Cop9 Signalosome Subunits

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Contributor: Amit Singh

The COP9 (Constitutive photomorphogenesis 9) signalosome (CSN) is a highly conserved protein complex that influences several signaling and developmental processes. The COP9 signalosome consists of eight subunits, among which two subunits, CSN5 and CSN6, contain an Mpr1/Pad1 N-terminal (MPN) domain and the remaining six subunits contain a proteasome, COP9 signalosome, and initiation factor 3 (PCI) domain. In plants, each MPN subunit is encoded by two genes, which is not the case in other organisms.

Keywords: COP9 signalosome ; CSN subunit ; hypomorphic mutants ; biotic stress ; abiotic stress ; hormonal signaling

1. Introduction

The COP9 (Constitutive photomorphogenesis 9) signalosome (CSN) is an eight-subunit protein complex which is conserved throughout evolution. It was first identified in Arabidopsis as a repressor of light-regulated development [1][2]. Regulation of protein degradation by deneddylation of cullin-RING E3 ligase (CRL) is the most studied biochemical function of CSN. CSN subunits are ordered from CSN1 to CSN8 on the basis of their descending molecular mass. Two of the subunits, CSN5 and CSN6, possess an Mpr1/Pad1 N-terminal (MPN) domain and the remaining six contain a proteasome, COP9 signalosome, and initiation factor 3 (PCI) domain. Both MPN and PCI domains are also present in lid subunit of 26S proteasome and eukaryotic translation initiation factor 3 (eIF3) [3].

Viable CSN mutants with reduced activity demonstrate that CSN plays a major role in the growth and development of the plant. RNA silencing of CSN5 subunit in Arabidopsis reduces auxin signaling [4]. Silencing of CSN3 and CSN6 subunits reveal the role of CSN in floral development. The various floral phenotypes (short stamens, less pollen grains and reduced number of petals) in these strains are suppressed by overexpression of the F-box protein UFO (unusual floral organs) hence, CSN is also linked with SKP1, Cullin and F-box-containing protein $(SCF)^{UFO}$ [5]. Further, it was found that Floral B domain transcription factor APETALA3 is down-regulated in strains with compromised CSN1 function [6]. CSN also regulates defense against pathogens by playing a crucial role in *N* gene-mediated resistance to tobacco mosaic virus [7], and in jasmonic acid-dependent plant defense responses [8]. Studies in human cells showed that the CSN also plays a vital role in the repair of double-strand breaks by getting recruited to double-strand break sites in a neddylation-dependent manner [9], and knockdown of CSN results in a defect of nucleotide excision repair [10]. In Arabidopsis, CSN also plays an important role in the DNA repair mechanism. *csn7* null mutants are tolerant to ultraviolet (UV)-C treatment due to constitutive expression of UV-induced genes as CSN negatively regulates ribonucleotide reductase activity [11]. Thus, the role of CSN in plants is not only limited to hormonal signaling and defense response but also has a critical impact in many cellular, developmental processes [12] as well as in cell cycle progression [13].

2. Role of CSN in Hormonal Signaling

The CSN plays a central role in hormonal signaling in plants, with the auxin-mediated response being a highly studied paradigm. The interaction of CSN with CRL E3 ubiquitin ligase was first identified in the study that focused on the role of CSN in SKP1, Cullin and F-box-containing protein- Transport Inhibitor response (SCF^{TIR1}) mediated auxin response [4]. In response to auxin perception, SCF^{TIR1} mediates degradation of the auxin/3-indoleacetic acid (AUX/IAA) transcriptional repressors. A transgenic version of the *Pisum*-AUX/IAA protein IAA6 introduced in Arabidopsis was partially stabilized in a transgenic Arabidopsis strain expressing a CSN5 antisense construct. This partial stabilization of psIAA6 impaired auxin signaling in these plants [4]. In addition to interacting with SCF^{TIR1}, the CSN also interacts with other CRLs such as the SCF specific for jasmonic acid signaling, SCF^{COI1}, gibberellic acid signaling, SCF^{SLY1}, flower development, SCF^{UFO}, as well as several other E3 ligases [6][14][15].

Similar to AUX/IAA protein stabilization in the absence of auxin, it was also found that DELLA proteins (repressor of gibberellic acid signaling) accumulate at a higher amount in the absence of gibberellic acid (GA). DELLA proteins interact with phytochrome interacting factor 3 (PIF3) and prevent the binding of PIF3 to the target gene promoters. In the presence

of GA, DELLA proteins are degraded releasing the PIF3 from the negative suppression of DELLA protein [16]. The significance of CSN in ethylene signaling was shown through the analysis of the Arabidopsis *eer5-1* mutant. These mutants have enhanced ethylene response in etiolated seedlings and are hypersensitive to ethylene. *eer5-1* is formed by amino acid substitution in a PCI-Associated Module (PAM). Enhanced ethylene response protein 5 (EER5) interacts with the C-terminal of ethylene-insensitive protein 2 (EIN2) and CSN, forming a bridge between EIN2 and transcriptional repressor during ethylene signaling [17]. CSN also regulates seed germination by promoting the degradation of RGL2 and ABI5 protein [18]. RGL2 is a GA signaling repressor whereas ABI5 is an effector of ABA response. *csn* mutants are impaired in the timely removal of RGL2 and ABI5. Arabidopsis contains five DELLA proteins (RGL1, RGL2, RGL3, GAI, and RGA) which negatively regulates the GA pathway; among these RGA and GAI functions mostly in dark to inhibit seed germination whereas, RGL2 works in light [19]. Seed germination inhibition by RGL2 is seed coat-dependent which probably involves CSN. RGL2 is regulated by SCF^{SLY1} E3 ligase-mediated protein degradation through proteasome [20].

3. Importance of CSN in Biotic and Abiotic Stress

Together with hormonal signaling, the CSN also plays a crucial role in plant defense response. The role of CSN in plant defense becomes evident during the studies of tomato defense response using tobacco rattle virus (TRV) based virus-induced gene silencing (VIGS). Expression of CSN5 was reduced using VIGS, and as a result, CSN5-VIGS plants were 50% stunted compared to control VIGS plants [8]. Reduced synthesis of jasmonic acid (JA) in CSN5-VIGS plants result in poor defense against *Manduca Sexta* and *Botrytis cinerea*. This shows the importance of CSN and SCF^{COI1} interaction, which is not only essential for plant development but also for plant defense. Although CSN5-VIGS plants show reduced resistance to herbivorous *M. sexta* and necrotrophic fungus *B. cinerea*, its susceptibility to tobacco mosaic virus (TMV) remains unaltered. Study of gene expression profiles revealed that JA-dependent wound genes are positively regulated by CSN, while salicylic acid (SA)-responsive pathogenesis related (PR) genes are negatively regulated by CSN. PR genes are constitutively high in CSN-VIGS plants which makes it less susceptible to TMV. This study showed that CSN is important for JA synthesis but not for the synthesis of SA [8]. Another study showed that gemini viral C2 protein compromised CSN activity on CUL1. C2 protein only affects CUL1 but not other cullins (CUL3 and CUL4) and this fact was confirmed by studying levels of neddylated cullin. The transgenic line containing C2 protein showed a higher level of neddylated CUL1 whereas, levels of CUL3 and CUL4 neddylation remains unaffected. Further, C2 protein interacts with CSN5 and alters several hormonal pathways (including auxin, JA, GA, ethylene, and ABA) which are regulated by CUL1-based SCF ubiquitin E3 ligases. Stabilization of GAI (substrate of SCF^{SLY1}) in the transgene containing C2 protein validates the impairment of SCF function. Transcriptomic data of this transgenic plant reveals that jasmonate response is most severely affected due to the impairment of SCF function by C2. Geminivirus infection gets diminished by exogenous application of JA indicating that inhibition of jasmonate response is important for infection [21].

Plants perceive biotic and abiotic signals differently. Biotic signals are receptor-mediated, whereas abiotic signals are mostly sensed by perturbation of the plasma membrane [22]. Plants utilize numerous hormonal signaling pathways in coping with abiotic stress. ABA and ethylene are the most frequent stimulant of abiotic stress which cross-talk with SA, JA, and auxin to change the gene expression profile for adaptive plant response against abiotic stress [23][24]. The role of CSN in abiotic stress is further supported by the study of differentially expressed proteins in heat-tolerant and heat-sensitive lines of rice. With the help of iTRAQ LC-MS/MS, it was found that 38 proteins are differentially expressed between heat-tolerant and heat-sensitive lines of rice. Among these, calcium-dependent protein kinase, COP9 signalosome, and bHLH transcription factor were up-regulated in heat-tolerant lines, suggesting further effects downstream during high night temperature response [25].

CSN hypomorphic mutants provided an important tool to understand the role of each CSN subunit in plant development, which could not have been possible with *csn* null mutants. Complete loss of any CSN subunit leads to seedling lethality in early stage, which obstructs further analysis of the role of subunits in plant growth and development. Phenotypic differences of CSN hypomorphic mutants on the basis of deneddylation, AUX/IAA degradation and auxin response have been represented in the Figure 1 [26]. In Arabidopsis, CSN5 is encoded by two genes CSN5A and CSN5B. T-DNA insertion lines in CSN5A and CSN5B produces *csn5a-1* (T-DNA inserted in the exon of At1g22920), *csn5a-2* (T-DNA inserted in the intron of At1g22920) and *csn5b-1* (T-DNA inserted in Atg71230). *csn5a-1* exhibits hyper-neddylation of CUL1, CUL3, and CUL4, whereas *csn5b-1* shows normal cullin neddylation similar to wild type. Using *csn5a-1* it was found that CSN5A is required for the recovery of AUX/IAA repressor levels following recurrent heat stress to regulate auxin homeostasis in Arabidopsis [27]. Further, it was identified that CSN5A is necessary for resetting transcriptional stress memory after recurrent heat stress in Arabidopsis [28].

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