Releasing the Brakes on Apoptosis: Peptide Antagonists Trigger Dimerization and Autoubiquitination of Cellular Inhibitor of Apoptosis Protein 1

Programmed cell death, or apoptosis, is a critical failsafe against uncontrolled proliferation. For this reason, apoptosis is frequently defective in cancer cells, allowing tumor growth to proceed unchecked. The inhibitor of apoptosis proteins, or IAPs, are some of the final "brakes" on apoptosis, directly inhibiting both caspases and their upstream activators (1,2,3,4). Thus it is unsurprising that IAP proteins are over-expressed in many human cancers (2,5).

The anti-apoptotic functions of IAP proteins are mitigated by the second mitochondrial activator of caspases (SMAC) (6,7). The N-terminal tail of SMAC, comprising the amino acid sequence AVPI, interacts with a peptide binding pocket on IAP proteins, preventing their association with caspases (8,9,2,4) and triggering the degradation of cellular IAP1 (cIAP1) (10-12).

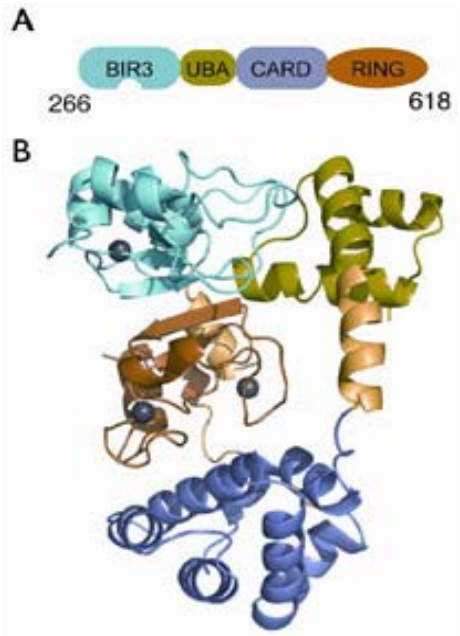

Figure 1. A) Domain structure of the cIAP1-B3R construct. B) Crystal structure of the monomeric form of cIAP1-B3R. Individual domains are colored as in the primary structure diagram at top. Linkers between domains are colored yellow. The RING domain (orange), which is responsible for homodimerization, is sequestered on three sides by the other globular domains.

cIAP1 (like several other IAP family members) is a ubiquitin ligase. It comprises three baculovirus IAP repeat (BIR) domains, a ubiquitin association (UBA) domain, a caspase association and recruitment domain (CARD), and a RING E3 ligase domain. A shortened construct consisting of only the BIR3-RING domains (cIAP1-B3R) is sufficient for antagonist-induced activation of ligase activity. Upon binding of the SMAC peptide or peptide mimetics to the BIR3 domain, we observed that cIAP1-B3R forms a RING-based dimer *in vitro*. This dimer form of the protein is active for ubiquitin ligation and leads to cIAP1's autoubiquitination and subsequent degradation by the proteasome. In this way, the release of SMAC from mitochondria abrogates the anti-apoptotic activity of cIAP1.

To understand the mechanism by which binding of a short peptide at the BIR3 domain triggers dimerization at the 300-amino-acid distant RING domain, we solved the structures of the monomeric and dimeric forms of cIAP1-B3R by X-ray crystallography and small angle X-ray scattering, respectively. Unexpectedly, the monomeric form of cIAP1 (Figure 1) adopts a wrapped conformation in which the RING domain is sequestered by the BIR3, UBA and CARD domains. This arrangement prevents the RING domain from adopting the conformation needed for homodimerization. Targeted mutagenesis of residues at the interface of the RING and BIR3 domains resulted in dimerization of cIAP1 even in the absence of peptide antagonists, confirming the inhibitory role of this conformation.

We were able to solve the structure of three dimeric fragments of cIAP1 using small angle X-ray scattering (SAXS): a UBA-RING truncation, cIAP1-B3R, and cIAP1-B3R with an N-terminal maltose binding protein (MBP) tag. *Ab initio* model calculation on all three of these constructs revealed extended, nearly planar dimeric structures (Figure 2). The long, flexible linkers connecting the globular domains of cIAP1 made the creation of a molecular model from these data challenging. Nevertheless, the relative size and shape of these structures made it clear that the RING-RING dimer is at the center of the complex, with the CARD, UBA and BIR3 domains extended out in a splayed "V."

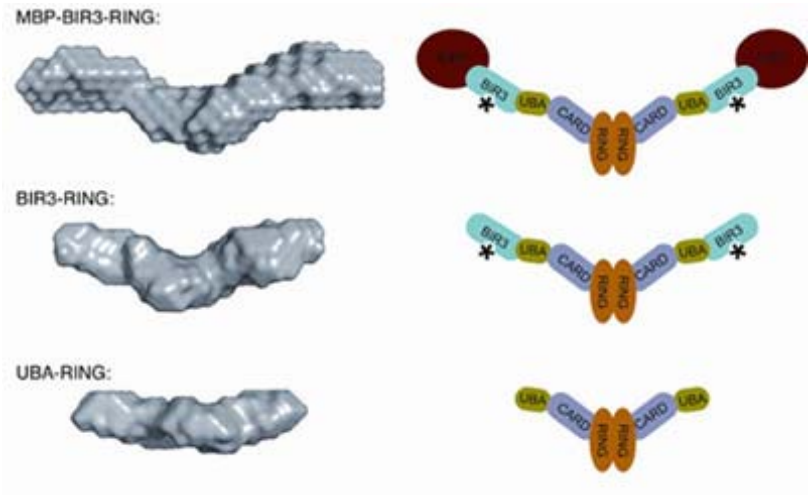

Figure 2. *Ab initio* SAXS envelopes of the dimeric forms of MBP-tagged cIAP1-B3R, cIAP1-B3R, and cIAP1 UBA-RING. Note the planar, extended shape of each construct. Proposed models for each construct are shown at right. Asterisks indicate bound antagonist.

The dramatically different monomeric and dimeric structures of cIAP1-B3R, along with *in vitro* and cellular data demonstrating the functional activity of the dimer, have allowed us to construct the following model for antagonist-based degradation of cIAP1. Initially, the protein exists in a compact, wrapped form incapable of dimerization. Upon disruption of the BIR3:RING interface—by SMAC peptide binding, drug binding, or mutation—the monomer unwraps, allowing RING:RING dimerization to occur. This form of the protein is active for ubiquitin ligation, and it rapidly ubiquitinates itself and is degraded by the proteasome. Apoptosis follows. Our understanding of cIAP1's autoubiquitination mechanism has significant implications for the development of anti-cancer therapeutics aimed at releasing the brakes on apoptosis.

Crystallography data was collected at the Advanced Light Source (ALS) and the Advanced

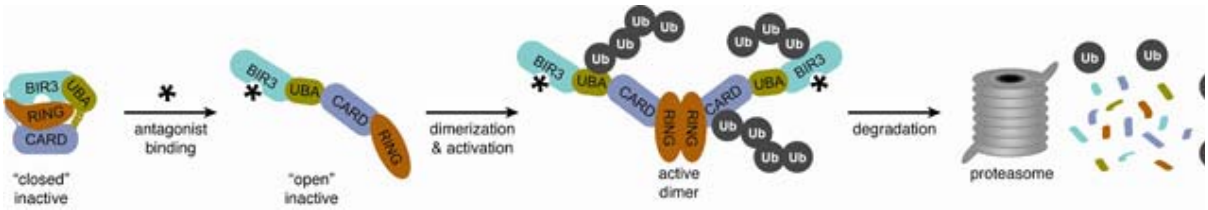

Figure 3. A model for antagonist-induced dimerization, activation, and degradation of cIAP1. In monomeric form, cIAP1-B3R exists in the closed, compact conformation. Binding of antagonist to the BIR3 domain disrupts the BIR3:RING interface, causing the compact structure to unwrap, making the RING domain available for dimerization. The dimeric form of the protein is an active ubiquitin ligase. It ubiquitinates itself, leading to its degradation by the proteasome.

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