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The COP9 signalosome-like complex in *S. cerevisiae* and links to other PCI complexes

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Abstract

The COP9 signalosome (CSN), the lid subcomplex of the proteasome and translational intitation factor 3 (eIF3) share structural similarities and are often referred to as the PCI family of complexes. In multicellular eukaryotes, the CSN is highly conserved as an 8-subunit complex but in *Saccharomyces cerevisiae* the complex is rather divergent. We further characterize the composition and properties of the CSN in budding yeast and its interactions with these related complexes. Using the generalized profile method we identified CSN candidates, four with PCI domains: Csn9, Csn10, Pci8/Csn11, and Csn12, and one with an MPN domain, Csn5/Rri1. These proteins and an additional interactor, Csi1, were tested for pairwise interactions by yeast two-hybrid and were found to form a cluster surrounding Csn12. Csn5 and Csn12 cofractionate in a complexed form with an apparent molecular weight of roughly 250 kDa. However, Csn5 migrates as a monomer in Δ*csn12* supporting the pivotal role of Csn12 in stabilizing the complex. Confocal fluorescence microscopy detects GFP-tagged Csn5 preferentially in the nucleus, whereas in absence of Csn12, Csn10, Pci8/Csn11, or Csi1, Csn5 is delocalized throughout the cell, indicating that multiple subunits are required for nuclear localization of Csn5. Two CSN subunits, Csn9 and Csi1, interact with the proteasome lid subunit Rpn5. Pci8/Csn11 has previously been shown to interact with eIF3. Together, these results point to a network of interactions between these three structurally similar, yet functionally diverse, complexes.

Keywords: Proteasome; Ubiquitin; COP9 signalosome; CSN; eIF3; PCI; MPN

1. Introduction

The PCI family of complexes, the proteasome lid, Cop9 signalosome (CSN), and eukaryotic translation initiation factor 3 (eIF3), are genetically and structurally homologous yet mediate different regulatory pathways (Bech-Otschir, Seeger, & Dubiel, 2002; Chamovitz & Glickman, 2002; Glickman & Ciechanover, 2002; Kim, Hofmann, von Armin, & Chamovitz, 2001; Schwechheimer & Deng, 2001). The lid is a peripheral yet essential subcomplex of the multisubunit, ubiquitin- and ATP-dependent, proteasome proteolytic complex (Glickman et al., 1998). eIF3 interacts with mRNA, the 40S ribosome and additional components, and is required for initiation of protein biosynthesis (Asano, Kinzy, Merrick, & Hershey, 1997; Hershey, Asano, Vornlocher, Hanachi, & Merrick, 1996). The CSN is a regulator of numerous

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signaling and developmental pathways (Chamovitz & Segal, 2001). In addition to sequence similarities, CSN, Lid, and most eIF3 subunits also share two signature structural motifs: the PCI (proteasome, CSN, eIF3) or MPN (Mpr1, Pad1 N-terminal) domains (Aravind & Ponting, 1998; Hofmann & Bucher, 1998; Maytal-Kivity, Hofmann, & Glickman, 2002). Both the lid and CSN normally contain two subunits with MPN domains, and six with PCI domains. The overall subunit composition and organization is also quite similar between these complexes (Fu, Reis, Lee, Glickman, & Vierstra, 2001; Glickman et al., 1998; Kapelari et al., 2000). Anecdotal evidence for interactions between these complexes has been published (Hoareau, Bochard, Retty, & Jalinot, 2002; Karniol et al., 1998; Kim et al., 2001; Kwok, Staub, & Deng, 1999; Lier & Paululat, 2002; Yahalom et al., 2001), though in light of their very diverse functions, the mechanistic reason for this is still an enigma.

The CSN is an 8-component complex, apparently found in all eukaryotes, and participates in a variety of signaling pathways. For instance, mutations in CSN subunits cause plant seedlings grown under darkness to mimic light development growth (Chamovitz et al., 1996; Wei, Chamovitz, & Deng, 1994), Drosophila mutants do not develop beyond larval stage (Freilich, Oron, Orgad, Segal, & Chamovitz, 1999; Lier & Paululat, 2002; Oron et al., 2002), while deletants in yeast are sensitized to mating pheromone (Maytal-Kivity, Piran, Pick, Hofmann, & Glickman, 2002). CSN components are also involved in AP-1, interferon and G protein-coupled receptor signaling pathways (Claret, Hibi, Dhut, Toda, & Karin, 1996; Cohen et al., 2000; Maytal-Kivity et al., 2002; Naumann, Bech-Otschir, Huang, Ferrell, & Dubiel, 1999; Spain et al., 1996; Tsuge, Matsui, & Wei, 2001). The mode of CSN action probably involves ubiquitin/proteasome-mediated degradation, though the exact mechanism is unclear (Bech-Otschir et al., 2002; Schwechheimer & Deng, 2001; Seeger, Gordon, & Dubiel, 2001). For instance, the CSN promotes the removal of Nedd8/Rub1 ubiquitin-like modification from the cullin subunit of E3 ubiquitin ligases (Lyapina et al., 2001; Maytal-Kivity et al., 2002; Zhou et al., 2001), and in general appears to be a negative regulator of the SCF (E3) ubiquitin ligase (Yang et al., 2002). The CSN has also been implicated in the phosphorylation of numerous regulatory proteins, many of which are targets of the ubiquitin/proteasome pathway, such as c-Jun, and p53 (Bech-Otschir et al., 2001; Cohen et al., 2000; Naumann et al., 1999; Seeger et al., 1998). Most subunits are present in *Schizosaccharomyces pombe*, with roles in cell cycle, UV damage response, and Cullin modification (Mundt, Liu, & Carr, 2002; Mundt et al., 1999; Zhou et al., 2001). Recently, evidence for a CSN-like complex in *Saccharomyces cerevisiae* has been produced, though its composition is quite different from that found in other eukaryotes and assignment of direct orthologs is not straightforward (Maytal-Kivity et al., 2002; Wee, Hetfeld, Dubiel, & Wolf, 2002). In this work, we further characterize the composition and properties of the CSN in budding yeast and its interactions with other members of the PCI family of complexes.

2. Materials and methods

2.1. Bioinformatics

Database searches were performed with a nonredundant data set constructed from current releases of SwissProt, TrEMBL, and GenPept. For the detection of PCI-domains, profiles calculated from previously published alignments (Hofmann & Bucher, 1998) were used (as stored in the PROSITE database under the accession numbers PS50249 and PS50250). Iterative profile refinement was performed (Hofmann, 2000), using the pftools package, version 2.1 (available from ftp://ftp.isrec.isb-sib.ch/sib-isrec/pftools/). Profiles were constructed using the BLOSUM45 substitution matrix and default penalties of 2.1 for gap opening and 0.2 for gap extension. The statistical significance of profile matches was derived from the analysis of the score distribution of a randomized database as described (Hofmann, 2000). Only sequence matches found with a probability of $P < 0.01$ were included into subsequent rounds of iterative profile refinement, the results of which are shown in Fig. 1.

2.2. Strains and plasmids

Csn5/Rri1 is encoded by YDL216c and is referred to as Csn5 according to standard nomenclature (Deng et al., 2000). Csn9, Csn10, Pci8/Csn11, and Csn12 are encoded by YDR179c, YOL117w, *PCI*8/YIL071c,

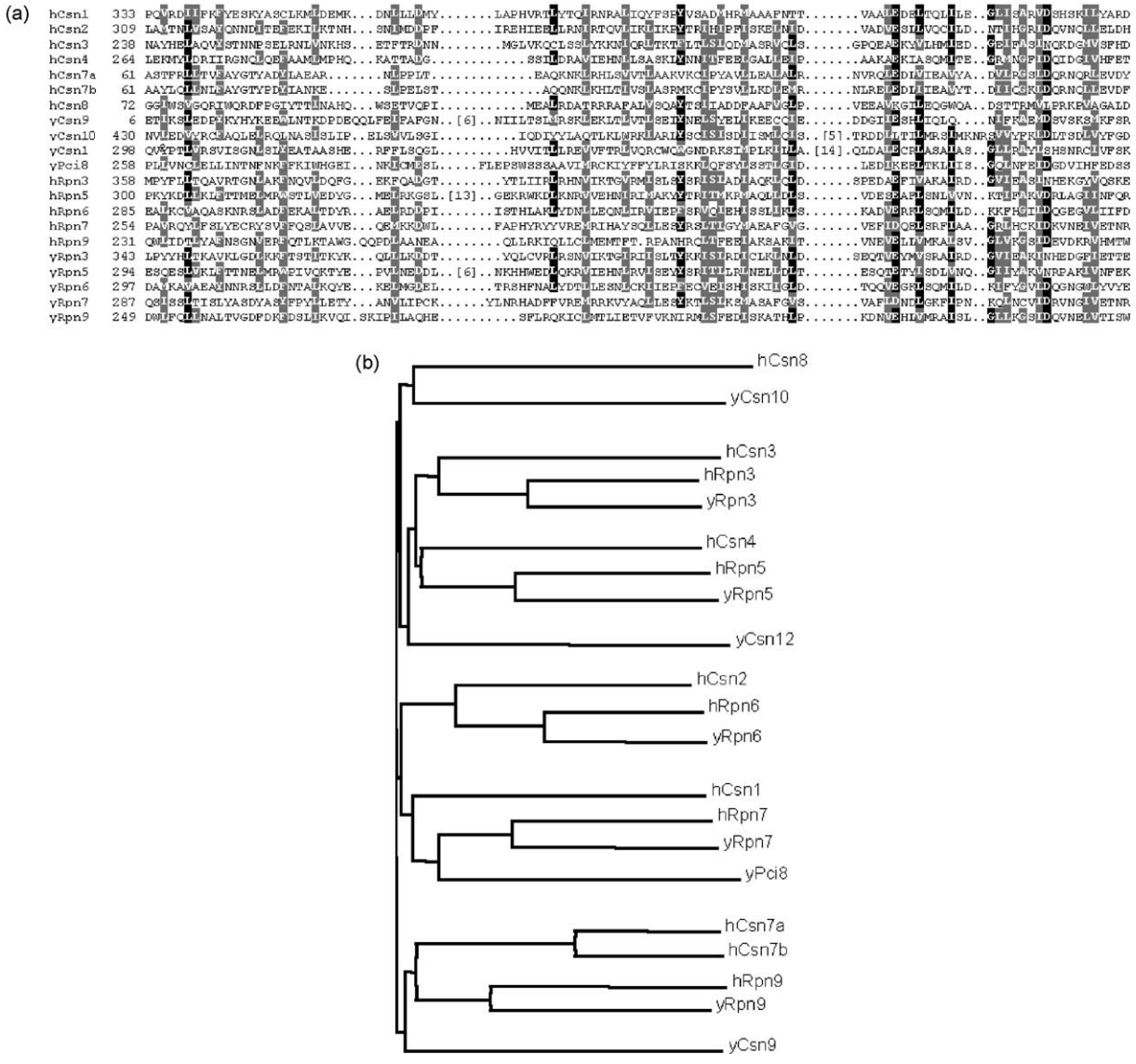

Fig. 1. PCI proteins in *S. cerevisiae* and their human counterparts. (a) Sequence alignment of the PCI region of CSN and proteasome lid subunits from yeast (y) and human (h). (b) Dendrogram analysis of yeast and human CSN and lid subunits showing evolutionary distance between the individual proteins.

and YJR084w, respectively. Csi1 (CSN interacting-1) is encoded by YMR025w. Deletant strains were purchased from EUROSCARF. Double mutants were made by mating haploids and sporulation, followed by tetrad dissection according to standard protocol (Burke, Dawson, & Stearns, 2000). *CSN* genes with their promoters were cloned by PCR and inserted into CEN plasmids (YCplac111). GFP, GST, or Myc epitopes (Longtine et al., 1998) were inserted in-frame before the stop codon for expression of tagged proteins. Unless other wise specified, CSN subunits were transformed into knockout strains for that gene.

2.3. Yeast 2-hybrid

Coding regions of CSN candidates were inserted into the Matchmaker III yeast 2-hybrid plasmids pGADT7 or pGBKT7 (Clontech). Y2H plasmids of lid Rpn subunits were a generous gift from Hongyong Fu and described elsewhere (Fu et al., 2001). All possible pairwise interactions among the six CSN candidates (Csn5, 9, 10, 11, 12, and Csi1) when expressed either as BD- or AD- fusions were reexamined (with the exception of the AD-Csn9 construct that was not available) for a total of $(6 \times 6) + (6 \times 5) = 66$ interactions. All 36 combinations between these six CSN subunits with the six PCI members of the lid (Rpn3, 5, 6, 7, 9, 12) were also tested in both BD- and AD-conformations (For an additional $(6 \times 6) + (5 \times 6) = 66$ transformants). Positive interactions were scored in strain AH109 (Clontech) for Ade and His autotrophy. Except for one negative example shown for comparison, double trasformants that did not yield growth within 5 days are not included in Fig. 2a. None of the constructs that are shown in Fig. 2a permitted growth on Ade-His media within 3–5 days when transformed alone.

2.4. Glycerol gradients

1 ml total yeast cell extract expressing tagged CSN subunits were separated on a 15–40% glycerol gradient in 100 mM NaCl, 50 mM Tris–HCl pH7.4 and protease inhibitor cocktail (Roche) for 20 h at 30 K RPM with SW41 rotor. 0.5 ml gradient fractions were resolved by SDS-PAGE and immunoblotted with the appropriate antibodies.

2.5. Fluorescence microscopy

Δ*csn5* and double deletions of Δ*csn5* with other CSN subunits were transformed with a CEN plasmid expressing Csn5-Gfp expressed from its own promotor. For direct fluorescence microscopy cells were washed with 70% (v/v) ethanol and incubated with 0.4 mg/ml RNAse A in 50 mM sodium citrate pH = 7 for 2 h and transferred to 50 mM sodium citrate pH = 7, 10 mM NaCl, 0.1% np-40 and 5 μg/ml propidium iodide (PI). Reconstructions of GFP and PI channels were made using an MRC-1024 laser confocal scanning microscope (Bio-Rad) with the objective Nikon Plan Apo 603/1.40. Immuno-fluorescence microscopy was performed as described (Enenkel, Lehmann, &

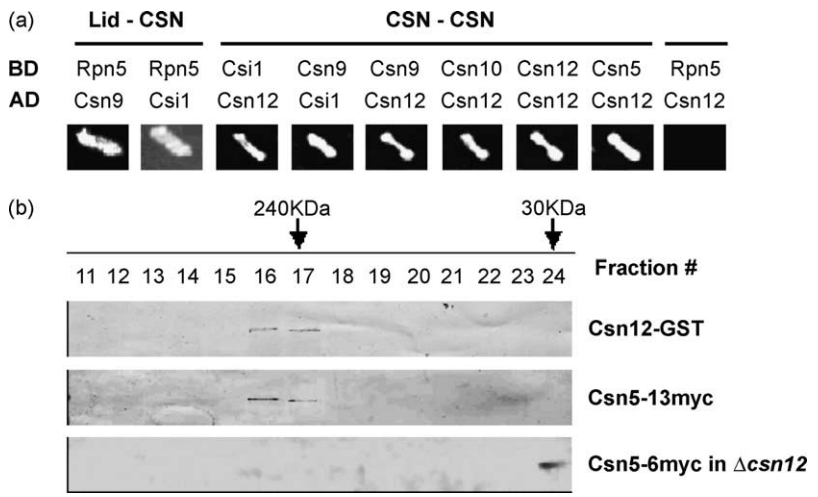

Fig. 2. Interactions of CSN subunits. (a) Y2H. We tested all possible pairwise interactions of CSN subunits from budding yeast among themselves or with PCI domain-containing lid subunits. Only positive interactions, as determined by growth on Ade-His selective media are shown. Single transformations as well as all other possible pairs did not sustain growth on selective media and are not included, except for one example that is shown for comparison. (b) Following glycerol gradient fractionation of yeast cell extract expressing natural abundance tagged Csn5 or Csn12, 0.5 ml fractions were tested with appropriate antibodies. Both Csn12 and Csn5 comigrate consistent with a 240MW complex. In extract from Δ*csn12* however, Csn5 is detected only in the lowest MW fraction consistent with its monomeric MW, indicating that incorporation of Csn5 into the complex is dependent on Csn12.

Kloetzel, 1998), using anti-GFP primary antibody (clontech).

3. Results

3.1. Interactions of CSN subunits in budding yeast

To characterize the PCI domain of yeast CSN candidates and to determine their relationship with CSN and Rpn subunits from other sources, we performed an extended search of the yeast genome using the 'generalized profile method' (Hofmann, 2000). Included in the profile construction were all PCI-containing CSN and Rpn subunits from both yeast and humans. Subsequently, iterative profile refinement (Hofmann, 2000) was used until a stable set of significantly matching *bona fide* PCI proteins in *S. cerevisiae* was identified. Alignment of the PCI domain of these CSN, and Rpn subunits is shown in Fig. 1a. By including a large set of proteins in dendrogram analysis a number of branches emerge, each with a subset of Rpn and CSN subunits (Fig. 1b). From this evolutionary tree, *sc*Csn9 may be closest to conventional Csn7; *sc*Csn10 to Csn8, Pci8/*sc*Csn11 to Csn1, and finally, *sc*Csn12 could stand in for either Csn3 or Csn4 (or both). Nevertheless, it must be stressed that the extremely low levels of overall sequence identity between subunits from budding yeast and those from other organisms (<16% in all cases (Maytal-Kivity et al., 2002)) makes these assignment tenuous at best. Only functional data could show whether specific roles are maintained between these highly divergent subunits from different organisms. In addition to these PCI subunits, an ortholog of the MPN subunit Csn5/Jab1 is present in *S. cerevisiae*, probably providing a catalytic hydrolytic activity to the complex (Cope et al., 2002; Maytal-Kivity, Piran, et al., 2002; Maytal-Kivity, Reis et al., 2002).

We reexamined pairwise interactions among these CSN candidate subunits. We then expanded this y2h screen to include all 36 possible interactions between the six CSN subunits (Csn5, 9, 10, 11, 12, and Csi1) and all six PCI components of the Lid complex (Rpn3, 5, 6, 7, 9, 12) in either direction (a total of 66 interactions were tested). Only positive interactions are shown in Fig. 2a. A cluster surrounding Csn12 incorporating Csn5, 9, 10, and Csi1 forms the skeleton of a yeast Csn5-containing complex. Pci8/Csn11 does not show pairwise interactions in our screen but does copurifies with the CSN (Gavin et al., 2002) and exhibits similar, though not identical phenotypes (Maytal-Kivity et al., 2002), suggesting that it too should be included in the composition of this complex. Interestingly, we found that two CSN subunits, Csn9 and Csi1, also interact with a PCI domain-containing lid subunit, Rpn5 (Fig. 2a). Indeed, Rpn5 copurified with other members of the CSN complex (Gavin et al., 2002), pointing to a stable link between CSN and lid in yeast. Interactions between PCI components of lid and CSN complexes has been documented in other organisms as well (Kwok et al., 1999; Lier & Paululat, 2002).

3.2. Endogenous yeast CSN subunits are present in a complexed form

Endogenously expressed Csn5-6myc migrates in a complexed form with an apparent MW of approximately 240 kDa, in agreement with the combined MW of the "core" CSN subunits. Similarly, Csn12 is also detected only in the same MW fractions, supporting its inclusion in the "core" of this CSN-like complex (Fig. 2b). Upon deletion of *csn12*, however, tagged Csn5 migrates in the lowest MW fraction, consistent with it being a monomer. This result signifies that incorporation of Csn5 into the complex is dependent on Csn12. The role of Csn12 in maintaining the integrity of the complex is in agreement with its y2h interactions (Fig. 2a). Previously, we showed that Csi1 also comigrates in similar MW fractions (Maytal-Kivity et al., 2002). Indeed, Csn5, 9, 10, Pci8/Csn11, and Csi1 coprecipitate together, indicating they form a stable complex (Gavin et al., 2002). Similarly, in other species, *at*Csn5 is also incorporated into the CSN only in the presence of *at*Csn1 (Kwok et al., 1998), while incorporation of *sp*Csn4 depends on *sp*Csn5 (Mundt et al., 2002). Also mammalian Csn1 is incorporated into the complex only if it properly interacts with Csn2, 3, and 4 via its intact PCI domain (Tsuge et al., 2001).

3.3. Cellular localization of Csn5 depends on complex integrity

Cellular localization of natural abundance Csn5 is predominantly nuclear (Fig. 3a). Nuclear localization

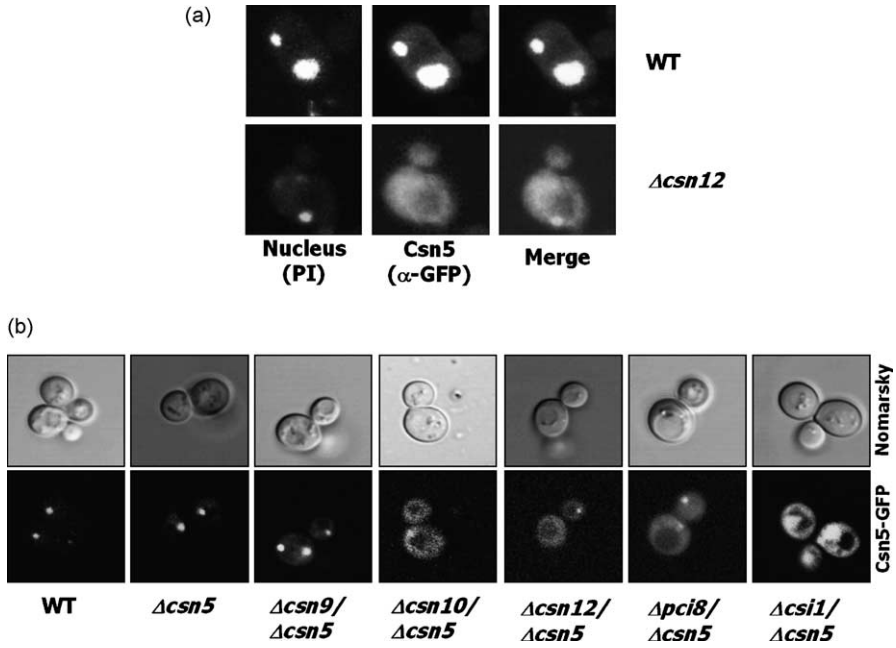

Fig. 3. Cellular localization of Csn5 is a function of complex composition. (a) Localization of naturally abundant Csn5 in WT and Δ*csn12* cells by immuno-fluorescence. Nucleus was visualized with propidium iodide (PI) and an overlay of the two channels in included. Csn5 is naturally localized to the nucleus. In absence of Csn12, Csn5 is delocalized throughout the cytoplasm. (b) Localization Csn5-GFP in WT and deletants for varius CSN subunits by direct fluorescence. Cells expressing GFP tagged Csn5 were monitored by confocal microscopy. Images show direct fluorescence of Csn5-GFP and a Nomarski optical image of the cell. Csn5 is localized to the nucleus in WT, while deletion of any CSN subunit other than Csn9 brings about delocalization of Csn5 throughout the cell.

has also been documented for most CSN subunits in plants, mammals and *S. pombe* (Kwok et al., 1998; Mundt et al., 2002; Seeger et al., 1998). Deletion of the pivotal Csn12 subunit causes dramatic delocalization of Csn5 throughout the cytoplasm (Fig. 3a). Csn12 is in direct contact with Csn5 and is necessary for maintaining Csn5 in a complexed form (Fig. 2). Thus, we can conclude that in budding yeast, monomeric Csn5 is delocalized whereas the complexed form is mainly nuclear. Interestingly, pervasive cytoplasmic localization of Csn5 is also apparent in Δ*csn10*, Δ*pci8/csn11*, or Δ*csi1* deletants (Fig. 3b) indicating that multiple subunits are required for nuclear localization of Csn5 rather than an interaction with a single specific subunit. Only deletion of Csn9 does not appear to alter the nuclear localization of Csn5 (Fig. 3b). Csn9 is the smallest identified subunit in the yeast complex, and may play only a peripheral role in maintaining complex stability. Nevertheless, Δ*csn9* does exhibit phenotypes regarding cullin modification and pheromone response (Maytal-Kivity et al., 2002), indicating that Csn9 plays a non redundant role in CSN function. Compromising complex integrity promotes delocalization of CSN subunits in other species as well. Thus, in *S. pombe*, Csn2 is delocalized in Δ*csn1*, (Mundt et al., 2002), while in plants, Csn5 is delocalized in a CSN2 mutation (Kwok et al., 1998). Together, it appears that the CSN is mainly a nuclear complex in budding yeast, with possible cytoplasmic localization for certain monomers or subcomplexes.

4. Discussion

4.1. Properties of the CSN-like complex in S. cerevisiae

As summarized in Fig. 4, based on the various protein interactions, a CSN-like complex is present in

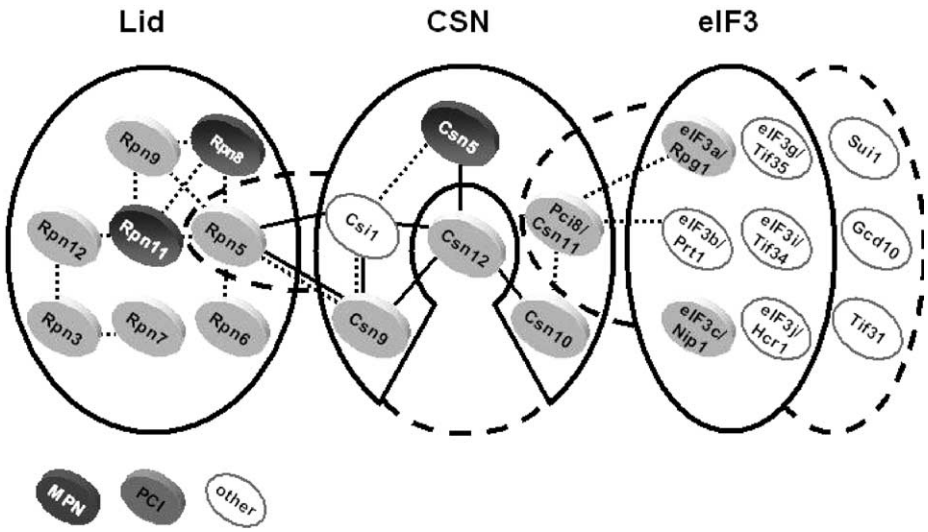

Fig. 4. The CSN complex in *S. cerevisiae*: at the crossroads of PCI complexes. Putative models of the three PCI complexes in *S. cerevisiae* and their mutual interactions. Three PCI complexes have been identified in budding yeast: proteasome lid, a CSN-like complex, and eIF3. MPN proteins are shown in dark grey, PCI proteins in light grey (and other subunits are clear). Subunits encircled by a solid line represent core subunits that copurify by conventional or affinity purification, or that have clear orthologs in other eukaryotes. Intimately associated proteins, which may be additional subunits or regulatory components, are encompassed within a hatched boundary. Pairwise interactions identified in this work are shown as solid links; protein interactions documented elsewhere are dotted lines. The structure of the lid is based on (Glickman et al., 1998; Fu et al., 2001; Davy et al., 2001). The outline of the CSN core complex in *S. cerevisiae* and the possible inclusion of Rpn5 are based on TAP affinity purification of Csn5 (Gavin et al., 2002). In addition to interactions of CSN subunits described herein, Csn9-Csi1, Csi1-Csn5, and Csn9-Rpn5 have also been shown in (Uetz et al., 2000), while Csn10-Csn11 is from (Ito et al., 2001). Interaction of Pci8 with eIF3a and eIF3b, and its identification as a possible eIF3e functional homolog is describe in (Shalev et al., 2001). For simplicity, only a gross structure of *S. cerevisiae* eIF3 is described in this figure; for additional details, readers are referred to (Hershey et al., 1996; Asano et al., 1997; Gavin et al., 2002; Shalev et al., 2001; Burks, Bezerra, Le, Gallie, & Browning, 2001; Phan et al., 1998).

budding yeast that includes four PCI proteins (Csn9, 10, 11, 12), one MPN protein (Csn5), and an additional interactor (Csi1). We cannot preclude association of additional subunits or interactors (CSIs), or, as Csn12 interacts with itself (Fig. 2), possible dimerization of this complex. So far, we do not find any evidence of free CSN subunits in yeast, though based on results obtained with Δ*csn1*2, certain conditions could promote disruption of the complex or release of uncomplexed subunits. For instance, some heterogeneity in inclusion of Csn11 or 12 with the CSN is evident from various publications (Fig. 4) suggesting that they may be labile under certain conditions. Since supra-stochiometric levels of Csn9 or 10, as well as presumably uncomplexed *hs*Csn1, can efficiently suppress G-protein signaling (Maytal-Kivity et al., 2002; Spain et al., 1996), they may be able to function as monomers. Interestingly, even the free N-terminal region of human Csn1 is functionally active although it is unable to interact with the complex (Tsuge et al., 2001), while overexpression of *hs*CSN5 stabilizes c-Jun (Naumann et al., 1999), suggesting that in other organisms as well, certain subunits may play a role in their free forms. These results indicate that some biological activities may lie with individual subunits or subcomplexes, rather than with the complex as a whole. We suggest that there may be distinct mechanistic roles for monomers versus complexed subunits in response to defined regulatory needs.

The CSN complex is also responsible for the hydrolytic removal of the Nedd8/Rub1 ubiquitin-like molecule from the cullin subunit of E3 ubiquitin ligases (Lyapina et al., 2001; Mundt et al., 2002; Maytal-Kivity et al., 2002; Zhou et al., 2001). A

similar deubiquitination activity is associated with the proteasome. Both these complexes contain two MPN proteins, one of which is of the MPN+ type that appears to be responsible for this proteolytic activity Maytal-Kivity et al. (2002): Rpn11 in the lid (Verma et al., 2002; Yao & Cohen, 2002), and Csn5 in the CSN (Cope et al., 2002). No hydrolytic enzymatic activity has been described for eIF3, and indeed the MPN subunits found in eIF3 from other species are not of the catalytic MPN+ type. This may indicate that mechanistically, eIF3 functions quite differently from the related complexes. In agreement, no MPN domain proteins have been identified in the eIF3 complex in budding yeast. It is important to note, however, that the biological phenotypes associated with the CSN cannot always be explained solely by a role in cullin modification (Maytal-Kivity et al., 2002; Yang et al., 2002).

4.2. CSN—at the crossroads of PCI complexes

Both Lid and CSN in other eukaryotes contain 6 PCI and 2 MPN subunits. Each subunit has a paralog in the related complex, indicating that the two may have diverged from a single complex (Fig. 1b). Subunit arrangement of these analogous components is also remarkably similar in the CSN and lid complexes of multicellular eukaryotes (Fu et al., 2001). The proteasome lid in yeast is highly conserved as a 6+2 (PCI/MPN) complex with clear similarities to the lid in other species (Fu et al., 2001; Glickman et al., 1998). The composition of the CSN complex in budding yeast, however, is quite different from the CSN in other species. The smaller number of identified subunits, along with the low level of sequence identity when compared to other CSN or lid subunits, highlights the divergence of this complex from its analogous counterpart in other species. The CSN-like complex in yeast is also set-aside within the PCI family; all CSN subunits identified so far are non-essential. Conversely, the core PCI components of the lid (Rpn3, 5, 6, 7, 12), or of eIF3 (Rpg1/eIF3a and Nip1/eIF3c) are all essential, as are the MPN components of the lid, Rpn8 and Rpn11. The most divergent PCI complex in yeast is eIF3. Only 5–6 subunits have been classified as core eIF3 components compared to the 11 or so in other species studied (Fig. 4). Furthermore, while eIF3 conventionally contains two MPN subunits (eIF3f/p47 and eIF3h/p40) no evidence for these subunits has been found in yeast.

The CSN in yeast shares links with other members of the PCI family of complexes. Pci8/Csn11 has been identified as an interactor of the eIF3 complex, specifically with eIF3a and c, and as a possible homolog of Int6/eIF3e (Shalev et al., 2001). Pci8 may link these two complexes. Furthermore, we identified pairwise interactions between both Csn9 and Csi1 from the CSN with the lid subunit Rpn5 (Fig. 4). Rpn5 also copurifies with the CSN, pointing to a stable interaction (Gavin et al., 2002). It may be of significance that both ‘linker’ proteins, Rpn5 and Pci8/Csn11, are PCI domain-containing proteins. In other organisms as well, interactions between the three PCI complexes have been documented, indicating that the association between them is a conserved property with a general functional role (Kim et al., 2001). It is worthy to note, however, that the documented interactions linking the PCI complexes in other eukaryotes are not between the direct orthologs of yeast subunits described here. For example, in mammals, Int6/eIF3e interacts with Csn3, 6, and 7a on the one hand and with Rpt4 on the other (Hoareau et al., 2002) in plants, Int6/eIF3e interacts with Csn7a, eIF3c also interacts with the CSN, (Karniol et al., 1998; Yahalom et al., 2001), and Csn1 interacts with Rpn6 (Kwok et al., 1999) while in Drosophila the interaction Csn2-Rpn6 has been reported (Lier & Paululat, 2002). The mechanistic meaning of these cross-complex interactions is still unclear. For instance, it is unclear whether these interactions represent direct docking of one complex onto another, or whether there is some promiscuity in incorporation of certain subunits to more than one complex.

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