al., 2000; Tekinay et al., 2003; Wang and Kuspa, 2002). Differentiation and morphogenesis of *Dictyostelium* are regulated by chemotactic and morphogenic signaling mediated by extracellular cAMP (Kimmel and Firtel, 2004; Kimmel and Parent, 2003). Here, using a yeast two-hybrid system, we first identified CSN5 in an interaction screen for a partner of the cell surface cAMP receptor 3 of *Dictyostelium* and further characterized the 7 other CSN subunits of *Dictyostelium*. Unlike the yeasts, each has a high degree of sequence relatedness to their metazoan counterpart. Additionally, we examined each for potential hetero- and homo-dimeric, pair-wise interactions. Finally, we conclude that CSN5 is required for cell proliferation in *Dictyostelium*, a function similar to that observed for the metazoa (Freilich et al., 1999; Lykke-Andersen et al., 2003; Tomoda et al., 2004; Yan et al., 2003), but largely distinct from the yeasts (Mundt et al., 1999, 2002; Wee et al., 2002).

Materials and methods

Cultivation, transformation and analysis of *Dictyostelium discoideum*

Dictyostelium AX3 cells were grown axenically in D3T medium (KD Medical) at 21 °C (Kim et al., 1999). To obtain cells at various developmental stages, exponentially growing cultures were cooled on ice, washed twice with ice-cold 17 mM phosphate buffer (pH 6.2), plated on non-nutrient agar, and allowed to develop at 21 °C with an overhead light source (Kim et al., 1999). *Dictyostelium* cells were transformed with 5–10 μg circular plasmid DNA by electroporation. Depending on the antibiotic resistance cassette present in the plasmids, the medium was supplemented with 20 μg/ml G418 (Invitrogen) and/or 10 μg/ml blasticidin S (Fisher). For Tet-OFF regulated overexpression (Blaauw et al., 2000), parental AX3 cells were co-transformed with the transactivator plasmid (MB35) and response plasmid (MB38) containing the appropriate gene construct. To silence expression of the gene construct, media were always supplemented with 30 μg/