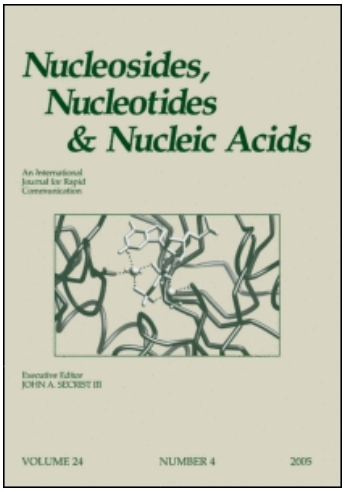

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Uptake of Oligonucleotides by Keratinocytes

P. Laktionov[a]; J-E Dazard[b]; J. Piette[b]; E. Vives[b]; E. Rykova[a]; V. Vlassov[a]; B. Lebleu[b]
[a] Institute of Bioorganic Chemistry, Siberian Division of Russian Academy of Sciences, Novosibirsk, Russia [b] Institute de Genetique Moleculaire de Montpellier, Montpellier, France

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UPTAKE OF OLIGONUCLEOTIDES BY KERATINOCYTES.

P. Laktionov*[1], J-E Dazard[2], J. Piette[2], E. Vives[2], E. Rykova[1], V. Vlassov[1] and B. Lebleu[2].

[1]Institute of Bioorganic Chemistry, Siberian Division of Russian Academy of Sciences, pr. Lavrentieva 8, Novosibirsk 630090, Russia
[2]Institute de Genetique Moleculaire de Montpellier, 1919 route de Mende F-34293 Montpellier, France

ABSTRACT. Oligonucleotides (ODNs) conjugated to rhodamin (Rh) and 4-[(N-2-chloroethyl-N-methyl)amino] benzylamine were used to investigate ODNs transport into keratinocytes. Affinity labeling of two proteins, 63 and 35 kDa, and the inhibition of the affinity labeling and ODNs uptake by the cells in the presence of nucleic acids, polyanions and trypsin suggest, that the proteins are involved in transport of nucleic acids in keratinocytes.

In contrast to many other cells, keratinocytes effectively take up oligodeoxynucleotides from the culture medium and accumulate the compounds in the nuclei. The uptake is unaffected by inhibitors of active transport such as sodium azide, monensin, or chloroquin suggesting the existence of a special mechanism for ODNs transportation into these cells [1]. The objective of this work was to study the interaction of oligonucleotides with proteins of keratinocytes, which may take part in the uptake and intracellular accumulation of the compounds.

The proteins of keratinocytes, involved in the interaction with oligonucleotides were investigated by affinity modification with ^{32}P-labeled 4-[(N-2-chloroethyl-N-methyl)amino] benzylamine conjugated oligonucleotides (ClR-ODNs) as it was described earlier[2]. Two proteins (m.w. 63 and 35 kDa) specifically interacting with oligonucleotides were detected (Kd 1 and 2 μM, respectively). In the cells A431 modified with [^{32}P]ClR-ODN and permeabilized by treatment with streptolysin O or

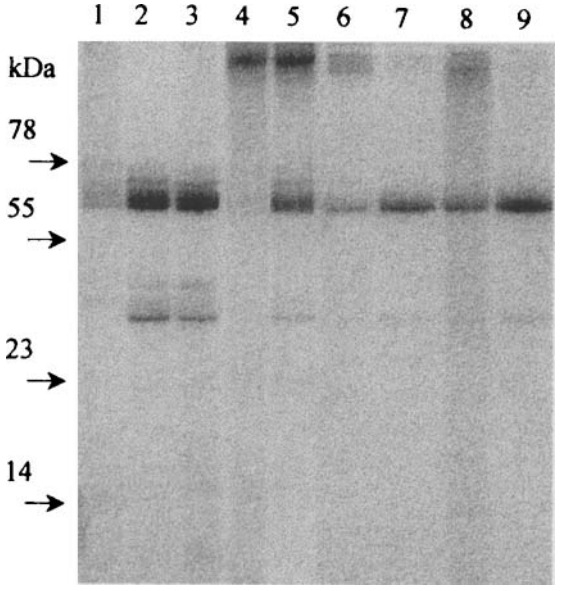

FIG. 1. Cellular proteins affinity labeled with [^{32}P]ClRp(N)$_{18}$. 10-20% SDS PAGE followed by autoradiography. The cells were incubated in PBS with 1 μM [^{32}P]ClRp(N)$_{18}$ for 1 hour at 37°C, washed, scraped from the plate, resuspended in lysis buffer with 1 mM PMSF, 10 μg/ml aprotinin and 150 μg/ml leupeptin containing 1% nonidet NP-40 and separated into the membrane-cytosolic (MC) and nuclear fractions (NF). Modified A431 cells were treated with streptolysine O (str O) (200U/5·10^6 cells), saponin (40 μg/ml) 10 min at room temperature, pelleted at 3000 rpm, supernatants were collected as cytosolic fraction (CF), cells were washed and separated into the MC and NF. 1 - Hela cells MC, 2 - HaCat cells MC, 3 - A431 cells MC, 4 - Hela cells NF, 5 - A431 cells NF, 6 - str O CF, 7 - str O MC, 8 - saponin CF, 9 - saponin MC.

saponin, the labeled proteins remain in the cell membrane, suggesting membrane localization of the proteins. The interaction of [^{32}P]ClR-ODN and uptake of Rh-ODNs by the cells were inhibited by ssDNA, dsDNA, oligonucleotides with phosphodiester and phosphorothioate backbone, and partially by poly IC and polyanions such as dextran sulfate and heparin. Trypsin treatment of the cells quenched the affinity labeling of the 63 kDa and 35 kDa proteins with alkylating ODNs and the uptake of Rh-ODNs. The affinity labelled 63 kDa and 35 kDa proteins were found in nuclear fraction of A431 cells.

This finding and the results of the experiments with inhibitors suggest that the 63 and 35 kDa proteins may be specific transporters of nucleic acids in the nuclei of keratinocytes.

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