Identification of mitochondrially-localized DNases responsible for nuclear apoptosis in *Tetrahymena thermophila*

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SUMMARY

The ciliated protozoan *Tetrahymena thermophila* has a unique mechanism of apoptosis-like nuclear degradation, called programmed nuclear death (PND). During conjugation, new micronuclei and macronuclei differentiate from a fertilized nucleus, whereas the parental macronucleus is eventually eliminated from the cytoplasm of exconjugants. In this process, the degenerating macronucleus and many mitochondria are taken in a large autophagosome together, where these sequestered mitochondria have lost their membrane potential. In animal apoptosis, mitochondrial factors such as apoptosis-inducing factor (AIF) and endonuclease G (EndoG) are involved in nuclear condensation and DNA laddering. Herein, we show that *Tetrahymena* AIF homolog is involved in PND. To elucidate the function of AIF in PND, we isolated mitochondria from wild-type and AIF deficient *Tetrahymena*. Comparison of both mitochondrial DNase activities revealed that the AIF deficient mitochondria showed drastically reduced activity, suggesting that AIF interacts with mitochondrial DNase, which plays a major role in PND. Furthermore, to identify nucleases localized in mitochondria further, we performed DNase assay on the polyacrylamide gel (SDS-DNA-PAGE) using mitochondrial proteins obtained from wild type. Some proteins showed DNase activity. Especially, a protein of approximately 15 kDa showed the highest activity. These nucleases might additionally support the PND progression.