Revisiting the COP9 signalosome as a transcriptional regulator

Daniel A. Chamovitz

Tel Aviv University, Tel Aviv, Israel

The COP9 signalosome (CSN) is a highly conserved protein complex that was originally described as a repressor of light-dependent growth and transcription in *Arabidopsis*. The most studied CSN function is the regulation of protein degradation, which occurs primarily through the removal of the ubiquitin-like modifier Nedd8 from cullin-based E3 ubiquitin ligases. This activity can regulate transcription-factor stability and, therefore, transcriptional activity. Recent data suggest that the CSN also regulates transcription on the chromatin by mechanisms that are not yet clearly understood. Furthermore, the CSN subunits CSN5 and CSN2 seem to act as transcriptional coactivators and corepressors, respectively. Here, I re-evaluate the mechanisms by which the CSN acts as a transcriptional regulator, and suggest that they could extend beyond the regulation of protein stability.

Introduction: the many functions of the CSN

The COP9 signalosome (CSN) is a highly conserved protein complex that consists of eight subunits known as CSN1 to CSN8 in higher eukaryotes (Fig 1; Deng *et al*, 2000). The complex was originally discovered as a repressor of light-dependent growth in *Arabidopsis* (Chamovitz *et al*, 1996; Wei *et al*, 1994). Subsequent work has identified and characterized the CSN in mammals, *Drosophila*, budding and fission yeast, fungi, *Dictyostelium* and *Caenorhabditis elegans*, highlighting its role as a general modulator of diverse cellular and developmental processes (reviewed in Wei *et al*, 2008).

The most studied function of the CSN is the regulation of protein degradation, which is carried out by several mechanisms (Table 1). The CSN removes Nedd8—a ubiquitin-like modifier—from cullin-based E3 ubiquitin ligases (Lyapina *et al*, 2001), thereby regulating ligase activity. The deneddylation activity resides in the JAMM/MPN+ domain of CSN5 (Cope *et al*, 2002;

Maytal-Kivity *et al*, 2002), and the intact complex is necessary and sufficient for deneddylation (Sharon *et al*, 2009). This activity of the CSN is well established, although not all cullin-based E3 ligase activities are sensitive to CSN-mediated deneddylation. For example, the signal-dependent degradation of IκB—which is mediated by SCF$^{β\text{-TrcP}}$—seems to be unaffected in mammalian cells after the knockdown of different CSN subunits (Harari-Steinberg *et al*, 2007; Menon *et al*, 2007; Panattoni *et al*, 2008; Schweitzer *et al*, 2007). Surprisingly, the TCR-stimulated downregulation of p27—mediated by SCF^{Skp2}—is also unaffected in CSN8-deficient T cells (Menon *et al*, 2007). Similarly, the signal-dependent degradation of the IκB homologue Cactus and the clock protein TIM—which is mediated by SCF^{Slimb}—is unaffected in *Drosophila csn5^{null}* mutants (Harari-Steinberg *et al*, 2007; Knowles *et al*, 2009). Several excellent reviews have elaborated on the role of the CSN as a deneddylase, which is therefore not covered here (Cope & Deshaies, 2003; Wei & Deng, 2003; Wolf *et al*, 2003).

However, CSN5-mediated deneddylation seems to be only one—albeit an extremely important one—of the CSN functions (Table 1). Indeed, if CSN5-mediated deneddylation were the only activity of the CSN, one would expect that loss of CSN5 function would be phenotypically equal to loss of the entire complex. This is indeed the case for null mutations in *Arabidopsis* and mice (Dohmann *et al*, 2005; Gusmaroli *et al*, 2007; Lykke-Andersen *et al*, 2003; Tomoda *et al*, 2004; Yan *et al*, 2003), whereas it is not the case in *Drosophila* and *Schizosaccharomyces pombe*. Although mutations in *Drosophila* CSN4, CSN5 and CSN8 all result in larval lethality, the mutants are characterized by overlapping—but unique—morphological phenotypes (Cope *et al*, 2002; Doronkin *et al*, 2003; Oren-Giladi *et al*, 2008; Oron *et al*, 2002). At a molecular level, the loss of the entire complex in *Drosophila csn4^{null}* mutants leads to more severe transcriptome changes than the loss of only CSN5 in *csn5^{null}* mutants, which maintain a complex that lacks CSN5 (Oron *et al*, 2007). Although this difference in transcriptomes might be explained by differences in the persistence of maternally contributed subunits, a similar picture arises from genetic studies in *S. pombe*. All the CSN subunit-null mutants of *S. pombe* that have been tested have impaired deneddylation of different cullins; however, only *csn1-d* and *csn2-d* mutants—and not *csn5-d* mutants—have obvious phenotypes (Mundt *et al*, 2002; Zhou *et al*, 2001). In addition, partial loss-of-function mutants of *Arabidopsis csn1* and *csn5* also show different growth phenotypes, as opposed to

Department of Plant Sciences, Tel Aviv University, Tel Aviv 69978, Israel
Tel: +972 3 640 6703; Fax: +972 3 640 8989;
E-mail: dannyc@tauex.tau.ac.il

the null mutants of each subunit, which are phenotypically equivalent (Gusmaroli *et al*, 2007; Wang *et al*, 2002, 2003). In summary, although CSN5-mediated deneddylation is clearly dependent on the entire CSN—and is essential in most organisms—the loss of the entire complex can have different consequences than the loss of only CSN5.

One of the first functions of the CSN to be identified in mammalian cells was regulation of the phosphorylation of ubiquitin-proteasome pathway substrates through the activity of CSN-associated kinases (Naumann *et al*, 1999; Seeger *et al*, 1998). These kinases act on several crucial transcriptional regulators and much of the effect of the CSN on transcription can be ascribed to this activity (reviewed in Harari-Steinberg & Chamovitz, 2004).

A second CSN-associated activity—deubiquitination—has also garnered much attention (Hetfeld *et al*, 2005; Zhou *et al*, 2003). This activity—mediated by the CSN-associated Ubp12/USP15—also influences protein stability and has been proposed to stabilize E3 ligase subunits by preventing their promiscuous autoubiquitination (reviewed in Wu *et al*, 2006). This was recently shown to extend to E3 ligase substrates, the stability of which is dependent on the CSN. For example, following signal-dependent degradation of IκBα and the subsequent nuclear localization of NF-κB, CSN-dependent USP15-mediated deubiquitination stabilizes newly synthesized IκBα, thereby attenuating NF-κB activity (Schweitzer *et al*, 2007).

The CSN also has poorly defined roles in regulating the subcellular localization of crucial signalling molecules, including the COP1 E3 ubiquitin ligase (Chamovitz *et al*, 1996; Wang *et al*, 2009), the cell-cycle regulator p27 (Tomoda *et al*, 2002), p53 (Oh *et al*, 2006) and the IκB homologue Cactus (Harari-Steinberg *et al*, 2007).

Although the CSN was originally identified as a transcriptional repressor involved in *Arabidopsis* photomorphogenesis (Wei *et al*, 1994; Wei & Deng, 1992), much of the subsequent work has concentrated on its role as a regulator of protein degradation. Several recent studies have emphasized the need to re-evaluate the role of the CSN as a transcriptional regulator.
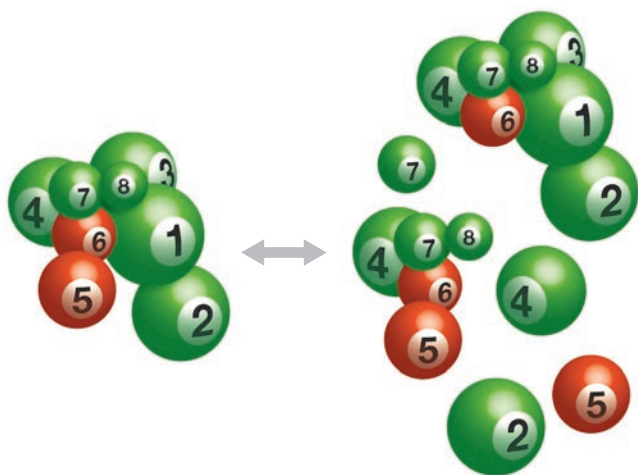
The CSN as a regulator of transcription

The CSN was originally described as a transcriptional repressor of a range of *Arabidopsis* genes that are normally repressed in the dark. As such, these 'light-activated' genes are expressed in darkness in null mutants of CSN subunits (Wei & Deng, 1992). This repression was subsequently ascribed to the role of the CSN in regulating the stability of the transcription factors that are normally unstable and degraded in darkness, but are stabilized in *csn* mutants (reviewed in Serino & Deng, 2003). This paradigm for the role of CSN in regulating the stability—and therefore the activity—of transcription factors and other signalling proteins has been shown in many systems (see Table 3 in Wei *et al*, 2008). In addition to light-regulated genes (Ma *et al*, 2003), numerous others are misregulated in *Arabidopsis* *csn* mutants, including those regulated by the phytohormones auxin (Dohmann *et al*, 2008a) and jasmonate (Feng *et al*, 2003), and those involved in cell-cycle regulation (Dohmann *et al*, 2008b).

The mutation of different CSN subunits in *Drosophila* leads to a misregulation of approximately 20% of the transcriptome during larval development (Oron *et al*, 2007). It is not surprising that mutations in a protein complex that lead to severe phenotypes and death also induce many transcriptome changes as an indirect

consequence. However, many of these changes are observed in early larval development (mid-second instar)—before the onset of visible mutant phenotypes, but at a stage where maternally contributed proteins have been depleted, rendering the larva true null mutants. This indicates that a primary effect of CSN perturbation is a change in gene-expression profiles. The most obvious among these changes in *Drosophila* is the achronic expression of numerous developmentally regulated genes (Oron *et al*, 2007), the transcription of many of which is usually limited to embryogenesis or metamorphosis. These results indicate that, in the absence of the CSN, the transcription of these genes is derepressed, similar to the derepression of light-activated genes in dark-grown mutants of the *Arabidopsis* CSN. However, although most of these early misregulated genes were derepressed, the transcription of another large set of genes was repressed in the mutants relative to the wild type (Oron *et al*, 2007), indicating a positive requirement of the CSN for gene expression.

Glossary

AP-1	activator protein 1
β-TrcP	β-transducin repeat containing protein
Cab	chlorophyll A/B binding protein
Ccnd2	gene encoding cyclin D2
CDK	cyclin-dependent kinase
Cdkn1a	gene encoding p21
ChIP	chromatin immunoprecipitation
CSA	Cockayne syndrome WD repeat protein
Cul1	cullin 1
DAX1	dosage-sensitive sex reversal-adrenal hypoplasia congenita critical region on the X chromosome protein 1
DDB	damaged DNA binding protein
E2f1	transcription factor E2f1
HEK	human embryonic kidney
IκB	inhibitor of κB
Jab1	Jun activation domain-binding protein 1/COP9 signalosome 5
JAMM	Jab1/MPN/MOV34 domain-containing metalloisopeptidase
MPR1p	multistep phosphorelay protein 1
MPN	MPR1p and PAD1p amino-terminal
NAP-1	nucleosome assembly protein 1
Nedd8	neural precursor cell expressed, developmentally down-regulated 8
NF-κB	nuclear factor-κB
PAD1p	phenylacrylic acid decarboxylase protein
PCI	proteasome-COP9-eukaryotic initiation factor 3
PCNA	proliferating cell nuclear antigen
Pdcd4	programmed cell death 4
*Pol*α	DNA polymerase α-catalytic subunit
*rbc*S	ribulose-1,5-bisphosphate carboxylase/oxygenase small subunit
Rbf	retinoblastoma family
RFX-1	MHC class II regulatory factor X, 1
SAP130	Sin3A-associated protein
SCF	Skp1–cullin–F-box
Skp2	S-phase kinase associated 2
Slimb	supernumerary limbs
TCR	T-cell receptor
TIM	timeless
Ubp12/USP15	ubiquitin specific protease

Fig 1 | Dynamic equilibrium among the eight-subunit CSN core complex, partial complexes and subunit monomers. Not all partial complexes or monomers are known or shown, and the composition of the partial complexes is probably both tissue and organism specific. The CSN4–CSN8 complex was described in human cells as the JAC (Tomoda *et al*, 2002). The size of each ball is proportional to the relative protein size. The subunit arrangement is shown according to Sharon *et al* (2009). The green balls represent PCI-containing subunits and the red balls represent MPN-containing subunits. CSN, COP9 signalosome; JAC, Jab1-associated complex; MPN, MPR1p and PAD1p amino-terminal; PCI, proteasome-COP9-eukaryotic initiation factor 3.

Table 1 | Activities ascribed to the CSN or CSN subunits

Activity	References
Deneddylation	Lyapina *et al*, 2001
Deubiquitination	Wu *et al*, 2006; Zhou *et al*, 2003
Kinase activity	Naumann *et al*, 1999; Seeger *et al*, 1998
Regulation of protein subcellular localization	Chamovitz *et al*, 1996; Tomoda *et al*, 2002; Wang *et al*, 2009
Transcriptional coactivation/ corepression	Claret *et al*, 1996; Dressel *et al*, 1999

CSN, COP9 signalosome.

This positive requirement is in agreement with the role of the CSN in regulating photomorphogenesis. Close re-examination of the early work on the *cop9* (*csn8*) mutant in *Arabidopsis* (Wei & Deng, 1992) reveals that the light-activated genes *cab* and *rbcS* are not only derepressed in the dark, but that their light-induced expression levels are lower than those of wild-type plants (N. Wei, personal communication; see Fig 5 in Wei & Deng, 1992). Similarly, although the expression of many auxin-induced genes is derepressed in *Arabidopsis csn* mutants, the induction of an auxin-responsive reporter gene in the presence of auxin is also reduced (Dohmann *et al*, 2008a), further illustrating a role of the CSN not only as a transcriptional repressor, but also as a transcriptional activator. This effect is mirrored in mice; during T-cell development, CSN5 is required for T-cell receptor-driven signals that are involved in positive selection (Panattoni *et al*, 2008), and the specific deletion of *Csn8* in T cells leads to an impairment of signal-induced expression of cell cycle-related genes (Menon *et al*, 2007). For example, in the absence of CSN8, the genes encoding cyclin E1, cyclin D2 and E2f1 are elevated relative to wild-type mice—consistent with the repressive role of the CSN. However, on stimulation for cell-cycle re-entry, the expression of these genes could not be upregulated to wild-type levels.

Despite these clear effects on transcription, a question remains as to whether the mechanism of this regulation is based exclusively on the CSN-dependent stability of transcription regulators, or whether other mechanisms are also involved. Some CSN functions are clearly cytoplasmic (Schweitzer *et al*, 2007; Tomoda *et al*, 2002); however, could the nuclear CSN (Chamovitz *et al*, 1996; Mundt *et al*, 2002; Tomoda *et al*, 2002) regulate transcription directly? An amino-terminal fragment of human CSN1 has been shown to translocate to the nucleus

and inhibit gene expression from several specific promoters (Tsuge *et al*, 2001). Although the mechanism of this inhibition is still unclear, it might be related to the fact that this part of CSN1 interacts with SAP130—a member of the DDB1 protein family and an established component of the transcription machinery (Menon *et al*, 2008).

Is the CSN a chromatin-based transcriptional regulator?

Several recent studies have shown that CSN subunits can localize to chromatin, further suggesting a direct role for the CSN in transcriptional regulation. The first indication that the CSN might be associated with chromatin came from the work of Groisman and colleagues (2003), who detected it in purified chromatin fractions from HeLa cells. They found that the CSN is bound to chromatin in association with both the CSA and DDB2-containing E3 ligase complexes, where it participates in regulating cellular responses to DNA damage.

A stable association between CSN subunits and chromatin was subsequently identified by ChIP experiments in both *Drosophila* (Ullah *et al*, 2007) and mammalian cells (Menon *et al*, 2007; Mori *et al*, 2008). Ullah and colleagues described a direct interaction between CSN4 and the *Drosophila* Rbf proteins, and showed that CSN4 could associate with Rbf-targeted promoters in both S2 cells and *Drosophila* embryos. CSN4 and Rbf simultaneously occupied the promoter region of the *Polα* and *PCNA* genes, suggesting that Rbf activity is regulated directly on the chromatin by the CSN, presumably through the modulation of Rbf stability.

The Groisman and Ullah papers are consistent with a role of the CSN in regulating transcription-factor stability and/or activity, albeit directly on the chromatin. The work of Menon *et al* (2007) further suggests that the CSN is a direct regulator of the transcription of cell cycle-related genes. By using Csn8-deficient T cells—which have impaired proliferation—the authors showed the normal, signal-induced degradation of several SCF substrates, including IκBα, p27 and Pdcd4. By contrast, the accumulation of G1 cyclins and CDKs was defective in these mutant cells, suggesting that the turnover of these proteins is affected by the loss of the CSN. Interestingly, treatment with the proteasome inhibitor MG132 did not restore the normal accumulation of G1 cyclins and CDKs in these Csn8-deficient cells, leading the authors to speculate that CSN-mediated transcriptional regulation—rather than direct regulation of protein turnover—might lead to the reduced levels of these proteins. Indeed, the expression of the genes encoding E2F1 and the G1 cyclins E1 and D2 could not be upregulated after TCR stimulation in the absence of Csn8, mirroring the corresponding protein-accumulation patterns. Both Csn1

and Csn8 were detected bound to the *Ccnd2*, *Cdk4* and *Cdkn1a* promoters by ChIP assays; the localization of two CSN subunits—and therefore probably the entire CSN complex—at the promoter regions of genes encoding cell-cycle regulators suggests that the CSN has the ability to regulate transcription directly, and this might be—at least partly—protease independent.

The caveat to this hypothesis is that it is partly based on negative results—the lack of MG132 rescue of G1 cyclin and CDK protein levels. Therefore, the mechanism by which the CSN regulates transcription on chromatin—apart from the regulation of transcription-factor stability—remains to be identified.

Most of these studies describe chromatin-associated roles for the CSN in the context of cell-cycle regulation, although they are probably not limited to this process. Indeed, Ullah *et al* (2007) claimed that CSN4 immunoprecipitated with the promoters of other important *Drosophila* transcription factors, such as the dorsal/ventral patterning gene *zerknullt*, the Gap gene *tailless* and the segmentation gene *fushi tarazu*, although the data were not shown. Therefore, the CSN probably associates with genomic regions that regulate many developmental processes.

Is the PCI domain a DNA-binding motif?

The crystal structure of CSN7, which was recently published, could shed new light on the role of the CSN in transcriptional regulation (Dessau *et al*, 2008). CSN7, similar to five other CSN subunits (CSN1, CSN2, CSN3, CSN4 and CSN8), contains a PCI domain, which is also common to six subunits of the proteasome lid and six subunits of eIF3. This domain is thought to mediate and stabilize protein–protein interactions within the complexes (Halimi & Chamovitz, 2008; Hofmann & Bucher, 1998).

The PCI domain is comprised of two subdomains, an N-terminal helical bundle and a carboxy-terminal winged helix, which are intimately connected through a central helix (Dessau *et al*, 2008). Importantly for the CSN-mediated transcription discussed here, the winged helix subdomain comprises a canonical helix-turn-helix that has a structure and electrostatic potential similar to those of winged helix nucleic acid-binding proteins, such as RFX-1 (Gajiwala *et al*, 2000). This suggests that an intact CSN would have six winged helix domains with the potential to bind to nucleic acids. Does the presence of multiple winged helix domains in the CSN imply that it has direct involvement—either as a complex or as individual subunits—in nucleotide binding? Although multiple CSN subunits have been shown to associate with chromatin, there is currently no evidence of direct nucleotide binding. However, two additional lines of evidence suggest that this possibility is worthy of further study. First, the CSN has been shown to bind to heparin, which is a highly sulphated glycosaminoglycan with the ability to bind to nucleic acid-binding proteins (Chamovitz *et al*, 1996). Second, theoretical modelling shows that the winged helix domain of CSN7 can dock with nucleic acids (see Supplementary Fig 11 in Dessau *et al*, 2008). Nonetheless, whether such a direct interaction occurs *in vivo* remains to be determined by further experimentation.

Which forms of the CSN regulate transcription?

The exact functional configuration of the CSN subunits is not always clear. Some of the eight subunits are detected only in a 'core complex' of approximately 500 kDa (also known as the COP9 signalosome), whereas others—such as CSN2, CSN4,

---

Sidebar A | In need of answers

(i) Is the COP9 signalosome (CSN) a transcriptional regulator? The activity of the CSN definitely influences both the repression and the activation of a range of genes. This activity was originally ascribed to CSN-mediated stability of transcription factors and other regulatory proteins. Recent work indicates that the CSN can associate with specific promoters to regulate transcription, although whether this interaction is direct—or mediated by other proteins—and the mechanism by which the CSN affects transcription on the chromatin are not known.

(ii) Where in the cell does the CSN regulate transcription? Probably both in the cytoplasm and in the nucleus. Individual subunits have been detected in various subcellular compartments, including the cell membrane, cytoplasm and nucleus. However, as outlined in the text, some of the CSN functions seem to be mediated in the nucleus directly on the chromatin.

(iii) Is CSN-mediated transcriptional control achieved through its regulation of transcription-factor stability? The CSN definitely has a crucial role in regulating the stability of specific transcription factors. However, some roles of CSN subunits in transcriptional regulation might be protease independent.

(iv) Does the entire CSN, or do individual CSN subunits, regulate transcription? Probably both, although we do not completely understand the relationship between individual subunits and the CSN complex.

(v) What is unique about CSN5 and CSN2? Both CSN5 and CSN2 have established functions that are independent of the CSN complex. Interestingly, CSN5 is a coactivator of transcription, whereas CSN2 is a corepressor. Could these subunits be the basis of CSN-mediated activation and repression? Both subunits have the weakest physical connection to the complex, and it is tempting to speculate that the CSN releases and rebinds to these proteins to regulate transcription differentially. Answering this question will require further study.

(vi) What regulates CSN-mediated transcription? We do not know how, which or even whether signals directly impinge on CSN activity. Certain CSN subunits can be phosphorylated (Fang *et al*, 2008; Harari-Steinberg & Chamovitz, 2004); however, we do not understand the implications of this modification for functionality.

(vii) What determines CSN specificity in transcriptional control? We do not have the answer to this question, although there are obviously tissue and environmental specificities that need to be considered both when analysing the primary literature and in planning future experiments.

CSN5, CSN6 and CSN7—are also detected independently of the core complex. The distribution of these subunit conformations might be dependent on the organism, tissue or specific conditions analysed, and several studies have suggested various equilibria between these different subunit forms (Fukumoto *et al*, 2005; Gusmaroli *et al*, 2007; Oron *et al*, 2002; Tsuge *et al*, 2001).

Deneddylation is clearly dependent on CSN5 within the context of the entire eight-subunit core complex (Cope *et al*, 2002; Sharon *et al*, 2009). A recombinant human CSN assembled in *Escherichia coli* shows deneddylation activity against Cul1, whereas a CSN complex that lacks CSN5 has no such activity (Sharon *et al*, 2009), although the complex can be readily detected *in vivo* and *in vitro* (Dohmann *et al*, 2005; Oron *et al*, 2002; Sharon *et al*, 2009).

Two subunits in particular—CSN2 and CSN5—seem to be stable in the absence of the CSN (Gusmaroli *et al*, 2007). In the model proposed by Sharon and colleagues (2009), these two subunits protrude from the core of the CSN and are each connected to it by a single interaction—CSN5 to CSN6 and CSN2 to CSN1—such that the loss of either subunit is not expected to affect the overall complex stability (Fig 1). CSN5 and CSN2 are the two subunits for which the most evidence exists regarding their roles independent of the CSN core complex. CSN5—also known as Jab1—was originally identified as a coactivator (with Jun1) of AP-1 transcription factor-binding sites (Claret *et al*, 1996), and has since been shown to interact with numerous proteins affecting diverse signalling pathways, both cytoplasmic and nuclear (Chamovitz & Segal, 2001; Richardson & Zundel, 2005; Zhang *et al*, 2008). CSN2—also known as Alien—was originally identified as a corepressor of steroid hormone signalling (Dressel *et al*, 1999). Alien interacts with a subset of nuclear hormone receptors—such as the human thyroid and DAX1 receptors and the *Drosophila* ecdysone and seven-up receptors—in the absence of hormone. The addition of the appropriate hormones leads to the dissociation of Alien from the receptor, allowing for hormone-mediated transcription (Altincicek *et al*, 2000; Dressel *et al*, 1999). Indeed, many of the physical phenotypes and transcriptome alterations identified in *Drosophila csn* mutants could be ascribed to the impaired function of the ecdysone receptor (Oren-Giladi *et al*, 2008; Oron *et al*, 2002, 2007). However, characterizing the function of CSN2/Alien in mammals is not as straightforward because CSN2 and Alien are two different splicing variants of the same locus. CSN2 is the full-length gene product, whereas Alien refers to the N-terminal 300 amino-acid residues of CSN2, which interestingly lack part of the PCI domain (Tenbaum *et al*, 2003). Whether the Alien form of CSN2 assembles into the CSN is not yet clear. Alien has also been recently shown to function directly on the chromatin, where it enhances NAP-1-mediated nucleosome assembly, thereby participating in gene silencing (Eckey *et al*, 2007). Although Alien was found to associate with chromatin in HEK293 cells in this study, both CSN8 and CSN2 were detected in the cytoplasm. The discrepancy between these results, and the nuclear localization of CSN8 and other subunits found in other studies and discussed above, remains to be resolved.

Outlook and perspectives

Seventeen years after the initial description of COP9 as a transcriptional repressor (Wei & Deng, 1992), the mechanisms by which the CSN regulates transcription are beginning to be understood. Recent studies have shed light on the roles of the CSN in regulating transcription directly on the chromatin, although many important questions remain regarding how this is achieved on a mechanistic level (Sidebar A). In contemplating a chromatin-based role, we must keep in mind that the CSN is not the only component of the ubiquitin-proteasome system to be implicated in transcriptional control on the chromatin. For example, the entire yeast proteasome is able to associate with regulatory sequences directly on the chromatin (Sikder *et al*, 2006). Therefore, it is conceivable that the CSN could participate in a chromatin-localized supercomplex with other components of the ubiquitin-proteasome system. Interestingly, although the proteasome obviously has a proteolytic role in regulating transcription-factor stability directly on the chromatin (Saccani *et al*, 2004), it is also thought to regulate transcription by nonproteolytic mechanisms

(Lassot *et al*, 2007). Therefore, although solid positive data are still lacking, a nonproteolytic role for the CSN—or at least for some CSN subunits—remains a possibility. Future work is expected to unravel the complex regulatory mechanisms of the CSN and its individual subunits in regulating gene expression.

REFERENCES
Altincicek B, Tenbaum SP, Dressel U, Thormeyer D, Renkawitz R, Baniahmad A (2000) Interaction of the corepressor Alien with DAX-1 is abrogated by mutations of DAX-1 involved in adrenal hypoplasia congenita. *J Biol Chem* **275:** 7662–7667

Chamovitz DA, Segal D (2001) JAB1/CSN5 and the COP9 signalosome. A complex situation. *EMBO Rep* **2:** 96–101

Chamovitz DA, Wei N, Osterlund MT, von Arnim AG, Staub JM, Matsui M, Deng XW (1996) The COP9 complex, a novel multisubunit nuclear regulator involved in light control of a plant developmental switch. *Cell* **86:** 115–121

Claret FX, Hibi M, Dhut S, Toda T, Karin M (1996) A new group of conserved coactivators that increase the specificity of AP-1 transcription factors. *Nature* **383:** 453–457

Cope G, Deshaies RJ (2003) COP9 signalosome: a multifunctional regulator of SCF and other cullin-based ubiquitin ligases. *Cell* **114:** 663–671

Cope GA, Suh GS, Aravind L, Schwarz SE, Zipursky SL, Koonin EV, Deshaies RJ (2002) Role of predicted metalloprotease motif of Jab1/Csn5 in cleavage of NEDD8 from CUL1. *Science* **15:** 15

Deng XW *et al* (2000) Unified nomenclature for the COP9 signalosome and its subunits: an essential regulator of development *Trends Genet* **16:** 202–203

Dessau M, Halimi Y, Erez T, Chomsky-Hecht O, Chamovitz DA, Hirsch JA (2008) The *Arabidopsis* COP9 signalosome subunit 7 is a model PCI domain protein with subdomains involved in COP9 signalosome assembly. *Plant Cell* **20:** 2815–2834

Dohmann EM, Kuhnle C, Schwechheimer C (2005) Loss of the CONSTITUTIVE PHOTOMORPHOGENIC9 signalosome subunit 5 is sufficient to cause the cop/det/fus mutant phenotype in *Arabidopsis*. *Plant Cell* **17:** 1967–1978

Dohmann EM, Levesque MP, Isono E, Schmid M, Schwechheimer C (2008a) Auxin responses in mutants of the *Arabidopsis* Cop9 signalosome. *Plant Physiol* **147:** 1369–1379

Dohmann EM, Levesque MP, De Veylder L, Reichardt I, Jurgens G, Schmid M, Schwechheimer C (2008b) The *Arabidopsis* COP9 signalosome is essential for G2 phase progression and genomic stability. *Development* **135:** 2013–2022

Doronkin S, Djagaeva I, Beckendorf SK (2003) The COP9 signalosome promotes degradation of cyclin E during early *Drosophila* oogenesis. *Dev Cell* **4:** 699–710

Dressel U, Thormeyer D, Altincicek B, Paululat A, Eggert M, Schneider S, Tenbaum SP, Renkawitz R, Baniahmad A (1999) Alien, a highly conserved protein with characteristics of a corepressor for members of the nuclear hormone receptor superfamily. *Mol Cell Biol* **19:** 3383–3394

Eckey M, Hong W, Papaioannou M, Baniahmad A (2007) The nucleosome assembly activity of NAP1 is enhanced by Alien. *Mol Cell Biol* **27:** 3557–3568

Fang L, Wang X, Yamoah K, Chen P-l, Pan Z-Q, Huang L (2008) Characterization of the human COP9 signalosome complex using affinity purification and mass spectrometry. *J Proteome Res* **7:** 4914–4925

Feng S, Ma L, Wang X, Xie D, Dinesh-Kumar SP, Wei N, Deng XW (2003) The COP9 signalosome interacts physically with SCF COI1 and modulates jasmonate responses. *Plant Cell* **15:** 1083–1094

Fukumoto A, Tomoda K, Kubota M, Kato JY, Yoneda-Kato N (2005) Small Jab1-containing subcomplex is regulated in an anchorage- and cell cycle-dependent manner, which is abrogated by ras transformation. *FEBS Lett* **579:** 1047–1054

Gajiwala KS, Chen H, Cornille F, Roques BP, Reith W, Mach B, Burley SK (2000) Structure of the winged-helix protein hRFX1 reveals a new mode of DNA binding. *Nature* **403:** 916–921

Groisman R, Polanowska J, Kuraoka I, Sawada J, Saijo M, Drapkin R, Kisselev AF, Tanaka K, Nakatani Y (2003) The ubiquitin ligase activity in the DDB2 and CSA complexes is differentially regulated by the COP9 signalosome in response to DNA damage. *Cell* **113:** 357–367

Gusmaroli G, Figueroa P, Serino G, Deng XW (2007) Role of the MPN subunits in COP9 signalosome assembly and activity, and their regulatory interaction with *Arabidopsis* Cullin3-based E3 ligases. *Plant Cell* **19:** 564–581

Halimi Y, Chamovitz DA (2008) The PCI complexes and ubiquitin proteasome system (UPS) in plant development. In *Intracellular Signaling in Plants*, Z Yang (Ed), Vol 33, pp 273–306. Oxford, UK: Blackwell

Harari-Steinberg O, Chamovitz DA (2004) The COP9 signalosome: mediating between kinase signaling and protein degradation. *Curr Protein Pept Sci* **5:** 185–189

Harari-Steinberg O, Cantera R, Denti S, Bianchi E, Oron E, Segal D, Chamovitz DA (2007) COP9 signalosome subunit 5 (CSN5/Jab1) regulates the development of the *Drosophila* immune system: effects on Cactus, Dorsal and hematopoiesis. *Genes Cells* **12:** 183–195

Hetfeld BK, Helfrich A, Kapelari B, Scheel H, Hofmann K, Guterman A, Glickman M, Schade R, Kloetzel PM, Dubiel W (2005) The zinc finger of the CSN-associated deubiquitinating enzyme USP15 is essential to rescue the E3 ligase Rbx1. *Curr Biol* **15:** 1217–1221

Hofmann K, Bucher P (1998) The PCI domain: A common theme in three multi-protein complexes. *Trends Biochem Sci* **23:** 204–205

Knowles A, Koh K, Wu JT, Chien CT, Chamovitz DA, Blau J (2009) The COP9 signalosome is required for light-dependent TIM degradation and *Drosophila* clock resetting. *J Neurosci* **29:** 1152–1162

Lassot I, Latreille D, Rousset E, Sourisseau M, Linares LK, Chable-Bessia C, Coux O, Benkirane M, Kiernan RE (2007) The proteasome regulates HIV-1 transcription by both proteolytic and nonproteolytic mechanisms. *Mol Cell* **25:** 369–383

Lyapina S, Cope G, Shevchenko A, Serino G, Tsuge T, Zhou C, Wolf DA, Wei N, Deshaies RJ (2001) Promotion of NEDD-CUL1 conjugate cleavage by COP9 signalosome. *Science* **292:** 1382–1385

Lykke-Andersen K, Schaefer L, Menon S, Deng X-W, Miller JB, Wei N (2003) Disruption of the COP9 signalosome Csn2 subunit in mice causes deficient cell proliferation, accumulation of p53 and cyclin E, and early embryonic death. *Mol Cell Biol* **23:** 6790–6797

Ma L, Zhao H, Deng XW (2003) Analysis of the mutational effects of the COP/DET/FUS loci on genome expression profiles reveals their overlapping yet not identical roles in regulating *Arabidopsis* seedling development. *Development* **130:** 969–981

Maytal-Kivity V, Reis N, Hofmann K, Glickman MH (2002) MPN+, a putative catalytic motif found in a subset of MPN domain proteins from eukaryotes and prokaryotes, is critical for Rpn11 function. *BMC Biochem* **3:** 28

Menon S, Chi H, Zhang H, Deng XW, Flavell RA, Wei N (2007) COP9 signalosome subunit 8 is essential for peripheral T cell homeostasis and antigen receptor-induced entry into the cell cycle from quiescence. *Nat Immunol* **8:** 1236–1245

Menon S, Tsuge T, Dohmae N, Takio K, Wei N (2008) Association of SAP130/SF3b-3 with Cullin-RING ubiquitin ligase complexes and its regulation by the COP9 signalosome. *BMC Biochem* **9:** 1

Mori M, Yoneda-Kato N, Yoshida A, Kato J-y (2008) Stable form of JAB1 enhances proliferation and maintenance of hematopoietic progenitors. *J Biol Chem* **283:** 29011–29021

Mundt KE, Liu C, Carr AM (2002) Deletion mutants in COP9/signalosome subunits in fission yeast *Schizosaccharomyces pombe* display distinct phenotypes. *Mol Biol Cell* **13:** 493–502

Naumann M, Bech-Otschir D, Huang X, Ferrell K, Dubiel W (1999) COP9 signalosome-directed c-Jun activation/stabilization is independent of JNK. *J Biol Chem* **274:** 35297–35300

Oh W, Lee EW, Sung YH, Yang MR, Ghim J, Lee HW, Song J (2006) Jab1 induces the cytoplasmic localization and degradation of p53 in coordination with Hdm2. *J Biol Chem* **281:** 17457–17465

Oren-Giladi P, Krieger O, Edgar BA, Chamovitz DA, Segal D (2008) Cop9 signalosome subunit 8 (CSN8) is essential for *Drosophila* development. *Genes Cells* **13:** 221–231

Oron E, Mannervik M, Rencus S, Harari-Steinberg O, Neuman-Silberberg S, Segal D, Chamovitz DA (2002) COP9 signalosome subunits 4 and 5 regulate multiple pleiotropic pathways in *Drosophila melanogaster*. *Development* **129:** 4399–4409

Oron E, Tuller T, Li L, Rozovsky N, Yekutieli D, Rencus-Lazar S, Segal D, Chor B, Edgar BA, Chamovitz DA (2007) Genomic analysis of COP9 signalosome function in *Drosophila melanogaster* reveals a role in temporal regulation of gene expression. *Mol Syst Biol* **3:** 108

Panattoni M, Sanvito F, Basso V, Doglioni C, Casorati G, Montini E, Bender JR, Mondino A, Pardi R (2008) Targeted inactivation of the COP9 signalosome impairs multiple stages of T cell development. *J Exp Med* **205:** 465–477

Richardson KS, Zundel W (2005) The emerging role of the COP9 signalosome in cancer. *Mol Cancer Res* **3:** 645–653

Saccani S, Marazzi I, Beg AA, Natoli G (2004) Degradation of promoter-bound p65/RelA is essential for the prompt termination of the nuclear factor kappaB response. *J Exp Med* **200:** 107–113

Schweitzer K, Bozko PM, Dubiel W, Naumann M (2007) CSN controls NF-kappaB by deubiquitinylation of IkappaBalpha. *EMBO J* **26:** 1532–1541

Seeger M, Kraft R, Ferrell K, Bech-Otschir D, Dumdey R, Schade R, Gordon C, Naumann M, Dubiel W (1998) A novel protein complex involved in signal transduction possessing similarities to 26S proteasome subunits. *FASEB J* **12:** 469–478

Serino G, Deng X-W (2003) The COP9 signalosome: regulating plant development through control of proteolysis. *Annu Rev Plant Biol* **54:** 165–182

Sharon M, Mao H, Erba EB, Stephens E, Zheng N, Robinson CV (2009) Symmetrical modularity of the COP9 signalosome complex suggests its multifunctionality. *Structure* **17:** 31–40

Sikder D, Johnston SA, Kodadek T (2006) Widespread, but non-identical, association of proteasomal 19 and 20 S proteins with yeast chromatin. *J Biol Chem* **281:** 27346–27355

Tenbaum SP, Juenemann S, Schlitt T, Bernal J, Renkawitz R, Munoz A, Baniahmad A (2003) Alien/CSN2 gene expression is regulated by thyroid hormone in rat brain. *Dev Biol* **254:** 149–160

Tomoda K, Kubota Y, Arata Y, Mori S, Maeda M, Tanaka T, Yoshida M, Yoneda-Kato N, Kato JY (2002) The cytoplasmic shuttling and subsequent degradation of p27Kip1 mediated by Jab1/CSN5 and the COP9 signalosome complex. *J Biol Chem* **277:** 2302–2310

Tomoda K, Yoneda-Kato N, Fukumoto A, Yamanaka S, Kato JY (2004) Multiple functions of Jab1 are required for early embryonic development and growth potential in mice. *J Biol Chem* **279:** 43013–43018

Tsuge T, Matsui M, Wei N (2001) The subunit 1 of the COP9 signalosome suppresses gene expression through its N-terminal domain and incorporates into the complex through the PCI domain. *J Mol Biol* **305:** 1–9

Ullah Z, Buckley MS, Arnosti DN, Henry RW (2007) Retinoblastoma protein regulation by the COP9 signalosome. *Mol Biol Cell* **18:** 1179–1186

Wang X, Kang D, Feng S, Serino G, Schwechheimer C, Wei N (2002) CSN1 N-terminal-dependent activity is required for *Arabidopsis* development but not for Rub1/Nedd8 deconjugation of cullins: a structure-function study of CSN1 subunit of COP9 signalosome. *Mol Biol Cell* **13:** 646–655

Wang X, Feng S, Nakayama N, Crosby WL, Irish V, Deng XW, Wei N (2003) The COP9 signalosome interacts with SCF(UFO) and participates in *Arabidopsis* flower development. *Plant Cell* **15:** 1071–1082

Wang X, Li W, Piqueras R, Cao K, Deng XW, Wei N (2009) Regulation of COP1 nuclear localization by the COP9 signalosome via direct interaction with CSN1. *Plant J* [doi: 10.1111/j.1365-313X.2009.03805.x]

Wei N, Deng XW (1992) COP9: a new genetic locus involved in light-regulated development and gene expression in *Arabidopsis*. *Plant Cell* **4:** 1507–1518

Wei N, Deng XW (2003) The COP9 signalosome. *Annu Rev Cell Dev Biol* **19:** 261–286

Wei N, Chamovitz DA, Deng XW (1994) *Arabidopsis* COP9 is a component of a novel signaling complex mediating light control of development. *Cell* **78:** 117–124

Wei N, Serino G, Deng XW (2008) The COP9 signalosome: more than a protease. *Trends Biochem Sci* **33:** 592–600

Wolf DA, Zhou C, Wee S (2003) The COP9 signalosome: an assembly and maintenance platform for cullin ubiquitin ligases? *Nat Cell Biol* **5:** 1029–1033

Wu JT, Chan YR, Chien CT (2006) Protection of cullin-RING E3 ligases by CSN-UBP12. *Trends Cell Biol* **6:** 362–369

Yan J, Walz K, Nakamura H, Carattini-Rivera S, Zhao Q, Vogel H, Wei N, Justice MJ, Bradley A, Lupski JR (2003) COP9 signalosome subunit 3 is essential for maintenance of cell proliferation in the mouse embryonic epiblast. *Mol Cell Biol* **23:** 6798–6808

Zhang XC, Chen J, Su CH, Yang HY, Lee MH (2008) Roles for CSN5 in control of p53/MDM2 activities. *J Cell Biochem* **103:** 1219–1230

Zhou C, Seibert V, Geyer R, Rhee E, Lyapina S, Cope G, Deshaies RJ, Wolf DA (2001) The fission yeast COP9/signalosome is involved in cullin modification by ubiquitin-related Ned8p. *BMC Biochem* **2:** 7

Zhou C, Wee S, Rhee E, Naumann M, Dubiel W, Wolf DA (2003) Fission yeast COP9/signalosome suppresses cullin activity through recruitment of the deubiquitylating enzyme Ubp12p. *Mol Cell* **11:** 927–938

Daniel A. Chamovitz