

APRIL 27, 2001

Molecular "Gateways to Death" Identified

Researchers have identified two key components of what are likely "gateways to death" in the membranes of the cell's mitochondria. When cell death signals are received by the mitochondria, these gateway proteins are believed to form pores in the mitochondrial membranes that allow molecules to pass into the cell, generating a cascade of biochemical reactions that causes cell death.

Programmed cell death, also called apoptosis, culls unneeded cells during development and growth. It also protects organisms by killing defective cells. Defects in apoptosis can be harmful—leading to extended cell survival, which may allow cancer cells to expand, for example. Conversely, faulty apoptosis can also accelerate the rate of cell death, and may contribute to neurodegenerative diseases, immunodeficiency disorders and infertility. The discovery of these gateway molecules could provide new molecular targets to improve therapy for a wide range of disorders, say the researchers.

"These studies show that BAX and BAK are the obligate gateway that clearly starts the process of apoptosis."

— **Stanley J. Korsmeyer**

In an article published in the April 27, 2001, issue of *Science*, researchers led by Howard Hughes Medical Institute investigator Stanley J. Korsmeyer show for the first time that two pro-apoptotic proteins, called BAX and BAK, constitute the critical initiating factors that launch the apoptotic process in mitochondria.

According to Korsmeyer and his colleagues at the Dana-Farber Cancer Institute at Harvard Medical School, a control protein called tBID triggers BAX and BAK to form pores in the cell membrane that allow cytochrome c to flow into the cell. This influx of cytochrome c launches the biochemical death cascade.

"Earlier work revealed that cells contained an anti-death molecule, BCL-2," said Korsmeyer. "But then we began finding these 'evil twins' such as tBID—closely related members of the BCL-2 family that were pro-death

molecules." The discovery of these molecules led Korsmeyer and his colleagues to propose the "rheostat" model of apoptosis, in which a balance of pro- and anti-apoptotic molecules determines whether a cell lives or dies in response to various stresses. The researchers also found that excess BAX and BAK induced cell suicide if a cell were to be stressed by chemicals or other apoptosis-inducing signals.

"We knew that further upstream, BID had to be activated by known death receptors on the cell surface. But then we began to ask how this activated tBID, and its cousin molecules, triggered the beginning of apoptosis in the mitochondria. We believed it had to cause a central initiating event that released cytochrome c from the mitochondria. We had evidence that tBID just couldn't do it by itself."

In their experiments, Korsmeyer and his colleagues used a retrovirus to insert activated tBID into mouse embryonic fibroblast cells. In those cells, which were engineered to lack single genes for either BAK or BAX, introducing tBID triggered normal cell death.

"So, then we wondered whether both downstream molecules were required, which is why we tested double knockout cells lacking both BAX and BAK," said Korsmeyer. "The combination of taking out both proteins was extremely synergistic, and these cells were highly resistant to apoptosis. It was a very impressive effect," he said. The scientists also found that in mouse liver, BAK and BAX were required for response to normal death signals.

According to Korsmeyer, detailed molecular studies of the behavior of the two proteins suggest that activated tBID can trigger BAK or BAX to change their conformations from receptors in the mitochondrial membrane to pores that allow cytochrome c, and perhaps other death-related molecules, to flow into the cell.

In additional experiments reported in the *Science* article, the researchers tested whether a broad range of death signals—ranging from DNA damage to radiation—required BAX and BAK to trigger apoptosis. They found that the two proteins were, indeed, required. Similarly, stress signals from the cell's endoplasmic reticulum, where protein transport takes place, failed to trigger normal apoptosis without BAX and BAK.

"These studies show that BAX and BAK are the obligate gateway that clearly starts the process of apoptosis," concluded Korsmeyer. He added that this apoptotic process involves disruption of function in the cell, and within the mitochondrion itself, in part because of the loss of cytochrome c from the energy-producing machinery.

Given the central roles of BAX and BAK in apoptosis, said Korsmeyer, "in neurodegenerative disorders involving accelerated apoptosis, inhibiting this pro-death step might prove to be therapeutic. And conversely, in diseases like cancer, accelerating the activation of BAX and BAK could be a potential therapeutic as well."