

PARTIAL APOPTOSIS? NUCLEAR “DEATH” IN LIVING CELLS

J Wolfe (P), SB Broder-Fingerhut, E Lu, S Singhal, and C Balane

Biology Department, Wesleyan University, Middletown, CT 06117

Aging is associated with reduction in tissue mass due to, among other things, regulated cell death, or apoptosis. Apoptosis is dependent on the activation of caspases, a family of aspartate-specific cysteinyl proteases. It is characterized by nuclear condensation, and inter-nucleosomal DNA cleavage (related to caspase cleavage of an endonuclease inhibitor), as well as cytoskeletal collapse and break-off of cellular fragments, or apoptotic bodies. During apoptosis nuclear demise and cellular death are closely coordinated. In certain situations however, such as during conjugation in the ciliate *Tetrahymena*, nuclear degradation occurs in a normal, healthy, living cell. The parental macronucleus, which is eliminated during sex-mediated genetic reorganization, becomes highly compact as chromatin condenses. Further, DNA is degraded by inter-nucleosomal cleavage in those macronuclei. In addition, caspase-like activity is increased during macronuclear elimination. Most significantly, macronuclear loss is arrested by millimolar concentrations of caspase inhibitors such as DEVD and zVAD. These data suggest that nuclear death in *Tetrahymena* is regulated by a mechanism related to apoptosis. In multicellular organisms apoptotic bodies are cleared by phagocytosis. We investigated the possibility that within a cell a “dying” nucleus is cleared by the equivalent, intracellular mechanism of autophagy, a lysosome-mediated process. We demonstrate that the degrading macronucleus accumulates acridine orange, a sign of acidification. Further, acid phosphatase, a lysosomal enzyme, co-localizes with the condensed macronucleus. Moreover, Texas Red-Dextran (TRD) pre-loaded into lysosomes becomes transferred into the condensed macronucleus. Finally, 3-methyladenine, an inhibitor of autophagy, blocks TRD transfer, and blocks macronuclear degradation. These data support a role for lysosomes in the elimination of the macronucleus, consistent with autophagy. Taken together, these results are consistent with a model of nuclear death involving regulation by apoptosis and elimination by autophagy. This model may also help to explain other systems of nuclear death such as occur during keratinocyte differentiation and muscle atrophy.