PCI Complexes: Beyond the Proteasome, CSN, and eIF3 Troika

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The bipartite PCI domain serves as the principal scaffold for proteasome lid, CSN, and eIF3, complexes that influence protein life span. PCI domains are also found in newly identified complexes directing nucleic acid regulation. The breadth of functions associated with the extended PCI family is a factor of shared subunits, among them a common factor Sem1/DSS1 that facilitates complex assembly.

The Three Canonical PCI complexes

Three distinct but homologous eukaryotic protein complexes, the 26S proteasome “lid,” the COP9 signalosome (CSN), and the eukaryotic translation initiation factor-3 (eIF3), collectively termed PCI complexes, regulate protein life span. The canonical version of these multisubunit assemblies, as found in multicellular organisms (Figure 1A), comprises six subunits bearing the hallmark PCI domain and two subunits carrying an MPN (Mpr1-Pad1-N-terminal) domain (Scheel and Hofmann, 2005). The PCI domain is defined by a conserved arrangement of curved bihelical repeats, terminating in a globular winged helix (Dessau et al., 2008; Scheel and Hofmann, 2005). PCI subunits might serve as structural scaffolds of the three parent complexes, thereby regulating proper complex assembly via interactions between each other as well as with other partners (Scheel and Hofmann, 2005). The MPN domain (also known as JAMM) is more conserved and might have originated from a metal-binding motif; indeed, subsets termed MPN+ are catalytically active metalloproteases (Chamovitz, 2009; Wei et al., 2008a).

Despite similar subunit composition, each complex performs a discrete cellular function. By structurally organizing translational components on the surface of the 40S ribosome, eIF3 plays a central role in protein synthesis (Shalev et al., 2001; Zhou et al., 2008). CSN components were first described as regulators of light-dependent growth in plants. The intact complex promotes recycling of cullin-RING ubiquitin ligases (CRLs) by removal of the ubiquitin-related Nedd8/Rub1 modifier from the cullin subunit (Wei et al., 2008a). The proteasome lid is the distal cap on the 26S proteasome megacomplex that carries out proteolysis of polyubiquitin-conjugated substrates (Sharon et al., 2006). The CSN and the proteasome lid complexes show the highest degree of analogy, with generally similar architecture and one-to-one subunit correspondence between structures (Figure 1). eIF3 activity requires a larger number of subunits, and its PCI and MPN subunits diverge from their paralogs in the lid and CSN (Figure 1).

By employing a unified approach to decipher subunit arrangement and general architecture of the three complexes, Carol Robinson and colleagues generated detailed interaction maps of the lid (Sharon et al., 2006), CSN (Sharon et al., 2009), and eIF3 (Zhou et al., 2008). Three-dimensional interaction maps were deduced by piecing together mass spectral information on fragments of intact complexes maintained in the gas phase. Within the lid, a module of three PCI components (Rpn12, Rpn3, and Rpn7) loosely associates with the complex (Sharon et al., 2006), as was previously predicted by Isono et al., 2004. Multiple interactions have been documented between the remaining five subunits (Rpn5, 6, 8, 9, and 11); however, Rpn11 dissociates more easily (Fu et al., 2001; Sharon et al., 2006). Similar dissection of eIF3 also identified an extending arm containing three PCI proteins, including paralogs of Rpn12 and Rpn3 (Figure 1). Even in the CSN, an analogous interaction surface is found between one module comprising CSN8, CSN3, and CSN1 and another unit containing CSN7, CSN4, and CSN6 (Figure 1A). Of note, the two MPN subunits are found in tandem in each complex.

PCI Protein Classification

The PCI domain was originally identified as a region of localized sequence similarity shared between several subunits of the lid, CSN, and eIF3 complexes. More recently, structural studies have shattered the view of PCI as a monolithic domain and have redefined the PCI region as two subdomains: N-terminal tetratricopeptide-like repeats followed by a winged helix (Dessau et al., 2008; Scheel and Hofmann, 2005; Wei et al., 2004). Our bioinformatics analysis suggests that there are three different PCI subclasses according to the region immediately upstream (Figure 1B).

Five subunits within each complex follow the “typical” PCI domain structure in which the PCI domain is preceded by additional bihelical repeats. This extension is occasionally referred to as PAM (PCI-associated module). The sixth subunit (Rpn12, Csn8, or eIF3k) harbors a more divergent PCI domain with barely detectable sequence similarity to the typical PCs and is hence classified into a separate class. This “Rpn12 subtype” also lacks the N-terminal helical extension, thereby resulting in substantially smaller proteins. A third PCI subclass is found in Sac3 and other proteins outside of the three canonical complexes. The sequences of these Sac3-like PCI domains share several features with the Rpn12 subtype. Together, these two subtypes
are considered “atypical PCI domains,” but due to a highly conserved nonhelical region preceding the PCI domain, Sac3 subtype proteins are much longer (Figure 1B).

Members of both Sac3 and Rpn12 atypical subtypes tend to form small complexes with a select typical PCI partner, specifically of the Rpn3/Csn3/elF3 subunit branch (Faza et al., 2009; Isono et al., 2004; Wilmes et al., 2008). Interestingly, such typical-atypical PCI dimers often bind an additional component, Sem1 (Faza et al., 2009; Sharon et al., 2006; Wilmes et al., 2008). Sem1/DSS1, a Multitasking Organizer of PCI/MPN Minicomplexes

Sem1 and its mammalian/plant ortholog DSS1 are short polypeptides of about 13 kDa with a highly conserved acidic stretch. In addition to interacting with a number of PCI proteins, including proteasome lid subunits (Faza et al., 2009; Wilmes et al., 2008), DSS1 also stabilizes the BRCA2-RAD51 interaction within a complex that exhibits homologous DNA recombination activity (Yang et al., 2002). The interacting surface of DSS1 complexed to BRCA2 is the acidic stretch alternating with aromatic residues; the complementary groove on BRCA2 is rich in basic and aromatic residues. Similar to histone chaperones, the high acidic and aromatic content of DSS1 might mimic oligonucleotides, thereby directing formation of nucleotide-binding protein complexes (Yang et al., 2002). This BRCA2-RAD51 minicomplex integrates into larger assemblies involved in homologous recombination and DNA repair. In a remarkable analogy to the proteasome regulatory particle, one of the ensuing complexes also
Figure 2. Sem1/DSS1, a Multitasking Organizer of PCI/MPN Minicomplexes

(A) A summary of Sem1/DSS1 participation in several assembly pathways (budding yeast in purple, human in green). Sem1 associates with typical (red) and atypical (blue) PCI subunits (inner ring). These minicomplexes incorporate into larger complexes, either with or without Sem1 (outer ring). Proposed links are shown as dashed arrows. Human DSS1 binds to the proteasome subunit RPN3 and has also been found with subunits of the BRCC and RNAPII-integrator complexes. Complexes are linked to the ubiquitin system via associated deubiquitinating enzymes (dUBs, shown as a yellow star) or other factors as detailed in the text.

(B) Putative musical chairs of yeast minicomplexes. Subunit compositions of yeast PCI complexes and their relationship to the newly identified Sem1-associated minicomplexes are shown (known paralogs are kept in the same position as in Figure 1). The Sc lid contains orthologs for all canonical PCI and MPN subunits. Ejection of Rpn12 and Rpn3 was observed in budding yeast proteasome that was mutated in RPN7, which suggested the existence of this minicomplex (Isono et al., 2004). The eIF3 core conserved in SceIF3 contains two PCI subunits (eIF3a and eIF3c), but appears to lack orthologs for eIF3e, eIF3i, and eIF3k. The loosely
contains two MPN subunits (Abraxas/ABRA1 and Brcc36), a vWA domain (NBA1) (Fu et al., 2001), polyubiquitin binding proteins (Rap80 and BRE), and an ubiquitin E3 ligase (BRC1-BARD1) (Wang et al., 2009). Independently, Sem1/DSS1 also interacts with other proteins through the same acidic BRC2-interacting stretch. One example is Rpn3, a PCI-containing subunit of the proteasome lid, through which DSS1 associates with the proteasome (Wei et al., 2008b).

New targets of Sem1 have been uncovered using an exhaustive epistatic miniarray screen of functionally related genes. A miniarray profiling of genes related to RNA processing in S. cerevisiae by Nevan Krogan and colleagues identified positive relationships between SEM1 and a number of genes encoding PCI proteins (Wilmes et al., 2008). Coimmunoprecipitation verified that Sem1 may in fact be a component of three distinct minicomplexes: Rpn3-Rpn12 of the proteasome lid, Csn12-Ypr045c, and Sac3-Thp1 (Wilmes et al., 2008). The Sem1-binding subunit in each dimer is a typical PCI protein, specifically from the Rpn3 branch, and the other is an atypical PCI (Figure 1B). A study of lid breakdown products identified Sem1 bound to Rpn3 as well as with Rpn7 (Sharon et al., 2006). Moreover, incorporation of Rpn3 and Rpn12 into the lid is hampered by a conditional mutation in RPN7 (Isono et al., 2004), emphasizing the structural link between these three lid subunits and Sem1 (Figure 1). SEM1 deletion also leads to pervasive proteasome defects, in line with Sem1 being essential for lid stability (Funakoshi et al., 2004). However, the role of Sem1 in balancing alternative assembly pathways remains unclear (Figure 2A).

Sem1 also associates with two PCI proteins involved in pre-mRNA splicing, Csn12 and the product of YPR045c (Wilmes et al., 2008). Interestingly, the spliceosome contains an MPN subunit, Prp8 (Grainger and Beggs, 2005); therefore, it is exciting to propose that the spliceosome also associates with this PCI minicomplex (Figure 2A). Independently, Csn12 was previously shown to interact with CSN subunits from budding yeast; however, other CSN components are not associated with the Csn12-Ypr045c minicomplex, and their mutant phenotypes are unrelated (Faza et al., 2009; Maytal-Kivty et al., 2002; Wilmes et al., 2008). Thp1 and Sac3, two other PCI proteins that coimmunoprecipitate with Sem1, are involved in a different aspect of mRNA processing. Mutations in SEM1, SAC3, or THP1 cause polyadenylated mRNA to accumulate in the nucleus and result in defective transcription-coupled mRNA nuclear export (Faza et al., 2009; Wilmes et al., 2008). The Thp1-Sac3 duo, along with Cdc31-Sus1, incorporates into a larger complex termed TREX-2 (Figure 2A). In turn, TREX-2 enters into even larger assemblies: in association with the polyubiquitin-binding export receptor Mex67, TREX-2 regulates gene gating and mRNA export, whereas in association with the SAGA complex, TREX-2 drives histone deubiquitination and acetylation (Chekanova et al., 2008; Faza et al., 2009; González-Aguilera et al., 2008; Köhler et al., 2008). It is still unclear whether Sem1 remains in these larger complexes.

Sem1-containing complexes appear in other organisms as well. DSS1, the human ortholog, associates with the proteasome, specifically via RPN3, and also copurifies with the integrator complex, a mediator of snRNA processing (Baillat et al., 2005). The integrator interacts with RNA polymerase II (RNAPII), mediates transcription, and drives snRNA processing. Two such cotranscriptionally processed snRNAs, U1 and U2, are components of the spliceosome complex, highlighting an interesting functional comparison to yeast Csn12-YPR045c (Figure 2A). Conserved orthologs of Thp1 and Sac3 are also found in the human genome (Figure 1B), although their interactions with DSS1 have not been confirmed. As mentioned above, DSS1 also directs BRCA2-containing complexes to sites of DNA damage (Figure 2A).

The compilation of these fascinating examples suggests that Sem1/DSS1 should not be considered a bona fide subunit of any single PCI or related complex; rather it serves as a molecular adhesive to recruit a number of different components into larger complexes. Sem1 might be required for stabilizing mini-PCI complexes in the absence of the entire hexameric PCI assemblage, perhaps by binding (and shielding) the interaction surface that would normally be used for attachment to the large complex. The requirement for Sem1 in the large complexes might be also a consequence of this role in stabilizing minicomplexes. By supplying intermittent acidic-hydrophobic surfaces able to compete for RNA or DNA binding, Sem1/DSS1 may underlie the common link between these complexes and nucleic acids (Figure 2A). Thus, proteasomes, normally involved in protein degradation, are found at sites of DNA damage and participate in various aspects of transcriptional activation, chromatin arrangement, and DNA repair (Krogan et al., 2004). It is interesting to consider whether Sem1/DSS1 directs proteasome components to these activities. Regardless, each megacomplex is related to the ubiquitin system, either through function or inferred from common domains/motifs (Figure 2A).

**Survey of PCI Associations in Budding Yeast**

How do the three Sem1-associated minicomplexes identified in S. cerevisiae relate to the PCI complexes present in this organism? The lid is well conserved in budding yeast; however, the other two PCI complexes deviate in architecture and subunit arrangement from the canonical complexes found in metazoa (Figure 2B). Specifically, ScCSN and ScElf3 lack an extended arm that contains Rpn3 and Rpn12 paralogs. It is tempting to suggest that several or all of the recently identified Sem1-associated PCI proteins could complement the missing module. By analogy to the configuration of the canonical complex, ScCsn11 would be expected to serve as the entry point for such a typical atypical PCI dimer (Figure 2B). As an indication that this may indeed be so, ScCsn12 interacts with individual ScCSN subunits; however, interaction with the intact complex remains an open question.

defined CSN in yeast also lacks two PCI subunits: apparent orthologs of CSN3 and CSN8. ScCsi1 bears neither a PCI nor an MPN domain (hence colored in black), yet is bioinformatically related to CSN6 (hence outlined in pink) (K.H., unpublished data). Note that ScCsn11 interacts with both ScCSN and ScElf3 as a possible eIF3e paralog (Shalev et al., 2001; Maytal-Kivty et al., 2002). Likewise, ScRpn5 was suggested as a common subunit of two complexes: ScCSN and proteasome lid (Gavin et al., 2002). Consequently, the three Sem1-associated PCI minicomplexes could theoretically complement the missing subunits in ScCSN or ScElf3.
ScElF3 lacks a few subunits integral to the canonical version from metazoa (Figure 2B), including three PCI subunits, elfI, elf3k, and elf3e (paralogs of Rpn3, Rpn12, and Rpn7). In an interesting case of molecular promiscuity, ScCsn11 also associates with the core ScElF3, directly interacting with elf3b. As ScCsn11 can be functionally complemented by human elf3e (Shalev et al., 2001), this subunit might double-up as the missing link between ScElF3 and one of the Sem1-associated PCI dimers (Figure 2B). Thus, the newly identified “orphaned” PCI proteins link between (Shalev et al., 2001), this subunit might double-up as the missing Sc

eIF3k, and eIF3e (paralogs of Rpn3, Rpn12, and Rpn7). In an interesting case of molecular promiscuity, ScElF3 and one of the Sem1-associated PCI dimers (Figure 2B). Thus, the newly identified “orphaned” PCI proteins expand the repertoire of PCI subunits in S. cerevisiae to more closely resemble the situation in metazoa. However, the situation is unlikely to be a simple one-to-one relationship, as some subunits appear to be present in more than one complex (Figure 2B).

Concluding Remarks

Our understanding of PCI-based complexes has broadened to include not only protein life span regulation, but also roles in chromatin dynamics, RNA processing, gene gating, and DNA repair. Interactions of PCI proteins with Sem1/DSS1 in minicomplexes on the one hand and with large protein assemblies such as the proteasome, TREX-2/MEX67, SAGA, or the spliceosome on the other indicate that PCI complexes are dynamic, with exchangeable compositions. Functional promiscuity associated with members of the extended PCI family is embodied by the multireactivity of Sem1/DSS1, a protein that moonlights as a factor of multiple seemingly unrelated complexes (e.g., proteasome, BRCC, integrator, etc). Through its highly acidic stretch, ScElF3 might mimic oligonucleotides, a property that could explain its presence in diverse DNA- and RNA-associated complexes. Indeed, in addition to functioning as a multicatalytic protease, the proteasome is also targeted to chromatin, transcriptional elongation activity, or sites of DNA damage. Guided by Sem1, might proteasome subunit composition be adjusted to alter its function or localization? As an initiator of RNA translation, elf3 fits the pattern for Sem1/DSS1-related complexes, although association with Sem1 has not yet been documented. Upon close inspection, the CSN is “not only a protease” (Wei et al., 2008a), but might also associate with chromatin and regulate transcription or gene expression (Chamovitz, 2009). Thus, in addition to their well-documented roles in enforcing cellular protein levels, the three canonical PCI complexes (and members of their expanding family) meet full circle at a variety of processes related to nucleic acid regulation.

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