

Focus on Apoptotic Signalling in Living Cells

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Apoptosis is a highly controlled cell death mechanism that is crucial for eliminating damaged or superfluous cells in multi cellular organisms. After the induction of apoptosis cells can die within hours or days depending on the stimulus used or type of cell. With the help of state-of-the-art live cell microscopy we now can dissect the signalling steps leading to apoptosis. Mitochondria are key organelles as they integrate death signals and determine the cells fate after outer mitochondrial membrane permeabilisation, while most of the mitochondrial signalling occurs in a limited time frame of less than 30 min. To resolve the sequence of signalling events we use GFP fusion proteins. Thereby, we can confirm the function of the involved proteins spatiotemporally under *in vivo* conditions. Exploiting live cell imaging we focus mainly on signalling processes involving mitochondria and the post-mitochondrial execution phase of apoptosis:

- The outer mitochondria membrane is permeabilised by pro-apoptotic Bcl-2 protein family members like Bax, which translocates to mitochondria after it's dimerisation. We detect this in cells expressing Bax-CFP and Bax-YFP fusion proteins when FRET occurs between the fluorescent proteins.
- Mitochondrial inter membrane proteins like cytochrome-c are released after the outer mitochondrial membrane permeabilisation to initiate the apoptotic execution. We use cytochrome-c-GFP or SMAC-YFP fusion proteins to observe this event [1, 2]
- After this release the inner mitochondrial membrane potential depolarises. We measure the mitochondrial membrane potential via the intensity of TMRM, that distributes across polarised membranes according to the Nernstian Equation [1, 2].

Subsequently, proteases of the caspase family get activated to execute the apoptotic cell death. This can be detected with FRET applying a CFP-DEVD-YFP fusion protein; while the DEVD sequence is a specific executioner caspase cleavage site [3]. Currently we use the mitochondrial membrane potential depolarisation as the temporal reference point for all other signalling events. Developing further experimental protocols including new fusion proteins especially for the detection of their intracellular interaction via FRET will allow us to extend the number of observable apoptotic signalling events, to detect them with higher precision, and to confirm signalling protein interactions in single cells.

References:

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