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Structural and Functional Characterization of Mitochondrial EndoG, a Sugar Non-specific Nuclease which Plays an Important Role During Apoptosis

Patrick Schäfer[1]†, Sebastian R. Scholz[1]†, Oleg Gimadutdinow[2] Iwona A. Cymerman[3], Janusz M. Bujnicki[3], Adolf Ruiz-Carrillo[4] Alfred Pingoud[1] and Gregor Meiss[1]*

[1]*Institute of Biochemistry Justus-Liebig-University Heinrich-Buff-Ring 58 D-35392 Giessen, Germany*

[2]*Department of Genetics Kazan State University Kremlevskaja 18, 420008 Kazan, Russian Federation*

[3]*Bioinformatics Laboratory International Institute of Molecular and Cell Biology Trojdena 4, 02-109 Warsaw Poland*

[4]*Department of Molecular and Cell Biology, Institute of Molecular Biology of Barcelona (IBMB), CID-CSIC Jordi Girona 18-26 08034 Barcelona, Spain*

**Corresponding author*

Combining sequence analysis, structure prediction, and site-directed mutagenesis, we have investigated the mechanism of catalysis and substrate binding by the apoptotic mitochondrial nuclease EndoG, which belongs to the large family of DNA/RNA non-specific ββα-Me-finger nucleases. Catalysis of phosphodiester bond cleavage involves several highly conserved amino acid residues, namely His143, Asn174, and Glu182 required for water activation and metal ion binding, as well as Arg141 required for proper substrate binding and positioning, respectively. These results indicate that EndoG basically follows a similar mechanism as the *Serratia* nuclease, the best studied representative of the family of DNA/RNA non-specific nucleases, but that differences are observed for transition state stabilisation. In addition, we have identified two putative DNA/RNA binding residues of bovine EndoG, Arg135 and Arg186, strictly conserved only among mammalian members of the nuclease family, suggesting a similar mode of binding to single and double-stranded nucleic acid substrates by these enzymes. Finally, we demonstrate by ectopic expression of active and inactive variants of bovine EndoG in HeLa and CV1-cells that extramitochondrial active EndoG by itself induces cell death, whereas expression of an enzymatically inactive variant does not.

Keywords: apoptosis; nuclease; endonuclease G; active-site; catalysis

Introduction

The mitochondrial endonuclease G (EndoG) is an enzyme involved in a variety of cellular functions, ranging from the generation of primers for mitochondrial DNA replication to the initiation of genomic inversion in herpes simplex type-1 virus (HSV-1).[1–3] One of the most recent findings is, that upon apoptotic stimuli, EndoG can be released from mitochondria and then contributes to the degradation of chromosomal DNA during apoptosis.[4–6] Database searches reveal that EndoG is a member of an ancient family of DNA/RNA non-specific nucleases. Nucleases of this family are found in a large number of pro- and eukaryotic organisms, including bacteria, protozoa, fungi and slime moulds, invertebrates and vertebrates.[7] The prokaryotic nucleases are usually secreted by their bacterial hosts and are likely to serve nutritional purposes, whereas in eukaryotic organisms these nucleases are targeted to mitochondria and, e.g. in *Caenorhabditis elegans* and mammals, contribute to apoptotic DNA degradation.[4,5,8,9] One of the structurally and mechanistically best characterized members of the DNA/RNA non-specific nucleases is the extracellular nuclease of *Serratia marcescens*.[10–12]

Mammalian EndoG is encoded in the nucleus and produced as a ~33 kDa preprotein with a mitochondrial targeting sequence at its N

† P.S. and S.R.S. contributed equally to this work.

Abbreviations used: EndoG, mitochondrial endonuclease G; HSV-1, herpes simplex type-1 virus; AIF, apoptosis inducing factor; tEndoG, truncated bovine EndoG; WAH-1, worm-AIF-homolog 1.

E-mail address of the corresponding author: gregor.meiss@chemie.bio.uni-giessen.de