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A PUMA mechanism unfolds

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Abstract

PUMA is a BCL-2 family protein that transmits stress signals to promote apoptosis. Upon DNA damage, a unique binding determinant within PUMA triggers partial unfolding of BCL-X_L, resulting in the release of sequestered p53 and commitment to p53-dependent cell death.

The BCL-2 family of apoptotic proteins arbitrates the cellular response to internal and external stress through a complex network of protein interactions that ultimately block or activate mitochondrial poration. Specialized stress sensors within the family communicate with downstream members via a conserved motif called the BCL-2 homology 3 (BH3) domain, which engages its targets as a single α-helix. These so-called 'BH3-only proteins' are otherwise heterogeneous, subserving discrete cellular functions and subject to distinct transcriptional and post-translational modes of regulation. PUMA, or 'p53 upregulated modulator of apoptosis', was first discovered as a p53 target that contributed to the apoptotic response at the level of the mitochondria[1,2]. PUMA has since been shown to engage the broad range of antiapoptotic BCL-2 family proteins through the now-classic interaction between its BH3 helix and a hydrophobic groove on the antiapoptotic surface[3,4]. Targeting antiapoptotic BCL-2 family proteins in this fashion lowers the apoptotic threshold by preventing sequestration of proapoptotic members and displacing them when already bound. In this issue, Follis *et al*.[5] uncover a unique BCL-X_L–binding feature of the PUMA BH3 domain ($PUMA^{BH3}$) and its mechanistic role in activating p53-dependent apoptotic signaling through induced unfolding of BCL-X_L and p53 release.

As guardian of the human genome, p53 has an essential tumor suppressive role in regulating the cell cycle and cell death in response to DNA damage. Whereas the transcriptional basis for p53 activity has been thoroughly defined in this context, transcription-independent signaling has also emerged as a contributory mechanism[6,7]. The localization of p53 in complex with BCL-X_L at the mitochondria of apoptotic cells implicated direct communication between the p53 and BCL-2 family pathways in regulating the cell's life-death rheostat[7]. Interestingly, the same basic domain within p53 that confers DNA binding functionality was shown to interact with an acidic surface formed by residues of the BCL-X_L α1 C terminus and the α3-α4 and α5-α6 loops[8]. That is, p53 engaged BCL-X_L at an interaction site distinct from that previously defined for the amphipathic BH3 helices, which bind a surface hydrophobic groove formed by the confluence of residues from BCL-X_L α-helices 2, 3, 4, 5 and 8. Here, Follis *et al*.[5] apply a comprehensive battery of structural, biochemical and cellular analyses to investigate the mechanism by which PUMA dissociates the p53–BCL-X_L complex to drive p53-dependent apoptosis.

Competing financial interests
The author declares no competing financial interests.

The first clue was derived from NMR experiments examining the influence of $PUMA^{BH3}$ titration on the resonances of a ^{15}N-labeled BCL-X_L construct termed BCL-$X_L\Delta L\Delta C$, which lacked the unstructured α1-α2 loop (whose resonances overlapped or interfered with those of the structured portions of the protein) and the C-terminal helix. Whereas $PUMA^{BH3}$ binding induced chemical shift changes in the canonical BH3-binding pocket of BCL-$X_L\Delta L\Delta C$, additional residues within α2, α3 and α4 were likewise perturbed (Fig. 1a), a phenomenon not observed, for example, upon titration with another BH3-pocket binder such as BAD^{BH3}. Structural analysis of the $PUMA^{BH3}$–BCL-X_L complex by X-ray crystallography further showed that Pro116 of the α3-α4 loop underwent a 5-Å displacement relative to its position in the uncomplexed and BAD^{BH3}–BCL-X_L structures. Taken together, these data suggested that, in addition to the expected interactions between $PUMA^{BH3}$ and BCL-X_L, unique allosteric changes in the structure flanked by α2 and α4 occurred that map to a portion of the region previously identified as the p53-binding site on BCL-X_L[8](Fig. 1a). Could $PUMA^{BH3}$ binding to BCL-X_L partially unfold and thereby alter the p53 binding site such that p53 dissociation ensues? Correlative biochemical studies indeed showed that preincubation of BCL-$X_L\Delta C$ with $PUMA^{BH3}$ or exposure of preformed p53–BCL-$X_L\Delta C$ complex to $PUMA^{BH3}$ abrogated the capacity of p53 to bind BCL-$X_L\Delta C$. Notably, no other BH3 domains tested recapitulated these effects, suggesting that $PUMA^{BH3}$ harbors a unique binding determinant that mediates its distinct functionality.

Alignment of BH3 sequences and inspection of both the NMR and crystal structures of the $PUMA^{BH3}$–BCL-X_L complexes provided a second mechanistic clue underlying the activity and specificity of $PUMA^{BH3}$-mediated partial unfolding of BCL-X_L. The $PUMA^{BH3}$ sequence contains a uniquely positioned tryptophan, N-terminal to the core BH3 consensus sequence, that was observed to engage in π-stacking interactions with His113 of BCL-X_L (Fig. 1a). To determine whether this unique interaction pair influenced the observed structural and biochemical effects of $PUMA^{BH3}$ on BCL-X_L and its association with p53, studies with the mutant constructs $PUMA^{BH3\ W71A}$ and BCL-$X_L\Delta C^{H113A}$ were undertaken. In each case, elimination of the π-stacking interaction by alanine mutagenesis completely eliminated the selective $PUMA^{BH3}$ activity, mechanistically linking the induced partial unfolding to a single complementary aromatic interaction. Importantly, the authors vetted their mechanistic conclusions in a cellular context, demonstrating that reconstitution of *Puma*$^{-/-}$ mouse embryonic fibroblasts with wild-type PUMA, but not $PUMA^{W71A}$, restored UV-induced apoptosis. Correspondingly, the UV-induced p53–BCL-X_L complex was effectively dissociated upon engagement by reintroduced wild-type PUMA but not by $PUMA^{W71A}$, as elegantly demonstrated by coimmunoprecipitation experiments. As even further evidence of mechanism-based specificity, both PUMA constructs fully restored TNF-induced apoptosis and were equally ineffective in triggering UV-induced apoptosis in the absence of p53.

The p53 and BCL-2 family pathways represent critical control points in the regulation of cellular life and death, and thus their intersection through direct interactions at the mitochondria seems logical, albeit noncanonical. BH3 domains are well known to be discriminating and, however homologous, encode sequence-based selectivity that dictates their unique and common functions. Here, one such determinant, a tryptophan within $PUMA^{BH3}$, confers a unique $PUMA^{BH3}$–BCL-X_L binding mode that selectively induces allosteric unfolding within a portion of the p53-binding site, rendering BCL-X_L incompatible for p53 interaction. As a consequence, cytosolic p53 is released to compound transcriptional activation and promote apoptosis through direct protein interaction with mitochondrial proapoptotic effectors, such as BAX[6] (Fig. 1b). Interestingly, a previous NMR study demonstrated that preincubation of BCL-$X_L\Delta C$ with BAD^{BH3} precluded p53 interaction[8]; inspection of the BAD^{BH3} sequence reveals an N-terminal tryptophan localized one residue upstream of $PUMA^{BH3}$ Trp71. Given the flexibility of the α3-α4 loop, it is

plausible that in discrete contexts, the regulatory mechanism elucidated here for PUMA^{BH3}–BCL-XL–p53 may translate to other multiprotein BCL-2 family complexes. Indeed, noncanonical BH3 interaction modes and their unique effects on target structure and function are emerging as important themes in BCL-2 family biology[9,10]. Rigorous multidisciplinary analyses, as showcased by the current study, are essential for both validating and integrating such discoveries, however unexpected, into the evolving and complex canon that defines the signaling mechanisms of life and death.

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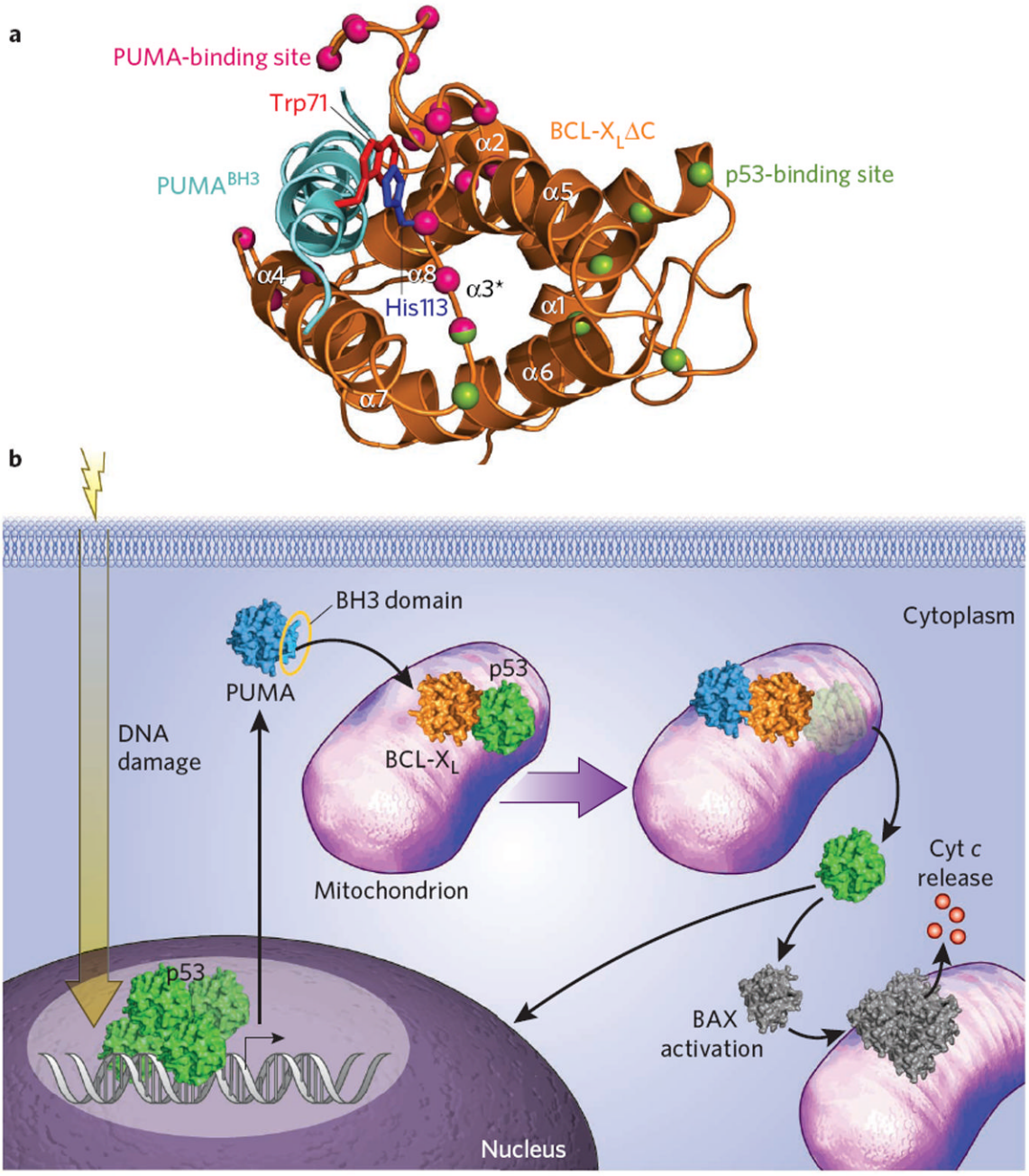

Figure 1.

Induction of p53-dependent apoptosis by selective PUMA^{BH3}-mediated dissociation of the mitochondrial p53–BCL-X$_L$ complex. (**a**) A unique π-stacking interaction between PUMA^{BH3} Trp71 (red) and BCL-X$_L$ His113 (blue) triggers partial unfolding of the BCL-X$_L$ α2-α3 region (α3*), a component of the previously defined p53 binding site on BCL-X$_L$. BCL-X$_L$ residues that undergo the greatest chemical shift change upon PUMA^{BH3} titration are depicted as pink balls, whereas those residues affected by p53 DNA-binding domain engagement, as defined by Petros *et al*.[8], are represented by green balls. Thr115 is dual-colored, reflecting an exemplary residue that is affected both by PUMA^{BH3} (ref. 5) and p53 (ref. 8) binding. The ribbon diagram of BCL-X$_L$ΔC protein and the PUMA^{BH3} helix are colored orange and cyan, respectively. (**b**) Irradiation-induced DNA damage triggers the p53

transcriptional response, resulting in the upregulation of PUMA, among other targets. $PUMA^{BH3}$ engages $BCL\text{-}X_L$ at the canonical binding site, resulting in structural changes that promote dissociation of p53. This liberation of cytosolic p53 is believed to contribute to p53-dependent apoptosis through amplification of the transcriptional response and direct activation of proapoptotic BAX. Cyt *c*, cytochrome *c*.