

2.1.2 Assays that measure apoptosis-induced proteases (caspases)

Several caspases (see Table 20, in the Appendix, page 123) are thought to mediate very early stages of apoptosis¹⁰. For instance, one of these, caspase 3 (CPP32) is required for the induction of apoptosis by certain effectors [especially tumor necrosis factor and the cytotoxic T cell ligand effector, CD95 (also called Fas)] Enari et al. (1996) , *Nature* **380**, 723–726.

These proteases cleave numerous substrates at the carboxy site of an aspartate residue. All are synthesized as pro-enzymes; activation involves cleavage at aspartate residues that could themselves be sites for the caspase family. As caspases are probably the most important effector molecules for triggering the biochemical events which lead to apoptotic cell death, assays for determination of caspase activation can detect apoptosis earlier than many other commonly used methods.

The most elucidatory assay for these caspases involves western blot detection of proteolytic cleavage products found in apoptotic cells. An antibody, Anti-PARP, sold by Roche Applied Science, can be used in such an assay. The antibody can detect intact and cleaved forms of Poly-ADP-Ribose Polymerase, a target for some caspases.

For specific and quantitative measurement of caspase activity Western blotting is not suitable. To quantify caspase activation enzyme activity assays based on detection of cleaved caspase substrates have been developed recently. However most of the caspase substrates are not exclusively cleaved by a specific caspase but only preferentially, while other members of the caspases family act on these substrates to a lower extent. Roche Applied Science offers a caspase 3 activity assay with highest specificity by the use of an immunosorbent enzyme assay principle.

