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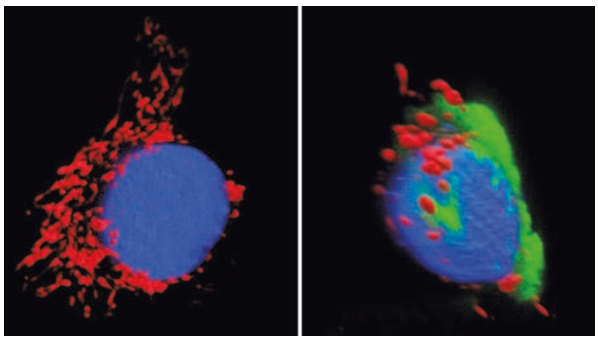
APOPTOSIS

Watching caspase 2 get active

> caspase 2 is activated upstream of MOMP, in the cell cytoplasm, in response to heat shock.

In the mitochondrial apoptosis pathway, cellular stress induces mitochondrial outer membrane permeabilization (MOMP). This results in the release of proteins from the mitochondrial intermembrane space that activate caspase cysteine proteases. So-called initiator caspases cleave and activate downstream caspases, which subsequently cleave other cellular substrates to trigger apoptosis. However, one initiator caspase, caspase 2, does not function in this way. Exactly where, when and how caspase 2 is activated in this apoptotic pathway has been elusive, partly owing to a lack of techniques for monitoring its activation. Douglas Green and colleagues now present a bimolecular fluorescence (BiFC) approach that enables the visualization of caspase 2 activation in single cells in real time. Using BiFC they firmly establish that caspase 2 is activated upstream of MOMP, in the cell cytoplasm, in response to heat shock.



Caspase 2 biomolecular fluorescence (BiFC) is undetectable in control HeLa cells (left image) but detectable in the cytoplasm when caspase 2 is activated by heat shock (green; right image). Nuclei are shown in blue and mitochondria in red. Images courtesy of D. Green, St. Jude Children's Research Hospital, Memphis, Tennessee, USA.

Caspase 2 dimerization induces its activation. Bouchier-Hayes *et al.* fused the carboxy-terminal and amino-terminal parts of venus, a yellow fluorescent protein derivative, to caspase 2 molecules and co-expressed them in human cells. Caspase 2 dimerization brings the venus protein parts into contact, resulting in a fluorescent signal that reflects real-time caspase 2 activation. Using this technique the authors demonstrate caspase 2 activation in response to heat shock, chemicals that disrupt the cytoskeleton, metabolic stress and DNA damage. As heat shock stimulates the most robust BiFC response, the authors characterized caspase 2 activation in response to heat-induced stress.

Although several reports suggest that caspase 2 is activated in the nucleus, three-dimensional images of cells expressing caspase 2 BiFC molecules show that caspase 2 activation occurs in the cytoplasm in response to heat shock. Furthermore, time-lapse microscopy shows that caspase 2 activation precedes the appearance of fluorescently labelled omi — one of the proteins released from mitochondria during MOMP — by at least 2 hours. This places caspase 2 activation upstream of MOMP.

Finally, the authors sought to identify the events that regulate caspase 2 activation. Heat induces the transcription of heat shock proteins, such as heat shock protein 90α (HSP90α), which promote cell survival. The authors find that inhibiting HSP90α using chemicals, or reducing its expression using short hairpin RNA, increases the number of BiFC-positive cells in response to heat shock. Thus, HSP90α might negatively regulate caspase 2 activation.

The authors therefore demonstrate and validate a valuable technique for studying the activation of initiator caspases in real time, and use this approach to show that heat shock-induced caspase 2 activation occurs upstream of MOMP in the mitochondrial apoptotic signalling cascade.

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ORIGINAL RESEARCH PAPER Bouchier-Hayes, L. *et al.* Characterization of cytoplasmic caspase-2 activation by induced proximity. *Mol. Cell* **35**, 830–840 (2009)

FURTHER READING Taylor, R. C. *et al.* Apoptosis: controlled demolition at the cellular level. *Nature Rev. Mol. Cell Biol.* **9**, 231–241 (2008)