

## Dun1, a Chk2-related kinase, is the central regulator of securin-separase dynamics during DNA damage signaling

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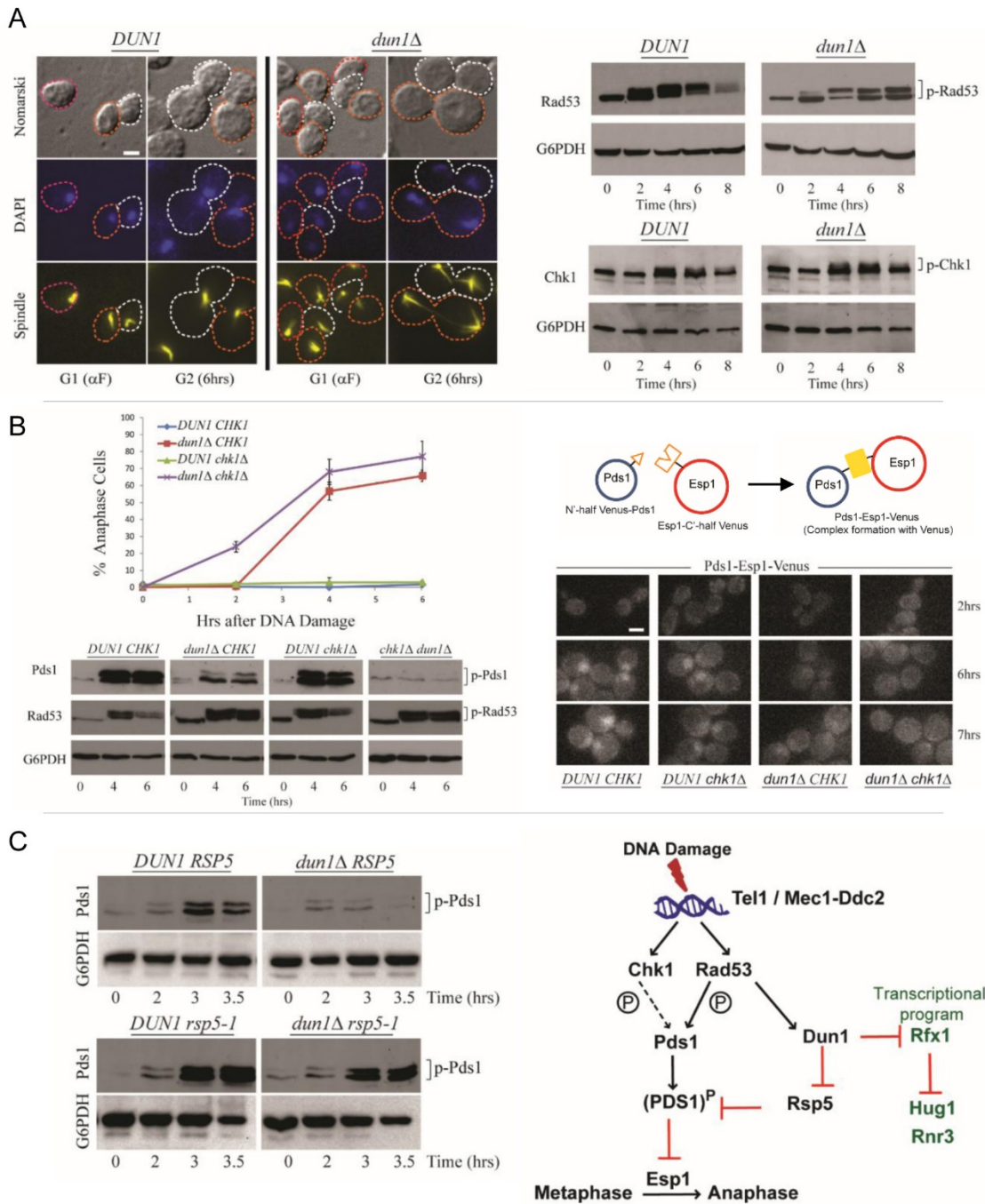
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## **Abstract**

The DNA damage checkpoint halts cell cycle progression in G2 in response to genotoxic insults. Central to the execution of cell cycle arrest is the checkpoint-induced stabilization of securin-separase complex (yeast Pds1-Esp1). The checkpoint kinases Chk1 and Chk2 (yeast Chk1 and Rad53) are thought to critically contribute to the stability of securin-separase complex by phosphorylation of securin, rendering it resistant to proteolytic destruction by the anaphase promoting complex (APC). Dun1, a Rad53 paralog related to Chk2, is also essential for checkpoint-imposed arrest. Dun1 is required for the DNA damage-induced transcription of DNA repair genes; however, its role in the execution of cell cycle arrest remains unknown. Here, we show that Dun1's role in checkpoint arrest is independent of its involvement in the transcription of repair genes. Instead, Dun1 is necessary to prevent Pds1 destruction during DNA damage in that the Dun1-deficient cells degrade Pds1, escape G2 arrest and undergo mitosis despite the presence of checkpoint-active Chk1 and Rad53. Interestingly, proteolytic degradation of Pds1 in the absence of Dun1 is mediated not by APC but by the HECT domain-containing E3 ligase Rsp5. Our results suggest a regulatory scheme in which Dun1 prevents chromosome segregation during DNA damage by inhibiting Rsp5-mediated proteolytic degradation of securin Pds1.

**Figure**



## Figure Legend

**(A) Left Panel:** Cell morphology and the state of mitotic spindle and the nucleus in both DNA damaged *DUN1* and *dun1Δ*. **Right panel:** Top panel - Western blot analysis of Rad53 dynamics in *DUN1* and *dun1Δ* cells; bottom panel - Chk1 dynamics in *DUN1 CHK1-MYC<sub>18</sub>* and *dun1Δ CHK1-MYC<sub>18</sub>* cells under DNA damage conditions. **(B) Left panel:** Onset of anaphase in *DUN1 CHK1*, *dun1Δ CHK1*, *DUN1 chk1Δ* and *dun1Δ chk1Δ* cells expressing *PDS1-HA<sub>3</sub>* during HO-mediated double-strand break. Lower panel: Western blot analyses of Pds1-HA<sub>3</sub> and Rad53. **Right panel:** Top: A schematic diagram depicting the use of BiFC to observe Esp1 and Pds1 interaction *in vivo*. Pds1 was N-terminally tagged with the 1<sup>st</sup>-half of Venus tag, (N'-half Venus-Pds1), while Esp1 was tagged at the C-terminus with the 2<sup>nd</sup>-half of Venus tag (Esp1-C'half Venus). Bottom: Venus-tagged Pds1-Esp1 interaction in *DUN1 CHK1*, *DUN1 chk1Δ*, *dun1Δ CHK1*, *dun1Δ chk1Δ* strains during DNA damage induced by HO expression. **(C) Left panel:** Western blot analyses to observe the state of phospho-Pds1. Top and bottom Western blots show dynamics of Pds1 during G1 arrest and after release at non-permissive temperature (37C). **Right panel:** A proposed regulatory scheme depicting the critical role of Dun1 kinase in DNA damage signalling pathway.