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Two Switches Turn on UV-Light-Induced Cell Death

Howard Hughes Medical Institute (HHMI) researchers have identified two molecular switches that stimulate programmed cell death induced by lethal doses of ultraviolet (UV) light.

Programmed cell death, also called apoptosis, is normally executed during development to prune unneeded cells or to eliminate cells that have been damaged by stresses, such as radiation or oxidation. If apoptosis is not working properly, however, tumors, immunodeficiency, autoimmune or neurodegenerative disorders may arise.

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Much research has focused on apoptosis because it is thought that learning how to modulate cell death pathways may provide promising new therapies for a number of diseases. Knowing how to switch off programmed cell death, for example, could allow physicians to preserve brain and heart tissue that would otherwise die from oxygen-deprivation during strokes and heart attacks.

In an article in the May 5, 2000, issue of the journal *Science*, HHMI investigator Roger J. Davis and colleagues at the University of Massachusetts Medical School in Worcester report that disrupting a pair of genes, *Jnk1* and *Jnk2*, in embryonic mouse cells allowed those cells to survive lethal doses of UV radiation. HHMI investigator Richard A. Flavell and colleagues at Yale University also aided in developing the *Jnk* mutant mice.

In earlier studies, Davis and his colleagues found that knocking out a similar gene, *Jnk3*, protected brain cells from cell death induced by chemical stresses.

"While we could show that the *Jnk* gene found in neurons was part of the apoptosis pathway, we had much more difficulty extending that finding to

other cells," said Davis. "The problem was that other cells possess the closely related genes, *Jnk1* and *Jnk2*, and knocking out only one produces no detectable effect. On the other hand, knocking out both genes causes death very early during embryogenesis."

In a five-year effort, the scientists solved the problem by learning to isolate and culture mouse embryonic fibroblast cells that lacked both genes.

"By showing that these cells were nearly completely protected from the effects of UV radiation, we clearly established that the *Jnk* genes are part of the stress-induced apoptotic pathway throughout the body," said Davis. "What's more, we were also able to take this finding a step further and establish how *Jnk* fits into the apoptosis pathway discovered by Xiaodong Wang."

Wang, an HHMI investigator at the University of Texas Southwestern Medical Center at Dallas, discovered that the release of an energy-carrying molecule called cytochrome c from the cells' mitochondria is the key molecular event that triggers cell death. Mitochondria are the energy-producing powerhouses of the cell. Scientists believe that mitochondria contain the cell's "sensors" that trigger apoptosis, since malfunctions in mitochondria indicate that cell viability is seriously compromised.

The *Jnk* genes code for proteins called c-Jun NH₂ kinases (JNKs) enzymes that switch on other enzymes by adding a phosphate to them. Biochemical studies by Davis and his colleagues revealed that JNK-deficient cells exposed to UV light failed to release cytochrome c from their mitochondria.

"Finding that JNK was necessary for proper functioning of the cytochrome c pathway was something we had not anticipated," said Davis. "It's important because JNK's role in apoptosis was controversial. This finding serves to fit JNK into an existing and established apoptosis mechanism."

The researchers are also exploring the differences between the enzymes produced by *Jnk1* and *Jnk2*, both of which seem to play roles in cell proliferation in addition to apoptosis.

"So far, the situation is confusing," said Davis. "The two genes are expressed throughout the body, and in most situations they seem to have a very similar function. When you knock out one, it appears as if one gene takes over the role of the other. Now, however, we hope to distinguish the two by taking the knockout cells that contain neither gene, and put one or the other gene back in, and observe whether there is any difference."

Even more complicating, said Davis, is that each of the two genes actually produces four different proteins, formed by alternative splices of different protein segments.

Despite such complications, said Davis, further study of the *Jnk* genes in particular to identify JNK's molecular target in the mitochondria promises not

only greater understanding of apoptosis, but also potential clinical applications.

"While we have much more work ahead to identify the relevant molecular mechanisms, this finding provides a critically important framework for future experiments," said Davis. "It also provides an extremely good opportunity for the development of drugs to turn apoptosis on or off."

In particular, said Davis, drugs that could block JNK to shut down apoptosis during strokes, heart attacks or organ transplantation would preserve cells that would otherwise be killed. Alternatively, drugs that activate JNK to turn on apoptosis selectively in tumor cells could prove highly effective in treating cancers.