ABSTRACT

Drosophila Retinoblastoma protein regulation by the COP9 Signalosome: A link between proteolytic destruction and activity of a transcriptional repressor?

By

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Mammalian retinoblastoma proteins and their *Drosophila* counterparts, Rbf1 and Rbf2, play key roles in cell cycle regulation and development through transcriptional regulation. In order to execute this function, retinoblastoma proteins associate with various cofactors, but the exact role of these cofactors and how they may be involved in development remains a mystery. We therefore set out to identify and characterize the functional significance of novel Rbf associated factors during development. In this work we show that the developmentally regulated COP9 signalosome (CSN) physically interacts with Rbf2 during embryogenesis. Our studies show that CSN plays an important role in stabilization of Rbf1 and Rbf2 against proteasome mediated turnover. In addition, COP9 and Rbf proteins co-occupy cell cycle regulated promoters, suggesting a role for COP9 in transcription. Consistent with a role in cell cycle regulation, depletion of CSN subunits results in altered cell cycle progression.

Recent studies indicate that proteolytic destruction of some transcription factors is important for their transcriptional activity. Interestingly, CSN has been shown to physically associate with the proteasome and the SCF E3 ligase. In addition, components of the proteasome have been localized to promoters. However, no link between destruction and activity has been established for repressors like Rbf. Consequently, we

set out to determine if Rbf protein instability is required for its activity. In this work we identify for both Rbf1 and Rbf2 a C-terminal proteasome mediated instability element. Furthermore, we identify a single lysine residue in the C-terminus of Rbf1 that is required for proteasome mediated destabilization of Rbf1, and may be a potential target for polyubiquitinylation. Interestingly, an Rbf1 mutant that lacks this lysine residue strongly represses a *PCNA* reporter gene. This finding indicates that Rbf1 turnover is not linked to its repressive activity in the context of the *PCNA* promoter.

Our findings suggest that gene repression may not be linked to protein turnover. Nonetheless, we propose that Rbf repression may involve the proteasome and the promoter-associated COP9 signalosome, serving to extend Rbf protein lifespan and enable appropriate programs of retinoblastoma gene control during development.