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Production and characterization of recombinant protein preparations of Endonuclease G-homologs from yeast, C. elegans and humans

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Abstract

Nuc1p, CPS-6, EndoG and EXOG are evolutionary conserved mitochondrial nucleases from yeast, Caenorhabditis elegans and humans, respectively. These enzymes play an important role in programmed cell death as well as mitochondrial DNA-repair and recombination. Whereas a significant interest has been given to the cell biology of these proteins, in particular their recruitment during caspase-independent apoptosis, determination of their biochemical properties has lagged behind. In part, biochemical as well as structural analysis of mitochondrial nucleases has been hampered by the fact that upon cloning and overexpression in Escherichia coli these enzymes can exert considerable toxicity and tend to aggregate and form inclusion bodies. We have, therefore, established a uniform E. coli expression system allowing us to obtain these four evolutionary related nucleases in active form from the soluble as well as insoluble fractions of E. coli cell lysates. Using preparations of recombinant Nuc1p, CPS-6, EndoG and EXOG we have compared biochemical properties and the substrate specificities of these related nucleases on selected substrates in parallel. Whereas Nuc1p and EXOG in addition to their endonuclease activity exert 5'-3'- exonuclease activity, CPS-6 and EndoG predominantly are endonucleases. These findings allow speculating that the mechanisms of action of these related nucleases in cell death as well as DNA-repair and recombination differ according to their enzyme activities and substrate specificities.

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Keywords

Apoptosis, DNA-repair, Endonuclease, Exonuclease, Mitochondria, Refolding