Cell Cycle 13:5, 685–686; March 1, 2014; © 2014 Landes Bioscience

DrICE resurrects Grim to antagonize DIAP1

Ting-Chun Yeh[1,2] and Shawn B Bratton[1,*]

[1]The University of Texas MD Anderson Cancer Center; Department of Molecular Carcinogenesis; Smithville, TX USA;
[2]Institute for Cellular and Molecular Biology; The University of Texas at Austin; Austin, TX USA

Caspases (cysteine proteases) have been studied extensively over the past 2 decades for their roles in mediating cell death in multicellular organisms. They are typically activated as part of a proteolytic cascade, in which an initiator caspase is recruited, through its association with an adaptor protein, to a large multimeric caspase-activating complex. Human caspase-9 and its *Drosophila* homolog, DRONC, for example, are activated within their Apaf-1 and DARK apoptosome complexes, respectively.[1] Once activated, these initiator caspases process downstream effector caspases, such as caspase-3 and DrICE, which, in turn, cleave numerous substrates, including those involved in transcription, translation, signal transduction, cellular structure, etc.[2] After cleavage, some proteins are inactivated; some exhibit an altered function due to the loss of a regulatory or binding domain; and some are rendered more active due to the removal of an inhibitory domain.[2] Many of these proteins also become susceptible to complete degradation, as in the case of the caspase inhibitor and E3 ubiquitin ligase, *Drosophila* inhibitor of apoptosis 1 (DIAP1), which is degraded via the N-end rule following removal of its N terminus by caspases.[3] More recently, we have described yet another regulatory mechanism whereby caspases extend, rather than decrease, the fate of a given substrate.[4] Indeed, the inhibitor of apoptosis (IAP) antagonist, Grim, which normally regulates caspase activity by displacing active DRONC and DrICE from DIAP1, is itself susceptible to both caspase cleavage and DIAP1-mediated ubiquitinylation, with the former subverting the latter (**Fig. 1**).[4]

Most IAPs, including DIAP1, contain a C-terminal RING domain that allows them to recruit E2 ubiquitin-conjugating enzymes, which subsequently mediate ubiquitinylation of the IAPs or their bound substrates. In our study, DIAP1 utilized UbcD1 to ubiquitinylate Grim at Lys136 in its C terminus. Notably, Grim, like all IAP antagonists, possesses an N-terminal IAP binding motif (IBM), through which it binds to baculovirus IAP repeat (BIR) domains. We discovered that Grim dimerizes, engages the BIR1 and BIR2 domains in DIAP1, and thus forms a tripartite complex.[4] Remarkably, however, DIAP1-UbcD1 ubiquitinylates only the BIR2-bound Grim, demonstrating unique structural selectivity, but also raising the possibility that BIR1-bound Grim may be degraded through an alternative mechanism. Regardless, Grim is also cleaved by DrICE at Asp132, which removes the lysine necessary for DIAP1-dependent ubiquitinylation of Grim (**Fig. 1**). DrICE cleaves Grim, either prior to or following ubiquitinylation, resulting in a form of Grim that is no longer ubiquitinylated or degraded. The accumulation of Grim in cells then displaces even more active caspases from DIAP1, initiating a caspase amplification loop that results in greater caspase activity and increased cell death. In short, our findings help demonstrate, for the first time, that an important crosstalk exists at the substrate level between caspases and the proteasomal system.

Of course, an obvious question is why should such a mechanism exist? One possibility is that it could serve as protection against unwanted low-level expression of Grim. In this scenario, DIAP1 would be expected to ubiquitinylate Grim and target it for destruction by the proteasome in order to prevent de-repression of caspases and apoptosis. On the other hand, when cell death is needed, higher levels of Grim expression would activate some caspases, which, in turn, should cleave Grim and initiate a caspase amplification loop, thereby ensuring that all cells that are supposed to die will do so. In this case, once a certain threshold is met, maximum caspase activity would rapidly ensue. Alternatively, this mechanism could also be amenable to finer control and could regulate cellular processes in which some caspase activity is desirable (e.g., in mediating differentiation), but is held in check in order to avert the onset of apoptosis. Indeed, Grim is present, and caspases are activated during the development of Malpighian tubules in the fly embryo, but they do not cause significant cell death.[5] Similarly, caspases play an essential role in spermatid individualization.[6] This type of regulatory control may determine a cell's fate through spatiotemporal control of caspase activity during different stages of development.[7]

It is worth noting that Grim is also ubiquitinylated by another *Drosophila* IAP, DIAP2.[4] Unlike DIAP1, DIAP2 plays a major role in innate immunity and nuclear factor kappa B (NFκB) signaling in flies. It often mediates K63-based ubiquitinylation of substrates, such as IMD,[8] and K63-linked ubiquitin chains generally prevent protein turnover, mediate the recruitment of other proteins, and/or alter subcellular localization. Whether Grim undergoes K63-based ubiquitinylation by DIAP2 or functions as a signaling molecule is currently unknown. However, Grim exhibits a distinct subcellular localization, and removal of K63-linked chains by caspases could alter its function in cells, perhaps converting it from a signaling

*Correspondence to: Shawn B Bratton; Email: sbbratton@mdanderson.org
Submitted: 10/24/2013; Accepted: 10/28/2013; Published Online: 01/21/2014
http://dx.doi.org/10.4161/cc.27857
Comment on: Yeh TC, et al. Proc Natl Acad Sci USA 2013; 110:14284-9; PMID:23940367; http://dx.doi.org/10.1073/pnas.1306179110

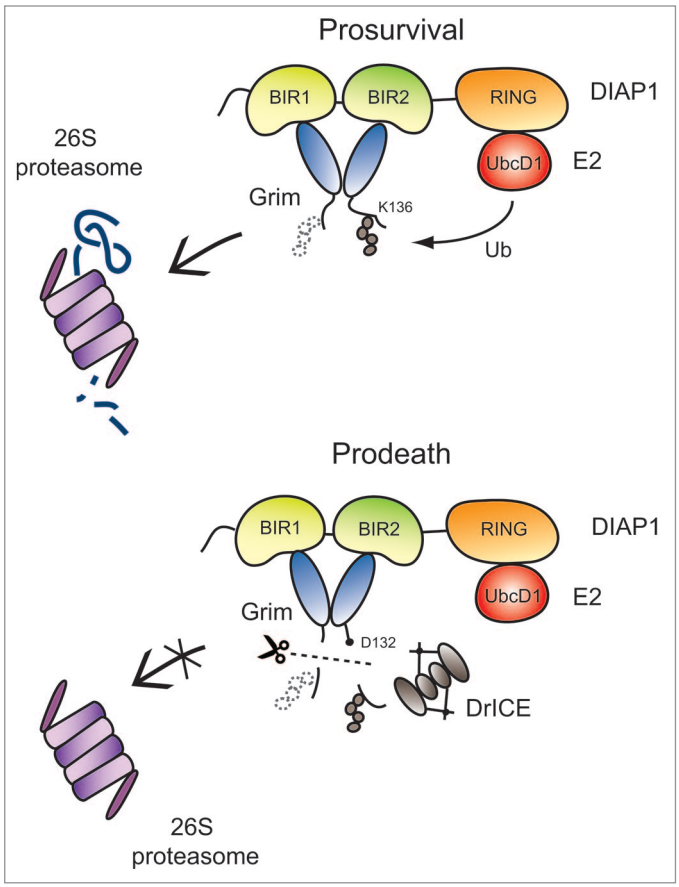

Figure 1. Regulation of Grim by DIAP1 and caspases. Grim interacts with DIAP1 through its BIR1 and BIR2 domains. BIR2-bound Grim is ubiquitinylated on Lys136, so that when Grim levels are relatively low, most Grim is ubiquitinylated and degraded by the proteasome (prosurvival). However, at higher Grim levels, DrICE is activated and cleaves Grim at Asp132, liberating its C terminus including any ubiquitin chains linked to Lys136 (prodeath).

molecule to a killer. Finally, we wish to stress that any protein with a single lysine (or a critical lysine responsible for its stability) could undergo a similar type of regulation as described here, if flanked by a caspase cleavage site. In fact, this dual regulation of a protein substrate by a protease and E3 ligase need not be limited to caspases or even cell death regulation. Moving forward, it will be interesting to discover additional examples of this unique interplay between caspases and the proteasome system.

References

1. Bratton SB, et al. J Cell Sci 2010; 123:3209-14; PMID:20844150; http://dx.doi.org/10.1242/jcs.073643
2. Lüthi AU, et al. Cell Death Differ 2007; 14:641-50; PMID:17273173; http://dx.doi.org/10.1038/sj.cdd.4402103
3. Ditzel M, et al. Nat Cell Biol 2003; 5:467-73; PMID:12692559; http://dx.doi.org/10.1038/ncb984
4. Yeh TC, et al. Proc Natl Acad Sci USA 2013; 110:14284-9; PMID:23940367; http://dx.doi.org/10.1073/pnas.1306179110
5. Tapadia MG, et al. J Biosci 2011; 36:531-44; PMID:21799264; http://dx.doi.org/10.1007/s12038-011-9092-3
6. Muro I, et al. Development 2006; 133:3305-15; PMID:16887831; http://dx.doi.org/10.1242/dev.02495
7. Takemoto K, et al. Proc Natl Acad Sci USA 2007; 104:13367-72; PMID:17679695; http://dx.doi.org/10.1073/pnas.0702733104
8. Paquette N, et al. Mol Cell 2010; 37:172-82; PMID:20122400; http://dx.doi.org/10.1016/j.molcel.2009.12.036