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Playing FullBAK

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BAK is a pro-apoptotic BCL-2 protein that forms toxic mitochondrial pores in response to cellular stress. A membrane-embedded protein historically refractory to recombinant expression in full-length form, BAK plays it close to the vest. We recently produced monomeric, full-length BAK (FL-BAK) for the first time,[1] providing new opportunities to interrogate its activation mechanism as compared with its close homolog BAX. Unlike BAX, soluble FL-BAK auto-translocates to isolated mitochondria, preferring the membrane environment, consistent with the relative subcellular distributions of BAX and BAK in vivo. FL-BAK translocation did not permeabilize the mitochondria unless a triggering ligand was present. Indeed, we measured direct, sequence-dependent interactions between FL-BAK and an activating BH3 helix. The BH3-only protein tBID, or a hydrocarbon-stapled version of its BH3 helix, oligomerized FL-BAK in the absence of any other factors, definitively demonstrating a direct activation mechanism.[1] Applying photoreactive BH3 helices and MS analysis, we mapped the trigger site for direct BAK activation to its canonical pocket at the C-terminal face, whereas the same ligands crosslinked to BAX at the novel N-terminal trigger site and, upon allosteric release of its C-terminal helix, to the exposed canonical pocket as well.[1] Our analyses suggest that activating BH3 interactions at the canonical pocket in the context of the mitochondrial membrane may represent a common mechanism for propelling the activation and oligomerization of BAK/BAX, with the N-terminal triggering mechanism of cytosolic BAX representing a unique afferent step required to initiate its activation and mitochondrial translocation. With new rules of the game continually revealed, the competitive interplay among BCL-2 family members has made for an exciting spectator sport that pits cellular life against cellular death.

BCL-2 proteins regulate apoptosis through complex and strategic plays among team members. On offense are BAK and BAX, two formidable teammates that, when activated by BH3 stress signaling, spring into action and score, forming a game-ending victory circle at the mitochondrial outer membrane—the end zone of the apoptotic pathway. The defense is comprised of nimble players like BCL-2 and MCL-1 that block the activated forms of BAK/BAX, stopping the death clock to keep the game alive. In large part, which team wins depends on who is carrying the ball, represented by the critical BH3-only proteins, an elite subgroup of pro-apoptotic BCL-2 family players whose only weapon for interaction is their single BH3 helix. Fortunately, during stressful circumstances, the quarterback has an arsenal of diverse BH3s to launch, some of which have the dual capacity to engage the offense and defense, further complicating the action.

The first structural studies of a BCL-2 protein created a player card for anti-apoptotic BCL-X_L, highlighting how its α-helices fold to form a surface pocket for intercepting the BH3 helix of pro-apoptotic members.[2] The interaction paradigm for how anti-apoptotics play defense was revealed: intercept the BH3 killer domain of the signaling BH3-only proteins (**Fig. 1**, Defensive play 1) and grab onto that of the activated forms of BAK/BAX for the block (**Fig. 1**, Defensive play 2). Fake passes from the quarterback (or drugs that neutralize the anti-apoptotic pocket), disorient the defensive players, liberating the offense to score a win for the home team (**Fig. 1**, Offensive play 1). Historically, knowledge of anti-apoptotic tactics for defending against cell death has exceeded our understanding of how the offense, represented by BAK/BAX, launch into action in the first place, making them rather controversial players.

It has been a challenge to study BCL-2 players in their full-length form, especially those like BAK that reside in the mitochondrial end zone. Preferring to play the cytosolic field, BAX has been amenable to analysis in full-length form, revealing many operational secrets, including its structure, transformational responses to direct engagement by BH3 domains and even localization of the BH3 point-of-contact for initiating BAX's sprint to the end zone[3] (**Fig. 1**, Offensive play 2). Some officials have long heralded a direct activation strategy for BAX[4] and BAK,[5] although a group of highly regarded referees questioned the maneuver, if not dismissed it outright by throwing a penalty flag.[6] However, the game charged on, and the direct activation play became such a crowd pleaser that even the naysayers ultimately joined the chorus of cheering fans.[7] Lurking at the end zone to catch the Hail Mary pass, BAK has avoided the spotlight, but biochemical and structural analyses of its truncated and full-length forms have now revealed that it too is a target for direct activation, faking out the defense by using the traditional interaction site for anti-apoptotic blockade of BH3 domains as its very own trigger site[1,8] (**Fig. 1**, Offensive play 3)! Indeed, once BAX reaches the mitochondrial end zone—its canonical site now available for engagement after allosteric release of the occupying C-terminal helix during upfield play[3] (**Fig. 1**, see Offensive play 2)—it may also engage in BH3-in-canonical

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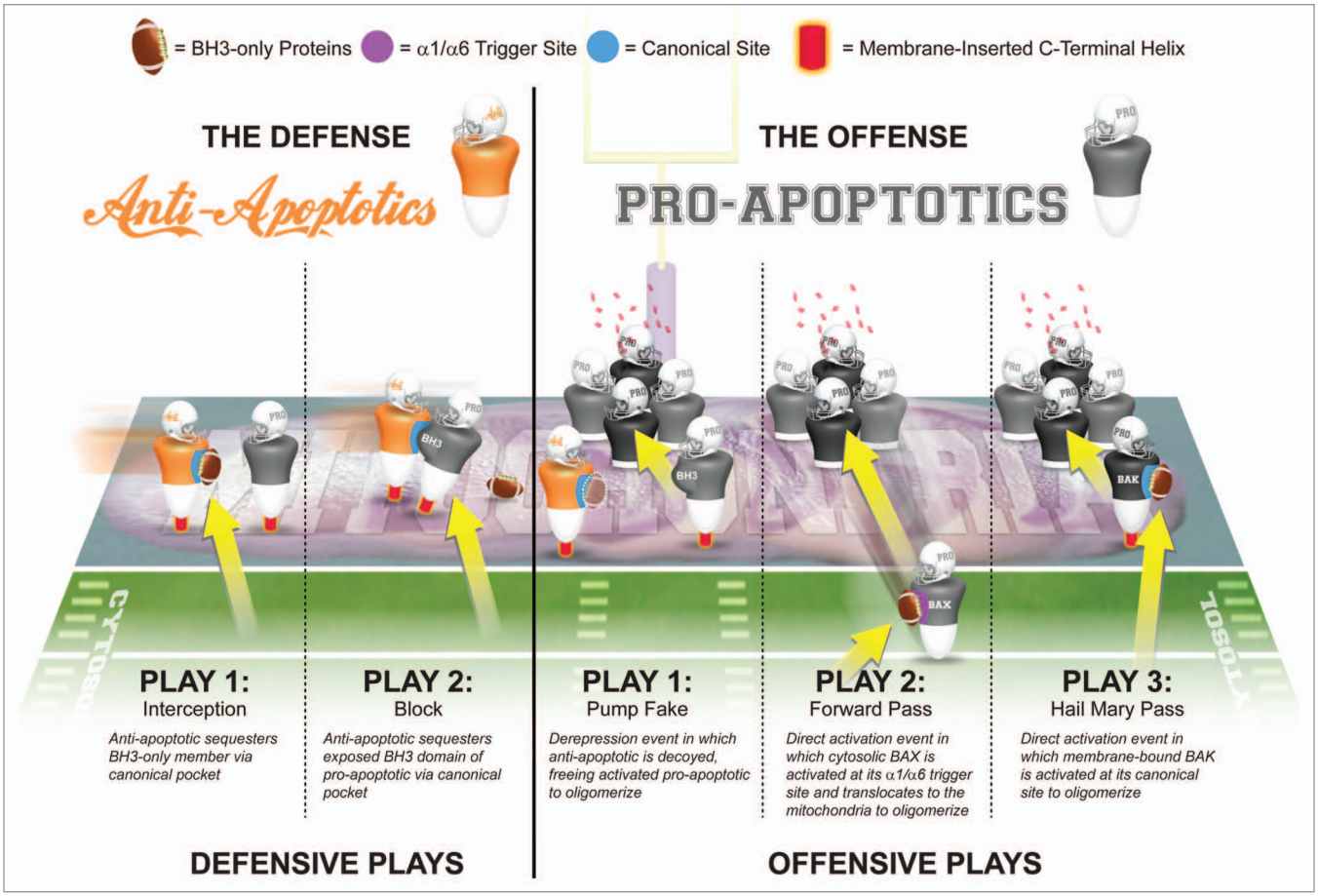

Figure 1. The contact sport of apoptotic regulation, played by the offensive and defensive teams of the BCL-2 family. Members of the anti-apoptotic team contain a surface pocket that can effectively intercept the killer BH3 domain helices thrown by the stressed-out quarterback (Defensive play 1, interception). Using the same strategy for tactical engagement, these defensive players can also effectively block the activated forms of BAK and BAX (Defensive play 2, block). However, when distracted by a fake pass from the quarterback, a disoriented defense can inadvertently release its grip on activated BAK or BAX, liberating the once-trapped offensive players to score (Offensive play 1, pump fake). The offensive running back, BAX, plays the cytosolic field and upon catching an activating BH3 helix in its N-terminal α1/α6 pocket, deploys its C-terminal helix and charges to the end zone (Offensive play 2, forward pass). BAK, with its C-terminal helix already firmly planted in the end zone, fakes out the defense by catching the Hail Mary activating BH3 helix in its C-terminal canonical pocket (a move that activated BAX, upon reaching the end zone, may also have in its playbook), catalyzing the formation of an oligomeric victory circle in celebration of the game-ending touchdown (Offensive play 3, Hail Mary pass). Figure acknowledgment: Eric D. Smith.

groove action to secure the oligomeric touchdown.[1,7] With these game-changing new surfaces for direct BAK and BAX activation revealed, coaches are already pondering whether new pharmacologic approaches might be permissible to power up their offense and stack the deck against notorious opponents like cancer, whose defenses must be overcome by any means necessary.

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