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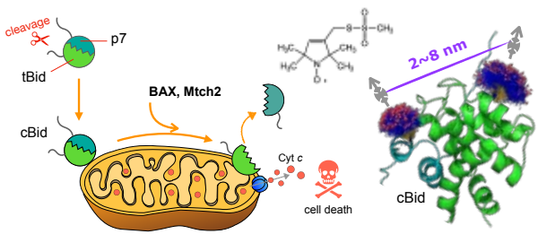
Electron Spin Resonance Reveals the Stepwise Apoptotic Activation on Mitochondrial Membranes

Bid protein cleavage by Caspase-8 represents a lethal event in the mitochondria-mediated apoptosis. Cleaved Bid (cBid) subsequently associates with BAX protein and membranes, leading to mitochondria outer membrane permeabilization (MOMP) and cell death. However, it remains largely unclear by what mechanism cBid interacts with BAX on mitochondrial membrane to bring about MOMP. The current structural models of Bid are established primarily based on the results of model membranes. The conformational changes in cBid during the association with real mitochondrial membranes have not been sufficiently characterized. Without the use of real mitochondria, the possible contributions of other factors that affect the regulation of Bid could be overlooked.

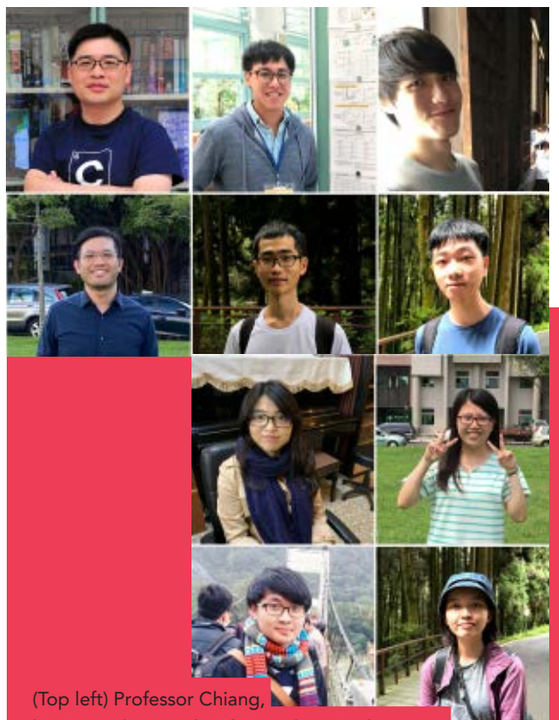
The team led by Prof. Yun-Wei Chiang at NTHU has recently made a breakthrough in identifying the conformational changes of cBid that occur during the process of cBid-induced BAX oligomerization and MOMP, using techniques including spin-label electron spin resonance (ESR) and PEGylation-based gel shift assay. The double electron-electron resonance (DEER) ESR technique is a powerful tool for determining protein structure and topology in a complex membrane environment. The DEER data provide interspin distance distributions in the range of 2–8 nm and the distance constraints that can be used to determine assembly and individual structures of protein subunits in a complex membrane environment. The team performed DEER to identify the conformational changes in cBid and explore the interactions of Bid with the mitochondrial carrier homolog 2 (Mtch2), a key protein known to play a role in facilitating the recruitment of tBid to mitochondria.



Stepwise activation of the pro-apoptotic Bid protein at mitochondrial membrane is revealed using electron spin resonance spectroscopy.

Various single-/double-/triple-cysteine variants of Bid were prepared for spin-label ESR as well as PEGylation studies. Triply spin-labeled samples were used to study the structural integrity of cBid during the cBid-induced BAX activation. Doubly spin-labeled cBid samples can be further divided into two groups: p7/tBid- breakup and intra-tBid groups; samples of the former allow one to explore when the breakup of cBid (i.e., p7/tBid) occur, and samples of the latter were used to identify the conformational changes in the tBid fragment at the mitochondrial membranes. New insights into the details of cBid-induced BAX activation at real mitochondrial membranes were thus revealed in this study.

In summary, the Chiang group reveals molecular details of the Bid activation at real mitochondrial membranes in a stepwise manner. The association of cBid with membranes and Mtch2 initiates structural rearrangements that unmask the BH3 domain, priming it for engagement with BAX to induce BAX-mediated MOMP. It is the interactions among cBid, BAX, and Mtch2 at mitochondrial membranes that further trigger the fragmentation of cBid and drive the conformation of tBid from a compact ensemble to an ensemble of highly extended conformations. However, the activated tBid fragments do not assemble into an aggregate in mitochondrial membranes. This study provides a better understanding of the cBid-induced BAX activation, and the refined model might lead to an effective treatment that can specifically target different functional states of Bid to suppress the cBid-induced BAX activation.



(Top left) Professor Chiang, his 3 postdocs, and 6 PhD students in the group.

> ***The study answers a long-standing question about how Bid triggers the BAX-mediated apoptosis on mitochondrial membranes, providing key information for the development of more efficient molecules to regulate apoptosis.***

Research Highlights

- Ta-You Wu Memorial Award Research Grant" by Ministry of Science and Technology (MOST), 2019-2022
- Outstanding Young Scholar Research Grant" by MOST, 2013-2019

Research Output

- C.L. Hung, H.H. Chang, S.W. Lee, Y.W. Chiang*, Stepwise activation of the pro-apoptotic protein Bid at mitochondrial membranes, Cell Death & Differentiation, 28 (2021) 1910-1925.
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