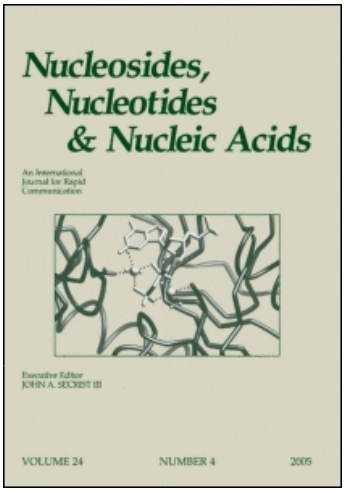

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Nuclear Accumulation of Microinjected Antisense Oligonucleotides

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To cite this Article Leonetti, Jean Paul , Mechti, Nadir , Degols, Geneviève and Lebleu, Bernard(1991) 'Nuclear Accumulation of Microinjected Antisense Oligonucleotides', Nucleosides, Nucleotides and Nucleic Acids, 10: 1, 537 — 539
To link to this Article: DOI: 10.1080/07328319108046520
URL: http://dx.doi.org/10.1080/07328319108046520

NUCLEOSIDES & NUCLEOTIDES, 10(1-3), 537-539 (1991)

NUCLEAR ACCUMULATION OF MICROINJECTED ANTISENSE OLIGONUCLEOTIDES

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ABSTRACT: Antisense oligomers conjugated with various fluorochromes were microinjected in the cytoplasm of cultured cells, and their distribution was followed by microscopy. A fast translocation in the nuclei and a concentration on nuclear structures was observed. Nuclear transport occurs by diffusion probably followed by binding on nuclear proteins.

Antisense oligodeoxyribonucleotides (oligomers) constitute an attractive new class of specific tools for genetic analysis and for potential therapeutic applications. Targets with different cellular locations have been described such as mRNA translation initiation sites, pre-mRNA splicing sites, or genes themselves (1). Oligomers might thus act in the cytoplasm and/or the nuclei. How oligomers internalized in the cytoplasm by receptor-mediated or by fluid-phase endocytosis (2, 3) reach their targets in these two cellular compartments remains an important question to improve the pharmacological properties of these molecules.

We therefore tried to ascertain the fate and the intracellular location of fluorescently tagged antisense oligomers directly introduced by microinjection in the cytoplasm of somatic cells. Fluorescence is essentially found associated with cell nuclei

30 min after microinjection. Nuclear membranes integrity was checked by coinjection of FITC dextran (70,000 mean Mw) which does not diffuse freely through nuclear pores microinjection never induces passage of FITC dextran through the nuclear membrane.

The mechanism of accumulation of oligomers in the nucleus of microinjected cells is independent towards temperature, ATP depletion, and competition with unlabeled molecules. This argue for a diffusion process, compatible with the small size of the oligomer (4). However these data are not sufficient to explain a nuclear accumulation, unless diffusion is followed by nuclear binding (5). In order to investigate potential oligomers binding sites, photosensitive radioactively labeled oligomers were incubated with isolated nuclei or nuclear extracts. Four major bands ranging from 36 kD to 50 kD were observed with intact nuclei. The binding of the oligomers to these proteins can be competed with unlabeled oligomers of the same sequence, with unrelated ones, or with heparin, a sulfated polyanion. However histones do not bind oligomers in intact nuclei, despite of their abundance and cationic charges.

Present data provide interesting prospects in the study of antisense oligomer mechanism of action if microinjection really mimics the step following oligomer escape from the endocytic pathway.

ACKNOWLEDGMENTS.

We are gratful to Pr J.L Imbach for the gift of FITC labeled Alpha-oligomers. J.P.L holds a fellowship of LNFCC and G.D from ARC. This work was supported by grants from ARC, INSERM and CNRS to BL.

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