Extracellular DNA in Culture of Primary and Transformed Cells, Infected and Not Infected with Mycoplasma

E. S. Morozkin, V. N. Sil'nikov, E. Yu. Rykova, V. V. Vlassov, and P. P. Laktionov

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The composition and kinetics of accumulation of extracellular DNA in cultures of primary human endotheliocytes, cervical adenocarcinoma, and mycoplasma-infected cervical adenocarcinoma cells were studied. The content of DNA bound to cell surface did not change during culturing. The concentration of extracellular DNA in culture medium increased during the lag phase and at the beginning of the exponential growth phase, which probably attests to active secretion of DNA by cells. Spontaneous extracellular DNA synthesis was observed only in cell culture infected with mycoplasma.

Key Words: *extracellular DNA; HUVEC; HeLa; mycoplasma*

Extracellular DNA is present in culture medium and on the surface of eukaryotic cells [6,10]. According to some data, the main sources of extracellular DNA are apoptosis and necrosis [5,12]. However, other data indicate that extracellular DNA appears in culture medium due to its active secretion by cells and its apearance is a homeostatic process [11]. Up to the present time, studies of extracellular DNA in cell culture were carried out on continuous cells or lymphocytes not dividing without stimulatory signals. These cells were not tested for mycoplasma infection, while according to some data, 10-87% cell cultures are infected with mycoplasma [2], which can lead to increase or decrease in the concentrations of extracellular nucleic acids.

We studied the levels of extracellular DNA in cultures of primary and transformed human cells and cells infected with mycoplasma.

MATERIALS AND METHODS

The study was carried out on HeLa cells (cervical adenocarcinoma), HeLa cells infected with mycoplasma, and HUVEC (human umbilical vein endothelial cells). The cells were cultured in DMEM with 10% FCS, 100 U/ml penicillin, and 100 μg/ml streptomycin at 5% CO_2 and 37°C. The HUVEC were obtained by treating the umbilical veins from 4 newborns with 0.1% collagenase-4 (Invitrogen); expression of von Willebrand factor and E-selectin was evaluated as described previously [4,7]. After incubation of the umbilical vein with collagenase, DMEM with 20% FCS without antibiotics was added. After 1.5 month of culturing, light microscopy showed a homogenous population of polygonal cells without long spindle-shaped cells. Mycoplasma was detected in HUVEC and HeLa cells by the PCR analysis of mycoplasma pRNA gene 16S DNA [3].

In order to obtain fractions of free and cell surface-bound extracellular nucleic acids, the cells were washed in phosphate buffer with 5 mM EDTA (PB-EDTA) and fresh culture medium was added. Culture medium was collected after different periods following washing in PB-EDTA and the cells were treated with PB-EDTA and then with 0.25% trypsin. Trypsin was inactivated by adding trypsin inhibitor. Culture medium, PB-EDTA eluate, and trypsin eluate were centrifuged for 10 min at 2000 rpm and filtered through a 1.2-μ filter. The resul-

Institute of Chemical Biology and Basic Medicine, Siberian Division of the Russian Academy of Sciences, Novosibirsk, Russia. ***Address for correspondence:*** morozkin@niboch.nsc.ru. E. S. Morozkin

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tant supernatants were used for isolation of DNA by adsorption on finely dispersed glass as described previously [8]. The concentration of DNA was measured using Hoechst-33258 fluorescent stain [9].

Incorporation of biotinilated dUTP analog into HUVEC extracellular DNA was studied by culturing myc⁻ (intact) and myc⁺ (mycoplasma-infected) HeLa cells with 0.15 mM biotinilated dUTP analog for 14 h. Fractions of extracellular DNA were obtained as described previously. The resultant DNA was separated in 1% agarose gel with ethidium bromide, transferred onto a caprone membrane, and visualized with avidin conjugate with alkaline phosphatase. Biotin-containing DNA was isolated using avidin sepharose. The resultant DNA fractions were tested by PCR analysis for mycoplasma pRNA 16S [3] and human pRNA 28S genes [1].

RESULTS

The process of DNA appearance in the extracellular medium was studied using HUVEC, cervical adenocarcinoma (myc⁻ HeLa) cells, and HeLa cells infected with mycoplasma (myc⁺ HeLa). The curves present the changes in DNA content in extracellular medium for HUVEC, myc⁻ HeLa, myc⁺ HeLa, and cell growth kinetics (Fig. 1). The content of DNA bound to cell surface was constant for all cell cultures irrespective of the period of cell growth. The concentration of DNA in HeLa culture medium increased over 24 h, this parameter being 6-fold higher in myc⁺ *vs.* myc⁻ HeLa cells (Fig. 1, *b*, *c*). In contrast to transformed cell culture, DNA content in primary culture increased only during the first 4 h, after which remained unchanged throughout the entire culturing process (Fig. 1, *a*). Analysis of DNA accumulation kinetics in culture medium

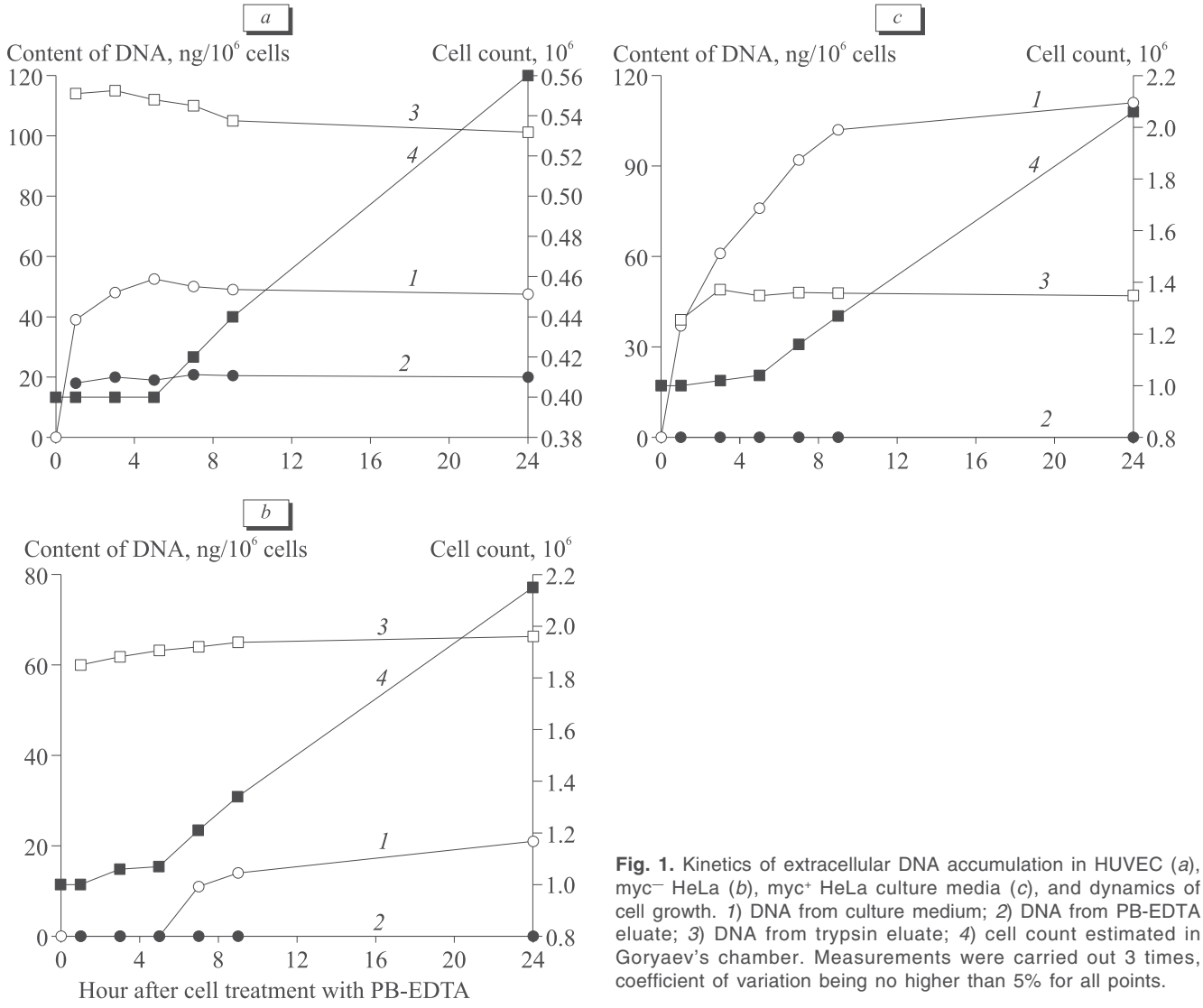

Fig. 1. Kinetics of extracellular DNA accumulation in HUVEC (*a*), myc⁻ HeLa (*b*), myc⁺ HeLa culture media (*c*), and dynamics of cell growth. *1*) DNA from culture medium; *2*) DNA from PB-EDTA eluate; *3*) DNA from trypsin eluate; *4*) cell count estimated in Goryaev's chamber. Measurements were carried out 3 times, coefficient of variation being no higher than 5% for all points.

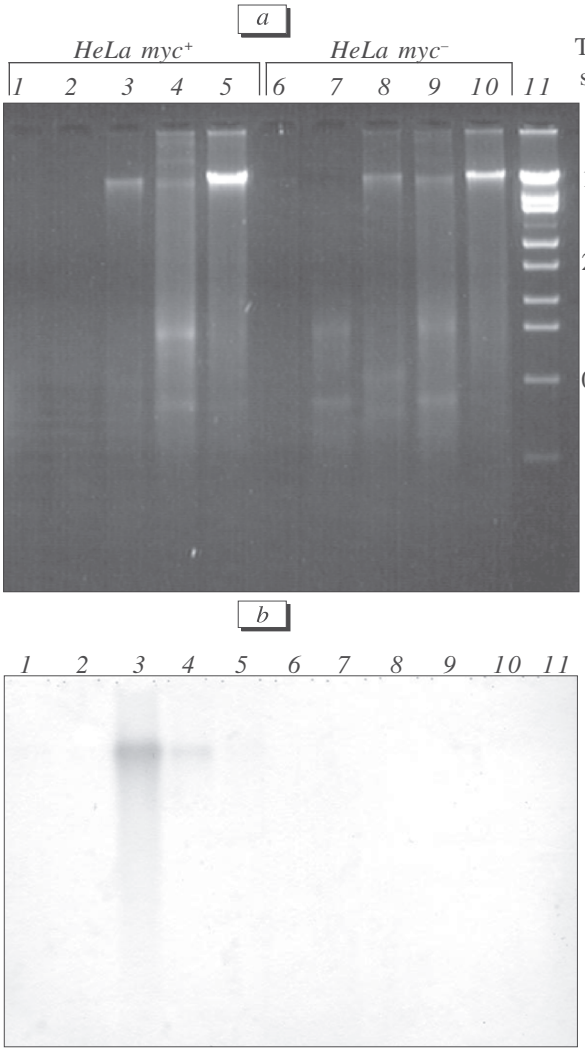

Fig. 2. Modification of extracellular DNA by biotinilated dUTP analog. Extracellular DNA was isolated from culture medium and eluates from cell surface, separated in 1.5% agarose gel, visualized with ethidium bromide (*a*), transferred onto caprone membrane, and visualized by biotinilated DNA with avidin conjugate with alkaline phosphatase (*b*). *1*, *6*) culture medium; *2*, *7*) PB-EDTA eluate; *3*, *8*) trypsin eluate; *4*, *9*) membrane cytosol fraction of cells; *5*, *10*) nuclear fraction; *11*) marker of DNA molecular weight.

and cell growth kinetics indicates that DNA appears in cell culture medium during the lag phase and at the beginning of the exponential growth phase, which suggests that extracellular DNA originates from a source other than apoptotic cells.

In addition to apoptosis or necrosis processes and active secretion of extracellular DNA, some scientists presented evidence of matrix synthesis of DNA in the extracellular medium [1]. We found that during cell incubation with biotinilated dUTP analog, DNA containing biotin residues was located mainly on the outer membrane of mycoplasma-infected HeLa cells (Fig. 2, *a*, *b*). Biotinilated DNA was found also in the membrane cytosol fraction of cells, presumably because of incomplete dissociation of DNA from the cell membrane surface during trypsin treatment of the cells. No biotinilated DNA in the extracellular DNA pool was detected in the cells not infected with mycoplasma (Fig. 2, *b*).

Deoxyribonucleic acid, isolated from trypsin eluate of myc+ HeLa cells cultured with biotinilated dUTP analog, was separated into biotin-containing and non-biotinilated fractions; both fractions were tested for human and mycoplasma genes. Analysis by the PCR method for detection of mycoplasma pRNA gene 16S and human pRNA gene 28S showed only mycoplasma DNA in the biotin-containing fraction and only human DNA in the DNA fraction containing no biotin.

Hence, the kinetics of accumulation of extracellular DNA attests to the mechanisms of active DNA secretion by cells. Synthesis of DNA on the outer cell membrane is realized only in a cell culture infected with mycoplasma, and the newly synthesized DNA is the mycoplasma DNA.

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