CELL DEATH

# DIAP1 puts ubiquitin on drICE

Caspase activity and apoptosis can be inhibited by members of the inhibitor of apoptosis (IAP) family. Selected IAPs are activated by effector caspase cleavage and recruitment of UBR-domain ubiquitin ligases (UBR-E3s). Caspases have been previously shown to undergo ubiquitylation, but the effect of this modification on caspase activity remained unclear. Ditzel *et al.* now report that caspase activity is regulated by a negative-feedback loop in which effector caspases activate *Drosophila* IAP1 (DIAP1) and are in turn inhibited by DIAP1-mediated non-degradative ubiquitylation.

Co-immunoprecipitations demonstrated that only cleaved DIAP1 ($DIAP1^{21-438}$) associates with caspases. Additionally, $DIAP1^{21-438}$, but not DIAP1 with a mutation in the RING finger or in the UBR-binding motif, can efficiently ubiquitylate effector caspases *in vivo*. These findings suggest that both the RING finger and UBR-E3 binding are necessary for DIAP1-mediated caspase ubiquitylation.

CORBIS

Eye-specific expression of the IAP antagonist Reaper (RPR) in *Drosophila melanogaster* causes apoptosis and a small-eye phenotype. Co-expression of RPR along with wild-type and mutant forms of DIAP1 revealed that both the RING finger and UBR-binding motifs are necessary for DIAP1-mediated inhibition of RPR-induced apoptosis and reversion of the small-eye phenotype. Moreover, DIAP1 mutants that are defective for caspase ubiquitylation cannot inhibit *D. melanogaster* effector caspase drICE (*Drosophila* interleukin 1β-converting enzyme; also known as ICE) activation, suggesting that ubiquitylation is necessary for DIAP1 inhibition of caspase activity.

Importantly, ubiquitylated drICE did not accumulate in cells that had been treated with proteasome inhibitors, nor did overexpression of DIAP1 lead to a decrease in drICE levels, which suggests that ubiquitylated caspases are not targeted for proteasomal degradation.

Furthermore, *in vitro* assays revealed that DIAP1 preferentially induces the formation of Lys63-linked polyubiquitin chains on drICE, a non-degradative form of ubiquitylation. Ubiquitylation of drICE by DIAP1 led to a decrease in drICE-mediated proteolysis, suggesting that ubiquitylation inhibits caspase activity directly. Mutagenesis of all exposed Lys residues of the large subunit of drICE (but not fewer) resulted in resistance to DIAP1-mediated ubiquitylation and inhibition.

These data contribute to a model of caspase regulation in which activated caspases cleave and activate DIAP1, which in turn inactivates caspases by polyubiquitylation, possibly through steric hindrance of the caspase substrate entry site. Under apoptotic conditions, however, DIAP1 destabilization or inhibition might lead to unrestrained caspase activation and apoptosis. These results describe a negative-feedback mechanism that allows low levels of active caspases to be kept in check in non-apoptotic cells.

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**ORIGINAL RESEARCH PAPER** Ditzel, M. *et al.* Inactivation of effector caspases through nondegradative polyubiquitylation. *Mol. Cell* **32**, 540–553 (2008)
**FURTHER READING** Vaux, D. L. & Silke, J. IAPs, RINGs and ubiquitylation. *Nature Rev. Mol. Cell Biol.* **6**, 287–297 (2005)