Yeast apoptosis and proteolysis in mitochondrial biogenesis

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Apoptosis is a genetically controlled process of cell suicide that plays a critical role in development and homeostasis. The apoptosis is a precisely regulated by the balance of proapoptotic and antiapoptotic factors and mitochondria represent the key regulation point. Disruption of apoptosis leads to development defects, cancer, neurodegenerative, and autoimmune diseases.

Yeast cell death with phenotype resembling mammalian apoptosis can be induced by various treatments including acetic acid, peroxide, or by ectopic expression of mammalian proapoptotic protein Bax. The cell death of *S. pombe* induced by these agents can be prevented by expression of antiapoptotic protein Bcl-X_L and also with an inhibitor of protein synthesis-cycloheximide, demonstrating the apoptosis-like features of these cell death-inducing processes. The deletion of mitochondrial ADP/ATP carrier, or the presence of inhibitors of respiration and oxidative phosphorylation, suppressed the protective action of Bcl-X_L. Our results demonstrate that functional mitochondrial oxidative phosphorylation is not required for Bax induced cell death, but it is essential for the Bcl-X_L protection against cell death induced by various agents. Apoptotic cell death in *S. pombe* is accompanied by changes in mitochondrial morphology. We observed the loss of mitochondrial tubular structure and the accelerated fragmentation of the mitochondria network, which can be prevented with expression of the antiapoptotic Bcl-X_L protein. These changes in mitochondrial morphology are not associated with a loss of mtDNA: expression of Bax actually leads to a moderate increase in the amount of mtDNA in *S. pombe* cells (1).

ATP-dependent proteases are crucial for cellular and mitochondrial homeostasis. By degrading short-lived regulatory proteins and abnormally misfolded proteins, they play an important role in the control of many cellular pathways. Disruption or disregulation of mammalian mitochondrial Lon protease leads to severe changes in the cell, linked with carcinogenesis, apoptosis, and necrosis. Although Lon is one of the least complicated ATP-dependent proteases, a structure of the full-length protein has been determined only just recently (Thermococcus onnurineus NA1 Lon). We determined the crystal structure of the human mitochondrial Lon protease (2). Although the overall structure is very similar to the *Ec*Lon one, the conformation around the active site more closely resembled that seen in the *Methanococcus jannaschii* Lon structure. A detailed analysis of these three structures led us to propose a mechanism by which hexamer formation is coupled to a conformational transition at the active site, which converts the inactive conformation seen in the *h*Lon structure to one resembling that seen in the *Ec*Lon one. To better understand the roles of the proteolytic domain in the overall functions of human Lon protease, we designed several point mutations in this domain based on the known Lon protease crystal structures. We then tested their influence on protease, peptidase, and ATPase activity as well as on oligomer formation and stability.

References

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(2) García-Nafría J. et al. (2010). Structure of the catalytic domain of the human mitochondrial Lon protease: proposed relation of oligomer formation and activity. *Protein Sci.* 19(5): 987-99.