Nature Reviews Molecular Cell Biology | AOP, published online 12 September 2013; doi:10.1038/nrm3664

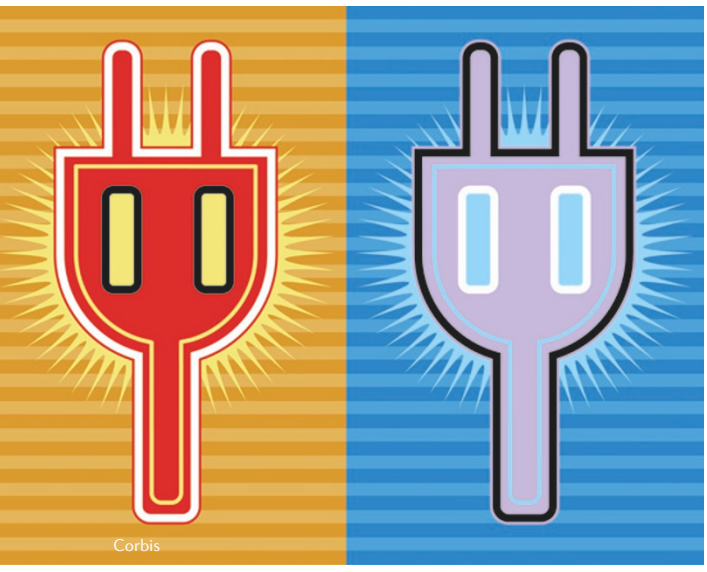
TRANSCRIPTION

Proteasome power to Def1

> transcriptional stress triggered the post-translational processing of Def1

Proteins that are ubiquitylated and targeted to the proteasome are not necessarily degraded — for example, it has now become apparent that proteasome-mediated removal of just a part of a protein might trigger its activation in response to transcriptional stress.

Transcriptional stress (for example, caused by DNA damage) can result in RNA polymerase II (Pol II) stalling, pausing or backtracking, which is overcome by degradation of the Pol II subunit Rpb1 (mediated by the elongin–cullin E3 ligase) and subsequent DNA repair. Def1 is known to be involved in this process in budding yeast, but its precise role had remained unclear. Svejstrup and colleagues observed that transcriptional stress triggered the post-translational processing of Def1, giving rise to a shorter form that lacked the carboxyl terminus. The appearance of this shorter protein correlated with Rpb1 polyubiquitylation and degradation, which indicates that Def1 processing might affect Rpb1 turnover. Indeed, when the authors constructed a yeast strain carrying a TEV (tobacco etch virus) protease cleavage site between amino acids 522 and 523 of Def1 (the predicted site of processing), they observed that artificial Def1 cleavage occurred concurrently with Rpb1 polyubiquitylation and degradation.



Corbis

Next, the authors sought to elucidate the mechanism of Def1 processing. Treatment of yeast cells with a proteasome inhibitor blocked the generation of processed Def1, and *in vitro* analysis confirmed the involvement of the proteasome. Def1 was found to be targeted to the proteasome following ubiquitylation by the E3 ligase Rsp5, with Lys281, Lys288, Lys328 and Lys329 being important for this process. Interestingly, although previous work had shown that Def1 is cytoplasmic, here the authors observed that transcriptional stress promotes relocalization of Def1 throughout the cell, including the nucleus, and that this is inhibited by treatment with a proteasome inhibitor. Further analysis suggested that the Def1 C terminus carries a motif that promotes nuclear export and that its removal following processing facilitates nuclear localization of the protein.

But how does processed Def1 promote Rpb1 degradation? Mutation of the Def1 CUE domain (which binds ubiquitin and is found in its amino terminus) blocked DNA damage-induced Rpb1 polyubiquitylation. This domain was necessary for Def1 binding to the elongin–cullin subunit Ela1 and for binding of Ela1 to Pol II, suggesting that Def1 bridges the interaction between elongin–cullin and Pol II. Consistent with this, the elongin–cullin components Elc1 and Ela1 bound poorly to Pol II in the absence of Def1. Furthermore, the authors showed that the Def1 CUE domain interacts with elongin–cullin via a newly identified ubiquitin homology domain in the Ela1 subunit.

So, in the presence of transcriptional stress Def1 is processed by the proteasome to promote its nuclear translocation, where it facilitates the association of elongin–cullin with the Pol II subunit Rpb1. This leads to Rpb1 ubiquitylation and degradation and consequent transcriptional arrest.

Rachel David

ORIGINAL RESEARCH PAPER Wilson, M. D. *et al.* Proteasome-mediated processing of Def1, a critical step in the cellular response to transcription stress. *Cell* **154**, 983–995 (2013)