The fission yeast COP9/signalosome is involved in cullin modification by ubiquitin-related Ned8p

ABSTRACT

Taken together, our data suggest that CSN is a global regulator of Ned8p modification of multiple cullins and potentially other proteins involved in cellular regulation.

INTRODUCTION

Background The cullin/RING family of ubiquitin ligases comprises a multifunctional set of enzymes controlling the stability and activity of cell cycle regulators, transcription factors, and signaling proteins (reviewed in ref.). Among the best studied examples of cullin/RING enzymes are the so-called SCF complexes. SCF complexes share homologues of the core components cullin 1 (CUL1), SKP1, and the RING domain protein HRT1/RBX1/ROC1, which associate with different F-box proteins and ubiquitin-conjugating enzymes. F-box proteins specifically bind substrates, following their phosphorylation in response to activation of various signaling pathways. Although substrate phosphorylation is one major determinant of SCF-mediated ubiquitylation, covalent modification of the CUL1 subunit through attachment of the ubiquitin-related peptide NEDD8 also regulates SCF activity. NEDD8 modification stimulates SCF-dependent substrate ubiquitylation in vitro, and mutant CUL1 resistant to NEDD8 modification is defective in vivo. In addition, fission yeast mutants deficient in the enzymes that attach Ned8p, or in the ned8 gene itself, are inviable. In addition to CUL1, the human genome contains at least five other cullins, CUL 2, 3, 4A, 4B, and 5. This diversity is partially recapitulated in S. pombe, which encodes pcu3 and pcu4, homologues of human CUL3 and CUL4, for which there are no direct correlates in budding yeast. All human cullins interact with HRT1/RBX1/ROC1, are modified by NEDD8, and have ubiquitin ligase activity in vitro. Similarly, the fission yeast cullins Pcu1p and Pcu4p are neddylated, and neddylation is critical for their function in vivo.. While it is clear that neddylation affects the activity of cullin/RING complexes, mechanisms controlling this modification are just emerging. A recent study by Lyapina et al. uncovered a novel connection between the COP9/signalosome (CSN) and cullin neddylation. CSN is a highly conserved multiprotein complex consisting of eight subunits, which have been implicated in a wide variety of regulatory processes, including, cell cycle control, signal transduction, transcriptional activation, and plant photomorphogenesis. To date, no distinct biochemical activity has been identified that can reconcile this diversity in CSN functions. The eight CSN subunits show a one-to-one relationship to components of the 19S proteasome lid complex and, like pcu3 and pcu4, at least seven of the eight human subunits are conserved in fission yeast, but not in budding yeast.. Lyapina et al. recently showed that disruption of S. pombe CSN subunit 1 (caa1/csn1) results in accumulation of Pcu1p in the neddylated state. However, it remained unclear whether this reflects global control of multiple cullins by Csn1p as part of the entire CSN complex. We have examined this question by determining the effect of disruption of several CSN subunits on Pcu3p neddylation, localization, and ubiquitin ligase activity. Our results indicate that the entire CSN complex is required for proper control of the neddylation state of multiple cullins.

CONCLUSION

Conclusions Our study reveals a general role of the S. pombe CSN

complex in the control of cullin modification by Ned8p. Regardless of the exact mechanism, CSN may regulate the activity of multiple cullin ubiquitin ligases through counteracting their stimulatory Ned8p modification. Future studies will reveal whether some of the other described functions of CSN also involve post-translational modification of regulatory proteins by ubiquitin-like modifiers.