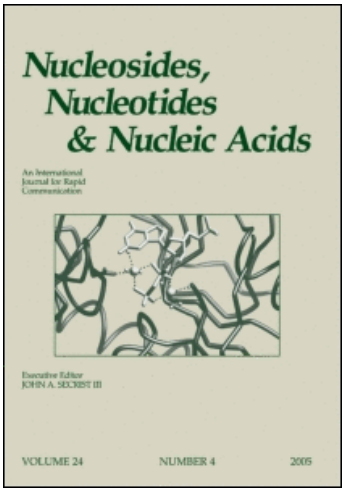


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# Release of Nucleic Acids by Eukaryotic Cells in Tissue Culture

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## ABSTRACT

Extracellular nucleic acids in cultures of A431 and HeLa cells were investigated. The data obtained demonstrate the presence of high weight DNA and RNA in the extracellular medium. Temporal changes of extracellular nucleic acids levels in growth medium were investigated.

*Key Words:* Extracellular DNA; Extracellular RNA; Secretion of DNA and RNA.

DNA and RNA are present in extracellular medium of eukaryotic cell cultures.[1,2] It was clearly demonstrated that neither cell necrosis nor apoptosis may fully explain the presence of extracellular nucleic acids,[3] although these processes are undoubtedly involved in the generation of extracellular nucleic acids.[4] Factors affecting release of extracellular nucleic acids from cells in culture medium and in organism are not understood to date.

We have investigated time course characteristics of release and composition of extracellular nucleic acids in cultures of A431 and HeLa cells. To investigate the kinetics

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of extracellular nucleic acids appearence in cellular supernatants HeLa and A431 cells were rinsed with phosphate buffered saline containing 5 mM EDTA (PBS-EDTA) 12 hours after seeding (zero point) with following cultivation. At different time intervals nucleic acids were isolated from growth medium and PBS-EDTA supernatant using quantitative guanidine thiocyanate/glass–milk method[5,6] and quantified using fluorescence-based assay, which allows the measurement of RNA and DNA content in probes containing both nucleic acids.[7]

The data obtained reveals the presence of both DNA and RNA in the growth medium and the only RNA in PBS-EDTA supernatants for both cell cultures (Fig. 1). Extracellular DNA (exDNA) concentration in growth medium constantly increases after PBS-EDTA treatment (Fig. 1, pannels A, C). Extracellular RNA (exRNA) concentration in growth medium increases after PBS-EDTA treatment during the first 3 hours with subsequent decrease up to zero during following 45 hours of cultivation for HeLa cells (Fig. 1, pannel B) and 24 hours of cultivation for A431 cells (Fig. 1, pannel D). Time dependence of RNA concentration changes in PBS-EDTA supernatants and in growth



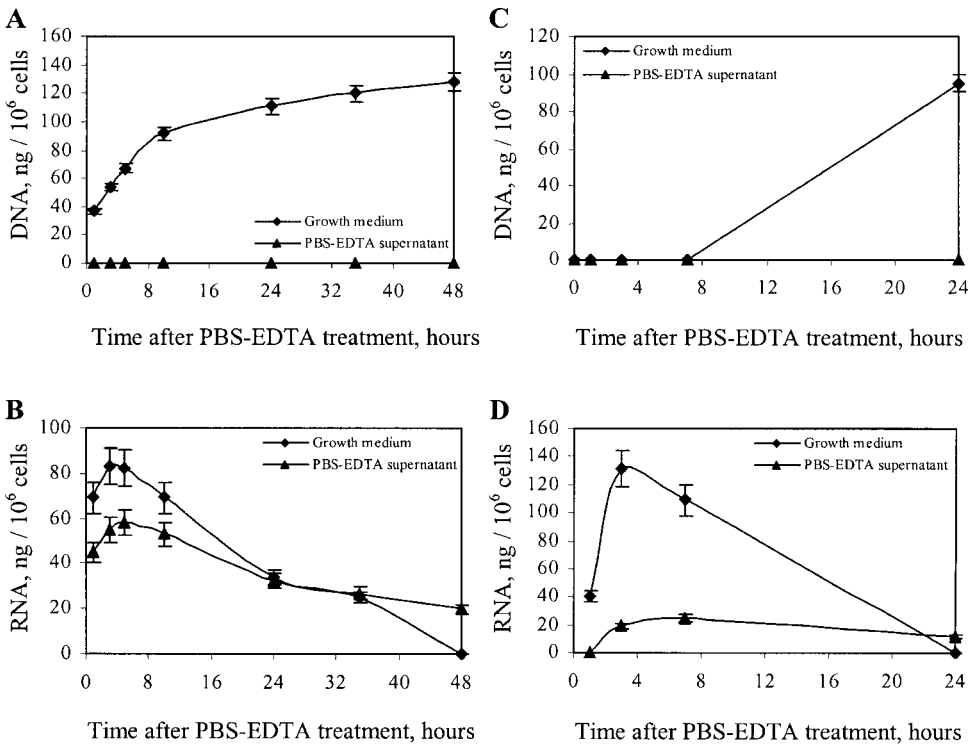


***Figure 1.*** Investigation of the time course of extracellular nucleic acids excretion. HeLa (Pannels A, B) and A431 (Pannels C, D) cells were seeded with following cultivation in DMEM culture medium supplemented with 10% FBS, at 37°C, 5% $CO_2$ at density of $5 \times 10^5$ per $100 \times 20$ mm culture dish. 12 hours after seeding cells were washed for 3 minutes at room temperature with phosphate buffered saline containing 5 mM EDTA (PBS-EDTA) (zero point) with following cultivation. At different time intervals growth medium was discarded and cells were rinsed with 2 ml of PBS-EDTA for 3 minutes. Cell viability was confirmed with trypan blue inclusion microscopy and MTT-tests. Nucleic acids from growth medium and PBS-EDTA supernatant were isolated and quantified.

medium were similar, but cell surface associated RNA was found to be more stable and was detected in this fraction even 48 hours after PBS-EDTA treatment (Fig. 1, pannels B, D).

Electrophoretic analysis of extracellular nucleic acids revealed that exRNA eluted with PBS-EDTA 3 hours after treatment is mainly represented as the clear band with the same electrophoretic mobility in 1% agarose gel as 10 kbp DNA (Fig. 2, pannel A), whereas exRNA isolated from growth medium is about 100–200 bases corresponding to 2,5–5S RNA which was found in culture medium by other investigators.[1,2] 24 hours after PBS-EDTA treatment only short RNA fragments about 100–200 bases were

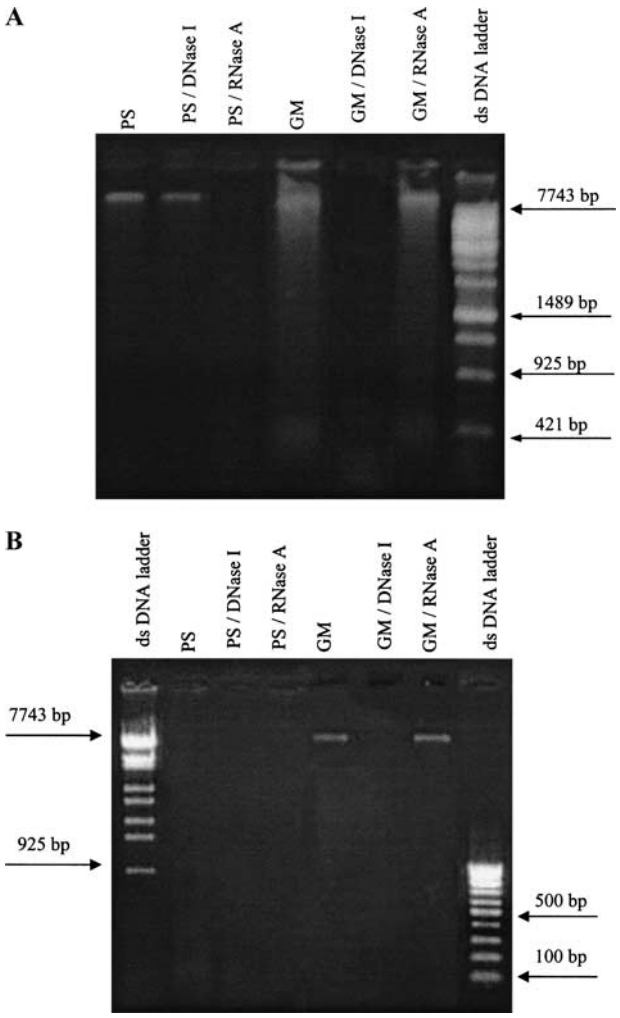


***Figure 2.*** Electrophoretic analysis of extracellular nucleic acids. Nucleic acids isolated from PBS-EDTA supernatant (PS) and growth medium (GM) of HeLa cells were electrophoresed on 1% agarose gels. To hydrolyze either DNA or RNA isolated nucleic acids were incubated with 0,1 mg/ml DNase I, 37°C, 2 hours in 10 mM Tris-HCl, 2,5 mM $MgCl_2$, pH 7,5 or 0,1 mg/ml RNase A, 37°C, 2 hours in 10 mM Tris-HCl, pH 7,5. Panel A—3 hours after zero point. Panel B—24 hours after zero point.

found in PBS-EDTA supernatant (Fig. 2, pannel B) suggesting that short-term secretion of high weight RNA after PBS-EDTA treatment is followed by degradation of secreted RNA. ExRNA eluted from cell surface is not protected from nuclease digestion and is highly accessible to nuclease treatment (data not shown).

3 hours after PBS-EDTA treatment 0,4 kbp, 0,85 kbp, 1,5 kbp, and 8,5–10 kbp DNA fragments were found in growth medium (Fig. 2, pannel A). Appearence of short DNA fragments in the growth medium may be explained by apoptotic process that can be related to PBS-EDTA treatment of cells. 24 hours after PBS-EDTA treatment clear DNA band about 8,5–10 kbp was found in growth medium (Fig. 2, pannel B). Disappearance of short exDNA fragments from growth medium is probably caused by their hydrolysis with extracellular DNases or by their penetration into cells.

The data obtained demonstrate prominent concentrations of cell surface associated exRNA and free exDNA in culture medium. Similar characteristics of extracellular nucleic acids concentrations and composition under cultivation of different cell lines suggest the importance of extracellular nucleic acids for normal cell growth.



## ACKNOWLEDGMENTS

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