DOI: 10.1017/S1431927605505956
Microsc Microanal 11(Suppl 2), 2005
Copyright 2005 Microscopy Society of America

Monitoring mitochondrial caspase-2 activity in intact cells by streak camera based fluorescence lifetime imaging microscopy

Brian A Herman, Jian-Hua Zhang, V Krishnan Ramanujan, Marisa Lopez-Cruzan, Salvador Medina Jr., Victoria E Centonze

Department of Cellular and Structural Biology, UT Health Science Center, 7703 Floyd Curl Drive, San Antonio, TX 78229, USA

Caspase-2 was the first mammalian apoptotic caspase to be cloned. Structural and functional studies showed that caspase-2, like caspase-8, -9 and -10, functions as an initiator caspase [1]. Accumulating evidence supports the hypothesis that caspase-2 plays an important role in the intrinsic pathway of apoptosis, in which mitochondrial outer membrane permeabilization (MOMP) occurs [2]. The intracellular localization and function of procaspase-2 has been debated in the literature. Initial reports placed caspase-2, along with caspase-9, in mitochondria. Other studies that used multiple antibodies to different epitopes of caspase-2 in conjunction with Western blot or immunostaining approaches, were unable to detect caspase-2 in mitochondria but found caspase-2 expression in nuclei, cytosol and Golgi [3]. In an effort to clarify these conflicting results and directly measure the activity of caspase-2 within mitochondria of cells undergoing apoptosis, we have genetically targeted a fluorescent caspase-2 substrate (VDVAD) to mitochondria in intact cells. The caspase-2 substrate is flanked by two fluorescent proteins that can undergo fluorescence resonance energy transfer (FRET) when the substrate is intact but lose the ability to undergo FRET when caspase-2 is activated and the substrate has been cleaved.

We recently developed a highly sensitive, multiphoton fluorescence lifetime imaging microscopy (FLIM) system using a streak camera that can achieve a time resolution ~ 50 ps in live cell imaging [4]. We employed this FLIM methodology for monitoring mitochondrial caspase-2 activity in intact cells in conjunction with the well-established acceptor photobleaching FRET method. Furthermore, we carried out cellular subfractionation and enzymatic assays to substantiate the results obtained from FRET imaging. The salient findings in our study are: (i) caspase-2 activity is localized in mitochondria upon the induction of apoptosis, (ii) the onset of caspase-2 activation following induction of apoptosis first occurs in the cytosol followed by its activation in the mitochondria, and (iii) absence of caspase-2 protects cells selectively from cell death induced by the mitochondrial oxidant stressor t-BOOH. In conclusion, our results support the hypothesis that caspase-2 is a critical element in oxidative stress induced cell death. Since reactive oxygen species play an important role in many pathological processes such as ischemia-reperfusion lesions, neurodegenerative diseases, and aging, it would be interesting to see if genetic modification of caspase-2 expression would have beneficial effects on these conditions.

[1] Troy and Shelanski, Cell Death Differ. 10: 101-107 (2003)
[2] Lassus et al, Science 297: 1352-1354 (2003)
[3] O'Reilly et al, Cell Death Differ. 9:832-841 (2002)
[4] Krishnan RV et al., Rev.Sci.Instrum. 74: 2714-2721 (2003).

https://doi.org/10.1017/S1431927605505956 Published online by Cambridge University Press

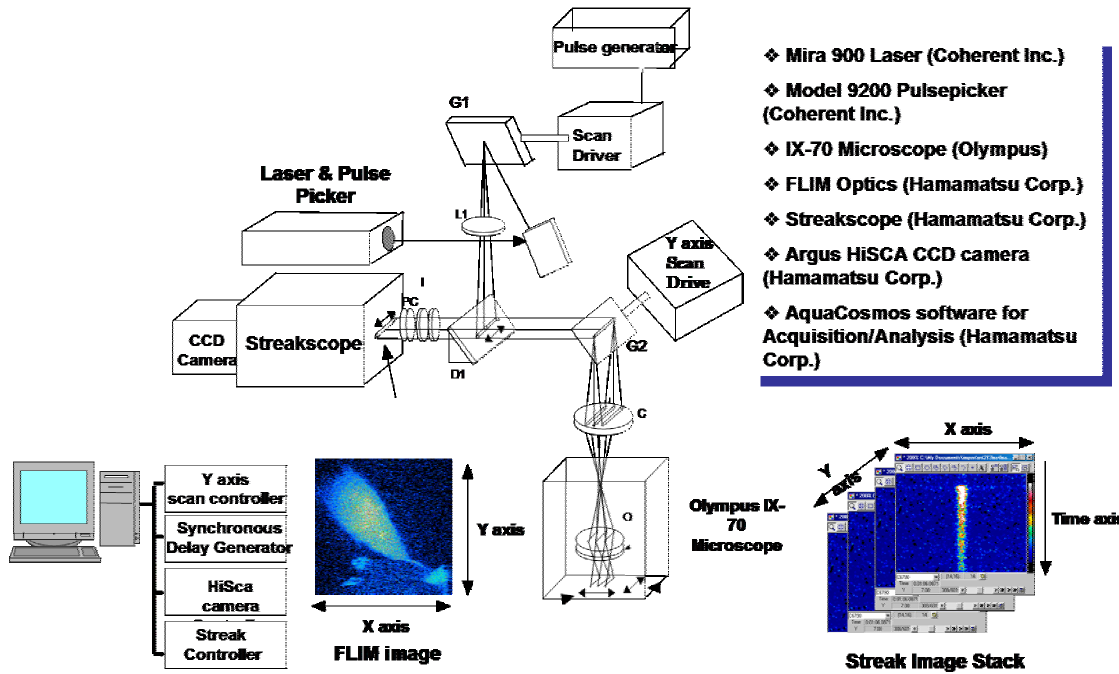

Fig 1: Schematic of StreakFLIM system. Fluorescence decay curves at every point in the specimen were collected by scanning the multiphoton excitation laser spot across (x,y) direction on the specimen. A numerical processing of these decay curves gives the fluorescence lifetime (τ) on a pixel-per-pixel basis and these values are reassigned to the corresponding (x,y) position on the specimen field of view to obtain the final fluorescence lifetime image (FLIM).

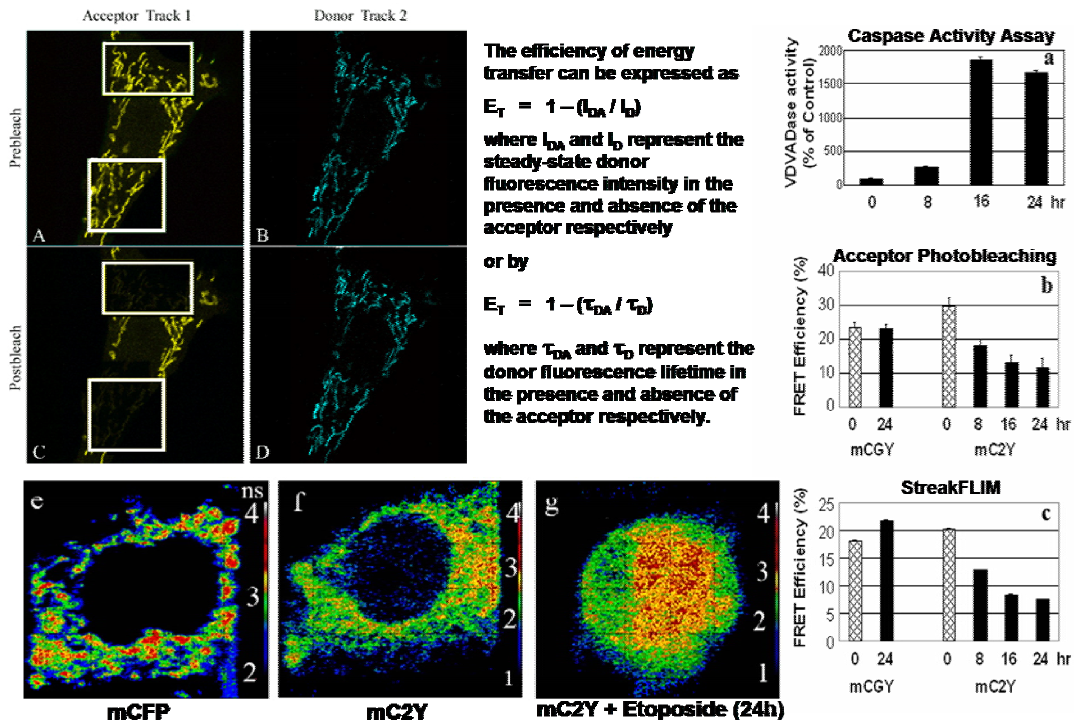

Fig 2: Oxidative stress-induced mitochondrial caspase-2 activity in intact cells as monitored by acceptor-photobleaching FRET (top panel), fluorescence lifetime imaging microscopy FRET (bottom panel) and biochemical assays (right panel).

https://doi.org/10.1017/S1431927605505956 Published online by Cambridge University Press