CHARACTERIZATION OF A NUCLEAR MATRIX-ASSOCIATED ENDONUCLEASE. Nevena Grdović and G. Poznanović, Laboratory of Molecular Biology, Institute for Biological Research "S. Stanković", 11060 Belgrade, Serbia and Montenegro.

The nuclear matrix is a proteinaceous network that serves as a structural framework of the eukaryotic nucleus. It was isolated by treating purified nuclei with nucleases, followed by extractions with high-salt and nonionic detergent (Bereznay and Coffey 1974). Aside from its well-defined structural role, the nuclear matrix is involved in many nuclear functions. It participates in chromatin organization and every aspect of DNA metabolism: from DNA synthesis, gene transcription to DNA degradation (Bereznay et al. 1995; Getzenberg 1994; Gromova et al. 1995).

DNA degradation during apoptosis starts with the release of high-molecular weight DNA fragments (50-300 kb) from their points of attachment on the nuclear matrix structure. Although the nuclease that catalyses this process is still unidentified, the biochemical requirements of initial apoptotic DNA degradation are well established (Sun and Cohen 1994; Kukleva 1995). Recently, it was shown that nonapoptotic cells contain a constitutive high-molecular weight fragmentation activity that is localized on the isolated nuclear matrix (Solovyann et al. 2002). In this paper we describe for the first time an endonuclease that is associated with the rat hepatocyte nuclear matrix. At present, a role for such a nuclear matrix-associated enzymatic activity is unclear. One possibility is that the nuclear matrix-associated endonuclease is involved in apoptotic DNA degradation. In order to verify this assumption, the biochemical requirements of the nuclear matrix-associated nuclease were examined.

Nuclear matrices were isolated from purified hepatocyte nuclei essentially as described previously (Poznanović et al. 1996) except that the nuclei were subjected to endogenous nuclease digestion. Endonuclease activities in the protein fractions were established by activity-gel analysis. The method is based on the electrophoretic separation of proteins in 10% SDS-polyacrylamide gels containing 100 μg/mL salmon sperm DNA (Auch et al. 1997). After electrophoresis the gels were washed in renaturation buffer (50 mM Tris pH 7.0, 1 mM DTT) overnight at 4°C, and incubated for 24 h in the same buffer with the addition of different cations in order to activate nuclease activity/ies. The gels were then stained with ethidium bromide and nuclease activities were detected as dark areas on a fluorescent background after transillumination of the gels with UV light.

Using this method we examined the presence of endonucleases in rat hepatocyte nuclei and their redistribution in protein fractions released during the isolation of the nuclear matrix (Fig. 1B). Initially, activity-gel analysis was performed at neutral pH and in the presence of Ca and Mg ions - the requirements for full rat hepatocyte endonuclease activity. Under given experimental conditions two enzymes were detected in the nuclear fraction (Fig. 1B, lane 1). The nuclease activity of the lower molecular weight protein was apparently higher. Although some of this nuclease activity could be detected in the protein fraction released after endogenous endonuclease digestion (lane 2), for the most it was resistant to extraction with high-salt and nonionic detergent (lanes 3 and 4) and predominantly partitioned in the nuclear matrix (lane 5). In order to establish its molecular weight, the dark area obtained after activity-gel analysis was excised and subjected to SDS-PAGE. After silver-staining it was estimated to be about 23 kD (results not shown).

The biochemical requirements of the 23 kD endonuclease were examined using the same method. First, the pH optimum was determined by performing activity gel analysis in the presence of Ca and Mg ions at different pH (Fig. 1C). The activity of 23 kD endonuclease was negligible at acidic pH values (lanes 1 and 2), maximal at neutral pH (lane 3), while basic pH led to its inhibition (lane 4).

Since most documented endonucleases require the presence of divalent cations for full activity (Zrichtoovsky et al. 1994), we examined the effect of Mg and Ca ions on the activity of p23. To that end, activity gel analysis was either performed in the presence of 5 mM MgCl₂ throughout with increasing concentrations of CaCl₂ (Fig. 1D, lanes 4-6) or in the presence of increasing concentrations of MgCl₂ (Fig. 1D, lanes 1-3). It was established that Ca²⁺ was not required for p23 activity, although its presence exerted a slight stimulatory effect. The optimal concentration of Mg²⁺ was between 5 and 10 mM. When activity gel analysis was performed with 5 mM MgCl₂ and 5 mM EDTA, p23 was completely inhibited (results not shown). On the basis of this finding we concluded that p23 is a Mg²⁺-dependent endonuclease. It is an established fact that the generation of high-molecular weight DNA fragments is a process with an absolute requirement for Mg²⁺ (Sun and Cohen 1994). Thus, the localization of p23 on the nuclear matrix, as well as its absolute requirement for Mg for its activity could qualify this enzyme as a potential participant in initial apoptotic DNA degradation.

We also examined the effect of Zn ion by performing activity gel analysis in the presence of 1 mM ZnCl₂ and 5 mM MgCl₂. Namely, it has been shown that Zn does not affect initial DNA degradation into large fragments, although this ion inhibits many described endonucleases (Kukleva 1995).
To summarize, a 23 kD nuclear endonuclease that is a constituent of the rat hepatocyte nuclear matrix has been characterized. It displayed an absolute requirement for Mg^{2+} for its activity, and was fully active in the absence of Ca^{2+} and in the presence of Zn^{2+}. Its optimal activity was observed at neutral pH in the absence of either Na^{+} or K^{+} ions. Our findings are in agreement with previously documented biochemical properties of the nuclease activity that is responsible for initial DNA degradation during apoptosis. Thus, the 23 kD endonuclease could qualify as the apoptotic endonuclease that is responsible for the first-stage of DNA degradation.

Further evidence for a role of p23 in apoptosis is provided by the observation that the enzyme retained its full activity in the presence of Zn (Fig. 1D, lane 7). It was also determined that p23 was inhibited by monovalent cations Na^{+} and K^{+} at physiologically relevant concentrations (Fig. 1E). Maximal activity of p23 was observed in the absence of either monovalent cation (lane 1), whereas increasing concentrations of NaCl (lanes 2-4) and KCl (lanes 5-7) led to a diminishment of its activity. Also, a cationic composition of the buffer that resembled the in vivo state in nuclei completely inhibited the enzymatic activity of p23 (results not shown). Our findings are in agreement with the hypothesis that physiological concentrations of K^{+} maintain apoptotic systems inactive while an efflux of K^{+} that is observed during apoptosis leads to its activation (Bortnere et al. 1997). Therefore, inhibition of the enzyme at "cellular" concentrations of cations could be responsible for its inactivity at nonapoptotic, physiological conditions.

To summarize, a 23 kD nuclear endonuclease that is a constituent of the rat hepatocyte nuclear matrix has been characterized. It displayed an absolute requirement for Mg^{2+} for its activity, and was fully active in the absence of Ca^{2+} and in the presence of Zn^{2+}. Its optimal activity was observed at neutral pH in the absence of either Na^{+} or K^{+} ions. Our findings are in agreement with previously documented biochemical properties of the nuclease activity that is responsible for initial DNA degradation during apoptosis. Thus, the 23 kD endonuclease could qualify as the apoptotic endonuclease that is responsible for the first-stage of DNA degradation.

Acknowledgement – This work was supported by the Ministry for Science, Technology and Development of the Republic of Serbia, Contract no. 1722