The COP9 Signalosome: Mediating Between Kinase Signaling and Protein Degradation

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Abstract: The COP9 Signalosome (CSN), a highly conserved eight-subunit complex, is found in all higher eukaryotes. It contains eight core subunits, named CSN1 – 8, in order of decreasing molecular weight. The CSN is structurally similar to the regulatory lid of 26S proteasome and the eukaryotic translation initiation factor eIF3. CSN is also now known to play an essential role in signaling processes controlling many aspects of plant and Drosophila development. Taken together, the various genetic studies demonstrate that the CSN is involved at the nexus between multiple signal inputs and a variety of downstream regulatory cascades controlling specific aspects of cellular differentiation. Research in various organisms has converged onto the notion that CSN is biochemically linked to ubiquitin-dependent protein degradation. Other proposed roles for the CSN include regulating eIF3 and kinase signaling. CSN is itself is both a target for kinase activity and associates with and coordinates activity of kinases. CSN-associated kinases. This kinase activity further regulates the ubiquitin-dependent degradation of various transcription factors. This review concentrates on the proposed activity of the CSN as a regulator of protein phosphorylation.

INTRODUCTION

The COP9 Signalosome (CSN), a highly conserved eight-subunit complex, was first identified in *Arabidopsis* as an essential regulator of light signal transduction about 10 years ago (reviewed in [1, 2]). It contains eight core subunits, named CSN1 – 8, in order of decreasing molecular weight [3]. The CSN is structurally related to the regulatory lid of 26S proteasome and the eukaryotic translation initiation factor eIF3 (reviewed in [4]). Common domains, the PCI motif (found in CSN1, 2, 3, 4, 7, and 8) and MPN motif (found in CSN5 and 6), found almost exclusively in subunits of these complexes suggest a common evolutionary origin. Indeed, subunits of the COP9 signalosome are paralogous in a one-to-one relationship to subunits of the proteasome lid, whereas eIF3 is more distantly related. Moreover, biochemical studies indicate physical interactions among these complexes [5-8].

However, CSN is also now known to play an essential role in signaling processes controlling many aspects of plant development, [9, 10] including cellular responses to plant hormones (e.g. auxin, [11]), environmental stress, and fungal and viral pathogens [12, 13]. The CSN has also been identified in mammals [14, 15], *Drosophila* [16], fungi [17], and fission yeast [18, 19], while *S. cerevisiae* contains a smaller related complex [20, 21]. The CSN is essential for development of *D. melanogaster* [16], and is involved in multiple developmental programs including oogenesis, axonal guidance, and steroid hormone signaling [22-24]. Taken together, the various genetic studies demonstrate that the CSN is involved at the nexus between multiple signal inputs and a variety of downstream regulatory cascades controlling specific aspects of cellular differentiation.

Research in various organisms has converged onto the notion that CSN is biochemically linked to ubiquitin-dependent protein degradation. The CSN regulates the activity of SCF-type E3 ubiquitin complexes by mediating the cleavage of NEDD8 from the cullin subunit [25]. In *Arabidopsis*, SCF-mediated degradation of PSIAA6 (i.e. a regulatory protein of auxin-response pathway) is compromised in *csn5* mutant plants. The mode of action of the CSN in regulating protein stability is probably complex, and indeed both intrinsic deneddylation and deubiquitination activities for the COP9 signalosome have been detected [26-28].

The connection of the CSN to ubiquitin-mediated protein degradation has been reviewed extensively [2, 29, 30]. Other proposed roles for the CSN include regulating eIF3 [4] and kinase signaling [31]. Kinase signaling and ubiquitin-mediated protein degradation are obviously not mutually exclusive, and indeed kinase signaling often regulates protein degradation. This review concentrates on the proposed activity of the CSN as a regulator of protein phosphorylation.

CSN-ASSOCIATED KINASES

When the CSN was first purified from mammalian cells, it copurified with a serine/threonine kinase activity against several important signaling molecules including I B, p105 and c-Jun [14]. Other CSN-associated proteins, such as p53, $p27^{kip1}$ and ICSBP, are also phosphorylated by the CSN associated kinases (reviewed in [31]). This CSN-associated kinase activity is inhibited by curcumin, a tumor suppressor and effector of AP-1 activation [32]. Recently, three different kinases were identified as co purifying with CSN from human cell culture.

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The first identified CSN-associated kinase was inositol 1,3,4-trisphosphate 5/6-kinase (5/6-kinase) [33]. 5/6-kinase was shown to interact with the CSN via CSN1, to phosphorylate some of the same substrates as those of the CSN-associated kinase activity, and 5/6-kinase is also inhibited by curcumin [34]. Furthermore, over-expression of CSN1 inhibited 5/6-kinase activity in cell culture studies. On the other hand, over-expression of 5/6-kinase led to an increase in the levels of free forms of CSN5, but not in the level of the CSN suggesting a possible regulatory mechanism for CSN5 activity in 5/6-kinase signaling.

Dubiel and co-workers have recently shown that the kinase activity that they originally identified in human erythrocytes is unique from the 5/6 kinase described above. They identified two protein kinases, CK2 and the PKD, that both associate with CSN and phosphorylate CSN subunits (see below) [35]. Furthermore, these two kinases act on both c-Jun and p53 and are inhibited by curcumin. They are probably recruited by the CSN in order to regulate ubiquitin conjugation of c-Jun and p53.

THE CSN AS A KINASE TARGET

In addition to its role in regulating kinases and/or kinase substrates, the CSN itself has been reported to be phosphorylated. Two dimensional gel electrophoresis of purified human CSN, followed by mass spectrometry, showed that all subunits are found in multiple forms with differing pI's, suggesting different grades of phosphorylation [32]. Among these, CSN2 and CSN7 especially showed multiple forms. This observation is in line with the fact that several CSN subunits contain canonical MAP kinase kinase (MAPKK) activation loop motifs [14]. Arabidopsis CSN7 contains several putative phosphorylation sites and indeed is phosphorylated *in vitro* by plant extracts [36]. The CSN-associated kinases CK2 and PKD bind CSN3 and phosphorylate CSN7, while CK2 also binds CSN7 and phosphorylates CSN2 [35]. Taken together, the associated kinase activity of the CSN and the post translational phosphorylation of CSN subunits, support the idea that the CSN is a central component of kinase-mediated signal transduction pathways.

CSN-DEPENDENT PHOSPHORYLATION REGU-LATES THE UBIQUITIN-DEPENDENT DEGRA-DATION OF TRANSCRIPTION FACTORS

c-Jun is Stabilized by CSN-Dependent Phosphorylation

c-Jun is a component of the AP-1 transcription complex, which is a regulator of many cytokine genes and is activated in response to environmental stress, radiation, and growth factors [37]. The c-Jun protein consists of a C-terminal basic region-leucine zipper (B-ZIP) DNA binding domain and an N-terminal transcriptional activation domain. Phosphorylation at the N-terminal activation domain, including Ser-63 and Ser-73, by JNK (c-Jun N-terminal kinase) under stress conditions, leads to the stabilization of c-Jun against the proteasome, leading to an elevation of AP-1 transcription activity, while phosphorylation of the C-terminal region down regulates c-Jun transcription ability.

The CSN appears to work as a positive regulator of AP-1 signaling by stabilizing c-Jun. Curcumin and other inhibitors known to control CSN associated CK2 and PKD activity induce the degradation of c-Jun in HeLa cells, suggesting that the kinase activity has a role in stabilizing c-Jun. The CSN associated kinase activity is specific for the amino-terminal activation domain of c-Jun [14], while JNK phosphorylates both sites of c-Jun, leading to the speculation that the CSN is specific for transcription regulation. Another observation supporting the CSN-dependant regulation of c-Jun signaling pathway is over-expression of hCSN2 and subsequent increase in overall CSN levels in HeLa cells resulted in c-Jun stabilization and increased AP-1 activity [38]. Taken together, these results lead to a model whereby the CSN kinase activity stabilizes c-Jun from degradation by the proteasome.

P53 is Destabilized by CSN-Dependent Phosphorylation

While CSN-dependent phosphorylation appears to stabilize c-Jun, CSN-dependent phosphorylation has an opposite effect on the tumor suppressor p53 – resulting in its degradation [39]. Under normal growth conditions p53 levels remain low due to continuous degradation by the proteasome. p53 becomes activated and stabilized when cells experience a variety of stress conditions. p53 is also a substrate of CSN-associated kinases CK2 and PKD. The CSN specifically mediates the phosphorylation of Ser149, Thr150 and Thr155 in the DNA-binding domain of p53. Mutational analysis indicates that Thr155 is the most important for p53 stability. This CSN-directed phosphorylation of Thr155 participates in maintaining low levels of p53 under normal conditions by increasing the binding of p53 to the ubiquitin ligase Mdm2, and thereby targeting the protein to degradation [40].

As the CSN-associated kinases activity appears to have opposite effects on two central transcription factors, and as these transcription factors themselves are known to be under intricate control mechanisms, the complexity of CSN function becomes obvious. The CSN-associated kinase was shown to be constitutively active in red blood cells, HeLa, HL-60 and MCF-7 cells [14, 40]. Whereas JNK is normally inactive and must be activated by a MAP kinase pathway [41], under these conditions, c-Jun would be continuously phosphorylated. It is not surprising then that reports have indicated crosstalk between JNK- and CSN-dependent c-Jun signaling. For example, transient expression of *Csn1* inhibits JNK and consequently Jun-dependent signaling [42, 43].

Both c-Jun and p53 bind CSN5 and are phosphorylated by CSN associated kinases. It is tempting to conjure up a model where the two transcription factors compete for interaction with the complex (Fig. **1**). Indeed CSN-directed p53 phosphorylation can be inhibited by c-Jun *in vitro* [44]. Under normal growth conditions, continuous CSN-dependent phosphorylation of p53 appears to cause its degradation by the ubiquitin system and keeps the tumor suppressor at low levels. At the same time, c-Jun, and perhaps specific AP-1 forms, are stabilized, supporting normal cell growth and differentiation. The c-Jun/p53 balance, controlled at least in part, by the CSN, is disturbed in many tumor cells. For
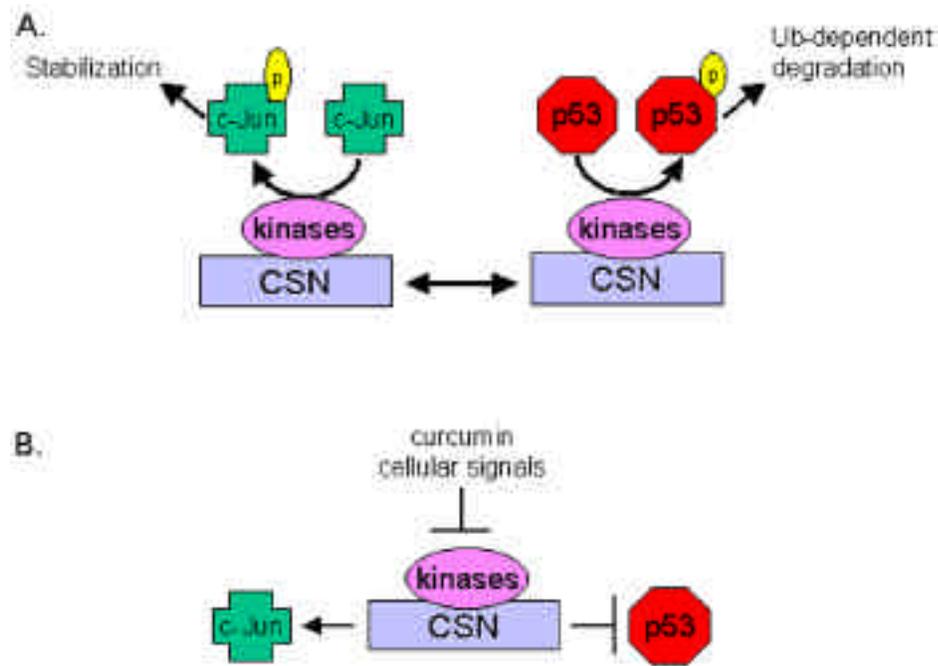
Fig. (1). The CSN is a negative regulator of p53 and a positive regulator of c-Jun. A model showing the CSN-associated kinase activity inhibiting p53 by promoting its degradation, while stabilizing and activating c-Jun. There is equilibrium of CSN kinase activity under normal growth conditions (A). Under specific cellular conditions such as found in certain tumors, or in the presence of the kinase inhibitor curcumin, the CSN-associated kinase activity is inhibited, releasing the inhibition on p53, while inducing the inhibition of c-Jun (B). According to this model, the inhibition of CSN kinase activity leads to increased apoptosis (through the stabilization of p53), and increased angiogenesis (through the degradation of c-Jun) (based on [44]). Lines ending in arrow heads – activation; Lines ending in a perpendicular line – inhibition. p – phosphate; Ub – ubiquitin.

example, in cervix carcinoma cells, p53 degradation is accelerated by the papilloma virus protein E6, which activates the p53. This correlates with increased c-Jun levels, which induces enhanced VEGF (vascular endothelial growth factor) production in these cells [44]. The basal VEGF production in HeLa and HL-60 cells is due mostly to the activity of c-Jun, which is stabilized by the CSN-specific phosphorylation that is sensitive to curcumin. p53 inhibits VEGF production, possibly by competition with c-Jun for CSN-specific phosphorylation. Therefore, in tumor cells where JNK signaling pathway is not active, CSN might be a potentially important target for tumor therapy.

THE CSN AS A GENERAL REGULATOR OF TRANSCRIPTION FACTORS?

In addition to c-Jun and p53, the CSN and/or its subunits has been reported to interact with a variety of other signaling molecules and transcription factors. CSN-dependant phosphoryation has been reported to be involved in the regulation of some of these. For example, the interferon consensus sequence-binding protein (ICSBP) is a constitutively expressed transcription factor in hematopoietic cells that confers resistance to pathogenic infections and acts as a tumor suppressor gene essential for proper differentiation of myeloid cells. Its transcriptional activity

and specificity is mediated via the interaction with various interferon regulatory factors (IRFs). ICSBP associates directly with CSN2 and is phosphorylated by a CSN-associated kinase at a unique residue in the domain that interacts with the IRFs. This phosphorylation is essential for interaction with IRF-1, and thus necessary for the repressive action of ICSBP on IRF-1. Thus the CSN-associated phosphorylation enhances the repressor activity of ICSBP upon pathogenic infection of immune cells [46]. It is not clear yet if this activity is related to any proteasome-related function.

CONCLUSION: THE CSN-DEPENDANT PHOSPHO-RYLATION AS A GENERAL REGULATOR OF SIGNALING?

The CSN has noted activities in regulating the E3 ubiquitin ligase activity controlling the degradation of central signaling molecules such as I B, $p27^{kip1}$, cyclin E and HY5. However it is important to consider that these proteins are also phosphorylated, and thus the CSN-dependant degradation of these and other signaling proteins may also involve CSN-associated kinase activities. For example, the CSN-associated kinase was active against I B [14], and CSN3 was reported to directly bind the regulatory -subunit of I B-kinase *in vitro* [47]. However a direct connection

between the CSN, associated kinases, and I B ubiquitin-dependent degradation has yet to be shown.

An additional level of complexity emerges if one considers that protein ubiquitination is now know to also have a nondestructive regulatory role in kinase signaling [48]. As the CSN was recently shown to associate with and regulate the activity of the deubiquinating enzyme Ubp12p [49], it is plausible that another function of the CSN lies in regulating kinase signaling through deubiquinition of signaling molecules, while simultaneously regulating the kinases themselves. An emerging view of the CSN thus has it acting as a master docking station, coordinating between specific kinases, substrates, E3 ligases and the proteasome (Fig. **2**). Recently the CSN has been shown in plants to associate *in vivo* with both the proteasome and specific SCF E3 ligase complexes [50]. Whether this mega complex also contains the kinases and their substrates remains to be shown.
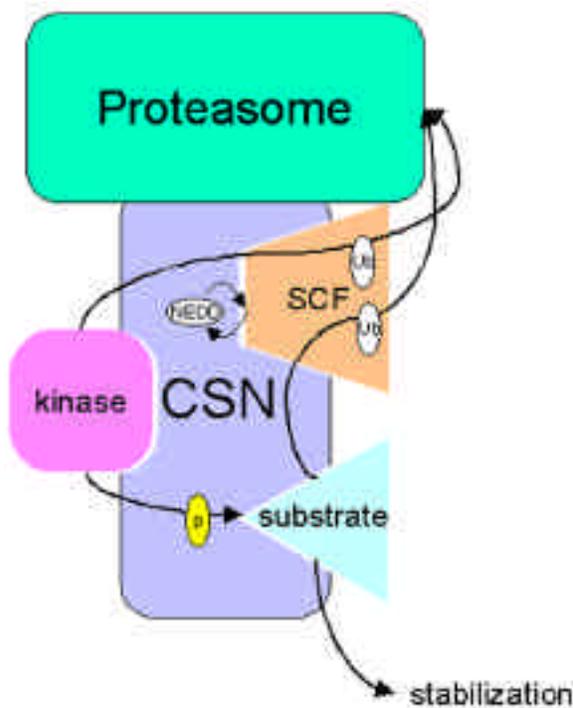

Fig. (2). The CSN as a master docking station. In this model, the CSN would organize a kinase, its substrate, and a specific SCF complex that would ubiquitinate the phosphorylated substrate, and pass it on for degradation by the proteasome. For certain substrates, the phosphorylation would lead to substrate stabilization rather then degradation. Furthermore, the kinases themselves may be marked by the SCF for degradation [51]. The SCF would be further regulated by the deneddylation activity in the CSN. The CSN also could regulate deubiquitination of additional substrates (not shown in the model). Arrows indicate protein interactions mediated through the CSN. SCF – Skip1-Cullin-F-Box complex; p – phosphate; Ub – ubiquitin.

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