COP9 signalosome: a provider of DNA building blocks

Nielsen, Olaf

Published in: Current Biology

Publication date: 2003

Document version
Early version, also known as pre-print

Citation for published version (APA):
COP9 Signalosome: A Provider of DNA Building Blocks

Olaf Nielsen

In fission yeast, the COP9 signalosome is required to activate ribonucleotide reductase for DNA synthesis. This is mediated via the ubiquitin ligase Pcu4, activation of which leads to degradation of the scaffold protein Spd1, which anchors the small ribonucleotide reductase subunit in the nucleus away from the large subunit in the cytoplasm.

The COP9 signalosome (CSN) is a highly conserved multiprotein complex, subunits of which show sequence similarity to components of the 19S proteasome lid complex. The CSN was originally identified as a negative regulator of photomorphogenesis in plants, and it has subsequently been found in mammalian cells, flies and fission yeast [1]. Genetic analyses have implicated the CSN in the control of developmental processes [2] and cell-cycle progression [3]. Although the precise biochemical function of the CSN has not yet been elucidated, a number of studies have suggested that it acts as a negative regulator of those ubiquitin ligases that have cullins as essential subunits.

It has been shown in vitro that purified CSN can cleave Nedd8 from cullins [4]. The addition to a cullin subunit of this ubiquitin-like protein — neddylation — is known to stimulate the respective ubiquitin ligase activity. In fission yeast cells lacking CSN subunits, the cullins Pcu1 and Pcu3 accumulate exclusively in their neddylated state [5,6]. Furthermore, both human and fission yeast CSN co-purify with a de-ubiquitinating activity [7]. A recent paper from Tony Carr’s group [8], however, reports that the two CSN subunits Csn1 and Csn2 are positive regulators of the ubiquitin ligase containing the cullin Pcu4 in fission yeast. The consequence of this activity is degradation of the small nuclear protein Spd1, previously found as a negative regulator of S-phase progression [9]. This effect can now be understood as an inhibition of the enzyme ribonucleotide reductase, which is responsible for the synthesis of the deoxynucleotide triphosphate (dNTP) building blocks of DNA.

Active ribonucleotide reductase is a heterotetramer, consisting of two large subunits (Cdc22) and two small subunits (Suc22). In the presence of the anchoring protein Spd1, Suc22 is kept in the nucleus, whereas Cdc22 is pan-cellular (Figure 1A). Thus, only a small fraction of the ribonucleotide reductase subunits is engaged in active complexes. This is the situation outside S-phase, where the need for dNTPs is limited. As cells enter DNA replication, Spd1 is ubiquitinated and degraded by the proteasome, and Suc22 is transported to the cytoplasm where it can form active complexes with Cdc22 (Figure 1B).

Mutant fission yeast cells lacking the CSN subunits Csn1 or Csn2, or the cullin Pcu4, fail to degrade Spd1 upon entry into S-phase, and hence Suc22 remains in the nucleus. This causes slow S-phase progression and activation of the DNA replication checkpoint kinase Cds1, presumably as a result of starvation for dNTPs. The S-phase phenotypes of csn1 and csn2 deletion mutants are completely reverted by either overproduction of Suc22 or deletion of the spd1+ gene, showing that they are indeed caused by a failure to activate ribonucleotide reductase.

Liu et al. [8] also found that the activation of ribonucleotide reductase by Csn1 and Csn2 is important when G2 cells experience DNA damage. Thus, csn1 and csn2 mutants are sensitive to UV and γ-radiation [3]. But unlike the activation at S-phase, this response requires the ATM-like checkpoint kinase Rad3 and its downstream effector kinase Chk1 (Figure 1C). The radiation sensitivity of csn1 and csn2 mutants is suppressed by deletion of the spd1+ gene or overproduction of Suc22, again arguing that the phenotype is caused by insufficient dNTP levels [8]. It is unclear how CSN-mediated degradation of Spd1 is triggered. Liu et al. [8] found no evidence of phosphorylation of the protein, and favour a model in which CSN and Pcu4 are activated to ubiquitinate Spd1. At S-phase this could be accomplished by cyclin-dependent kinase activity, whereas the Chk1 protein kinase may be responsible for the activation following DNA damage [8].

Why is ribonucleotide reductase so tightly regulated? In the distantly related budding yeast, dNTP levels are also elevated in response to DNA damage, but apparently by a different mechanism as this species appears to have lost the CSN during evolution [10]. Elevated nucleotide pools may be important for repair DNA synthesis, which involves conventional as well as special ‘trans-lesion’ synthesis polymerases. Down-regulation of ribonucleotide reductase outside S-phase also seems to be a general feature [11]. Perhaps low cytoplasmatic dNTP levels help to reduce the activity of transposons and viruses that bring along their own polymerase.

Curiously, while csn1 and csn2 mutants display slow S-phase progression and radiation sensitivity, fission yeast cells deleted for the other CSN subunits show no discernible phenotype [5]. The effect of mutating the different CSN subunits is mirrored by the neddylation-status of Pcu4; only cells depleted for Csn1 or Csn2 appear to be defective in de-neddylation of Pcu4 [8]. This is in contrast to the situation for the other two fission yeast cullins, Pcu1 and Pcu3, which are constitutively neddylated when any CSN subunit is deleted [5,6]. This suggests that the entire CSN regulates the cullins Pcu1 and Pcu3, in a way that remains to be elucidated (note that pcu1+ is an essential gene). The cullin Pcu4 on the other hand is regulated mainly by the CSN subunits Csn1 or Csn2, and this interaction
activates ribonucleotide reductase via ubiquitination and consequent degradation of Spd1.

It may appear as a paradox that cells deficient for Csn1 or Csn2 are unable to de-neddylate Pcu4 — and so would be expected to have increased activity of Pcu4’s associated ubiquitin ligase — yet they cannot perform Pcu4-dependent ubiquitination of Spd1. This suggests that Csn1 and Csn2, in addition to inhibiting Pcu4, are required to modulate the spatial and temporal activity of this ubiquitin ligase, perhaps by functioning as an assembly platform as some inhibitors of cyclin-dependent kinases are known to do [12].

In this context it is noteworthy that Pcu1 and Pcu3, which require the entire CSN — at least for de-neddylation — are expected to form canonical ‘SCF’ ubiquitin ligase complexes via Skp1-interacting domains, whereas Pcu4, activity of which depends on only Csn1 and Csn2, does not appear to have such a domain [8]. Pcu4 may, therefore, function as a ubiquitin ligase in a different context. It is unclear whether Csn1 and Csn2 induce Pcu4-dependent ubiquitination of Spd1 when integrated into the CSN, or whether they form a subcomplex on their own. When Liu et al. [4] purified a tagged form of Csn2 from cells, they recovered the entire CSN complex together with Pcu4 [8], supporting the former possibility. Curiously, neither Pcu1 nor Pcu3 were recovered in these complexes. Clearly, additional biochemical analyses are required to clarify this issue.

Interestingly, a protein Ddb1 also co-purifies with the CSN from fission yeast cells [8]. This protein was recently identified as the fission yeast homolog of the human protein p127/DDB1 [13], which together with the WD-repeat protein p48/DDB2, binds to UV-damaged DNA and is implicated in general genomic nucleotide excision repair. Xeroderma pigmentosum group E patients, which are hypersensitive to UV-light, lack this complex as a result of mutations in the gene for p48 [14]. Fission yeast ddb1 mutants show slow S-phase progression [13] and presumably this is also caused by failure to activate ribonucleotide reductase.

Groisman et al. [15] very recently showed that human DDB1 similarly participates in a large protein complex that, in addition to DDB2, contains the entire CSN, cullin 4A and the Roc1 protein. When human cells are exposed to UV-radiation, the CSN leaves this complex, which then becomes tightly associated with chromatin. Concomitantly, cullin 4A is activated by neddylation and presumably this leads to ubiquitination of chromatin-bound substrates important for the execution of nucleotide excision repair. At later time points the CSN re-associates with the complex, and cullin 4A is de-neddylated again. Removing CSN subunit 5 by RNA interference (RNAi) significantly decreased nucleotide excision repair, suggesting a positive role for CSN in ubiquitination which would be consistent with the assembly platform model discussed above.

Groisman et al. [15] also found a similar protein complex that contains another WD-repeat protein, CSA, instead of DDB2 [15]. The gene for this protein is mutated in Cockayne syndrome group A patients, who are defective in transcription-coupled nucleotide excision repair. In this case, however, the CSN is recruited to the complex following UV-irradiation, thereby inactivating cullin 4A, suggesting that suppression of ubiquitination is important for transcription-coupled nucleotide excision repair. Clearly, a view of the CSN as a hit man armed with cullin ubiquitin ligases is emerging: CSN brings the ubiquitin ligase
to its targets, where the ubiquitination can be switched on and off again in response to specific stimuli.

References


